Abstracts

2

HUGH DINGLE and ROY L. CALDWELL, University of Iowa.

Aggressive, territorial, and reproductive behavior of the mantis shrimp *Gonodactylus oerstedi* (Stomatopoda).

Extensive laboratory and field observations were carried out on the stomatopod *Gonodactylus oerstedi* in Bermuda. When two individuals of the same sex encounter each other, they exhibit marked aggressive interaction with one animal soon becoming dominant. Dominance is influenced, although not necessarily determined, by size, stage in the reproductive cycle (females), and individual behavioral characteristics. Aggressive behavior involves a variety of fixed motor actions including an elaborate threat display making extensive use of color and pattern, antennular flicking, flexing of the abdomen, and attacking and striking with the highly modified second maxillipeds. Because of structural modifications of the cuticle and of body positions assumed during aggression, the strikes of attacking animals are seldom severely injurious. Rather than being destructive, the strike seems to be a highly vigorous "display."

In nature, *Gonodactylus* occupies cavities in coralline rocks, and the aggressive behavior is well adapted for defense of these cavities. The threat display, for example, usually fills the entrance to a cavity and effectively blocks encroachment by an intruder. The occupant may leave briefly to strike another animal or to seize prey, but quickly returns to its home. Territory thus seems to include the occupied rock plus a small area surrounding it. A resident animal undertakes several "housekeeping" activities including cleaning its chamber and closing the entrance at night with small bits of debris and reopening it the following morning. Only a particularly aggressive animal is capable of dislodging a cavity occupant, but eviction takes place rapidly when it does occur.

In the case of interaction between two individuals of opposite sex, the course of behavior is primarily determined by the female. If she is receptive, courtship follows, leading to copulation. If she is not, mutual aggression results, leading to the usual dominant-subordinate relationship.

The only times cavities were found containing more than one adult animal were instances of shared occupancy by a male and female immediately prior to egg-laying. Courtship and copulation in nature presumably take place in a chamber; a few hours before egg-laying the female evicts the male. She then remains in the cavity with the eggs and young, cleaning the egg mass at intervals and picking it up if disturbed. When defending against an intruder she usually leaves the egg mass behind. The larvae remain in the chamber with the mother until the molt to the 4th stadium; following this molt, all leave the chamber within about 2 hours to enter the plankton.

3

JOHN R. HUNTER, U. S. Bureau of Commercial Fisheries.

Light and schooling behavior of juvenile jack mackerel, *Trachurus symmetricus* (Ayres).

Schools of six jack mackerel each were photographed with infrared film at eight different levels of photometric brightness from 8.1 foot-lamberts to 6×10^{-7} foot-lamberts and also in darkness (an unknown level below the sensitivity of the photometer). Three indices were used to measure the behavior of a school from motion pictures. Two of the indices, mean distance to nearest neighbor and mean separation distance, were measures of the distances among individuals in a school and the other, mean angular deviation, was a measure of differences in orientation among individuals (Hunter, J. R., J. Fish. Res. Bd. Can. 23:547, 1966). A value for each index was calculated for each motion picture frame analyzed.

From 8.1 foot-lamberts to 6×10^{-6} foot-lamberts no differences existed in the angular deviation of the school or in the distances among fish. At 6×10^{-7} foot-lamberts the intervals among fish were much larger than at higher levels of brightness and groups showed little uniformity in their orientation. Below 6×10^{-7} foot-lamberts (darkness) schools were dispersed and values of angular deviation for the schools did not differ from a random distribution. Comparison of these data to measurements of light in the sea indicates that sufficient light is available at night for the maintenance of jack mackerel schools.

4

BRIAN A. HAZLETT, University of Michigan.

Inter- and intra-specific aggressive behavior in hermit crabs.

Hermit crabs fight one another for gastropod shells for inhabitation. Exchanges are effected through species-specific behavior patterns which act at a signal level. Experiments in Sweden showed that both *Pagurus bernhardus* and *P. cuanensis* will fight conspecific individuals more often than members of the other species.

However, this preference is based on a shell-inhabitation preference in the case of *Pagurus cuanensis* and a preference for fighting conspecific individuals in *P. bernhardus*. The latter may be due to the differences in the behavioral repertoires of the two species.

5

JOHN J. MAGNUSON, Bureau of Commercial Fisheries Biological Laboratory, Honolulu.

Continuous locomotion in scombrid fishes.

Typical swimming speeds maintained day and night by various scombrids rangs from one to two body lengths per second for fish of 35 cm fork length. Among the functions of locomotion in scombrids are maintenance of hydrostatic equilibrium, gill ventilation, search for food, and simply moving from one place to another. Only the first two require continuous activity. At least two

lines of evidence suggest that maintenance of hydrostatic equilibrium sets the lower limit on swimming speed. First, pectoral fins (the hydrofoils) are almost always extended and the mouth gap is usually slight (Pacific bonita, *Sarda chiliensis,* even close the mouth as much as 42 per cent of the time). Second, a negative relation exists between typical speed and the size of gas bladder and pectoral fins. The larger the gas bladder the less the fish weighs in water; the larger the pectorals the less is the speed required to generate given amounts of lift. Many species have no gas bladder. It is interesting to speculate how its absence in a pelagic fish, with the price of additional expenditures of energy for continuous locomotion, can be adaptive. Near the sea surface scombrids without a gas bladder might be more successful in pursuit of prey fishes, because rapid ascents would not increase the volume of gas so rapidly as to harm the fish. Deeper swimming scombrids would not be so hindered because sudden vertical movements at greater depths have little effect on gas volumes. Species that feed wholly on plankton or typically pursue their prey downward would also not be hampered by a gas bladder.

6

DAVID HUGHES, University of Miami.

The tidal movements of *Penaeus duorarum* Burkenroad (Penaeidae: Crust.). (Introduced by Arthur J. Myrberg, Jr.)

The movements of postlarval shrimp into their shallow water "nursery areas," and the subsequent offshore movements of juveniles and subadults, is apparently largely effected by their passive displacement by the tide. Whereas postlarvae are predominantly collected from flood tides, juveniles are most abundant in ebb tide samples. The method whereby postlarvae and juveniles discriminate between the tides and the manner in which they embark and disembark from them is under investigation.

The perception of very small salinity differences by postlarvae has been shown to be very acute. The changes in salinity incurred with change in tide influences the shrimps' activity levels and orientation. The interaction of these behavioral responses with a biological rhythm controlling activity levels and orientation can largely explain the method whereby the tidal movements are carried out.

7

D. M. ROSS, University of Alberta.

Behavior patterns in sea anemones in relation to commensal habits. (Motion picture)

Sea anemones in a number of families live as commensals with other invertebrates, especially with hermit-crabs and molluscs. Some of these associations have been studied by Ross (Proc. Zool. Soc. Lond., 134:43, 1960; Science, 148:527, 1965) and by Ross and Sutton (Proc. Roy. Soc. B. 155:266, 1961). The anemones *Calliactis parasitica,* a commensal with pagurids, and *Stomphia coccinea,* which lives on the mussel *Modiolus modiolus,* show behavior patterns which depend on responses to organic components of the shells. These "shell-factors" act as releasers for complex sequences of movements showing features which might be described as "perseverance" or "adaptability" if they occurred in higher animals. Cinematographic films and experimental trials with shells (empty, occupied, treated in various ways, etc.) have given information about these behavior patterns. Next steps are to identify the "shell-factors" and to study other such associations. (Supported by Operating Grant A-1445 of the National Research Council of Canada.)

8

ERNST S. REESE, University of Hawaii.

The study of the development of behavior of marine animals. (Motion picture)

Behavior is studied from a conceptual basis: descriptive and comparative, physiological, developmental, and ecological. Evolutionary implications must be appreciated in each. Generally, development of behavior of marine animals has been neglected because of the difficulty of working with marine larvae. However, techniques now available make the rearing of some species possible, and the recent studies on development and early behavior are discussed in reference to the questions asked below.

Organisms represent incredibly complex and highly adapted response systems. Highly adapted behavior need not necessarily be complex, however, and although the two qualifications often are inclusive it is well to recognize their independence when working with larvae. A number of questions may be asked. How does behavior develop in terms of time, mutual interactions, and environmental experience? On what kinds of information is the development based? To what extent can genetic, maturational and experimental factors be studied? What is learned from deprivation experiments and what other experimental approaches are available? What is learned from the comparative approach? When development is delayed, have "subsystems" of behavior evolved in response to the very different needs ecologically and behaviorally of the larvae? Can such sub-systems be used to understand the development of the "adult" specific patterns of behavior? For example, stimuli which act as specific releasers during one stage may not be effective during a subsequent stage, and on the motor side structures used for one function may serve another one later. (Supported in part by grant GB-3651 from the NSF, and by the Eniwetok Marine Biological Laboratory.)

9

JOHN MARK DEAN, Battelle Memorial Institute, Pacific Northwest Laboratory.

The metabolism of intermediary metabolites by tissues of temperature acclimated trout.

One of the interesting aspects of temperature acclimation in poikilotherms is the metabolism of intermediary metabolites and the possible shifting of metabolic pathways with acclimation. This study was concerned with the utilization of acetate and palmitate by rainbow trout (*Salmo gairdneri*) which were acclimated at 5° and 18°C for 3 weeks. Acetate or palmitate labeled with C^{14} was added to homogenates prepared from either liver,

red, or white muscle tissues. They were then incubated at 5°, 11.5° or 18°C for 3 hours when acid was injected to kill the reaction and drive the $C^{14}O_2$ out of solution. The respired $C^{14}O_2$ was trapped in ethanolamine and counted by a standard liquid scintillation method. In addition, the total lipids in the incubation mixture were extracted and the C^{14} incorporated into the total lipids was counted.

The liver of fish acclimated at 5°C had a higher yield of labeled $C^{14}O_2$ when incubated with acetate at 5°, 11.5° and 18°C than did the liver of warm fish. There was also a higher incorporation of labeled acetate into the total lipids of the cold fish than the warm fish when they were incubated at the intermediate temperature of 11.5°C. We have also observed that the red muscle of the trout has a higher level of oxygen consumption, total lipids, cytochrome oxidase and lipase than white muscle at the same temperature. Our present results would indicate that red muscle can oxidize acetate and palmitate at a higher rate than can white muscle. Temperature acclimation may be an important factor in the utilization of acetate and palmitate by msucle tissue. These results will be discussed as they relate to temperature acclimation and the ecology of the rainbow trout. (Work performed under United States Atomic Energy Commission Contract AT (45-1)-1830.)

10

HUGH E. VROMAN, JOHN N. KAPLANIS and W. E. ROBBINS, Insect Physiology Laboratory, Entomology Research Division, ARS, USDA, Beltsville, Maryland.

Cholesterol turnover in the house fly.

The cholesterol turnover in an insect having a short life-span was studied by following the change in specific activity of free and ester 4-^{14}C-cholesterol in male house flies, *Musca domestica* Linnaeus, for 15 days.

Larval house flies were reared aseptically on a semidefined diet containing 4-^{14}C-cholesterol. Male flies were segregated at eclosion and fed a diet containing unlabelled cholesterol. At 5-day intervals 50 flies were removed and the free and ester cholesterol was separated by chromatography of lipid extracts; the specific activity of each fraction was determined.

The specific activities of the free and ester cholesterol declined linearly over the 15 days, and the specific activity of the ester cholesterol remained very close to that of the free cholesterol. A rapid equilibrium between the two pools was indicated. However, the concentration of ester in tissue decreased rapidly over the first 5 days and then remained constant; the concentration of free cholesterol did not decrease until after 5 days.

Apparently the dietary cholesterol is absorbed by the fly, but an overall loss of cholesterol from the tissues of the adult male house fly occurs over 15 days. Additionally, this insect seems to have a "surplus" of cholesterol ester which decreases rapidly to two-thirds the initial concentration.

11

A. E. BARNITT, JR. and C. S. HAMMEN, University of Rhode Island.

Fumarate reductase and coupled phosphorylation in the American oyster, *Crassostrea virginica* (Gmelin). (Introduced by R. W. Harrison)

The American oyster, *Crassostrea virginica* (Gmelin) is a facultative anaerobe, surviving prolonged anaerobiosis, yet consuming oxygen rapidly when it is available. During oxygen lack the oyster accumulates succinate, and probably not lactate. Fumarate reductase activity was investigated as a possible mechanism for the accumulation of succinate and the accomplishment of a coupled phosphorylation. Fumarate reductase activity in a 10% mantle homogenate was determined spectrophotometrically at 25° by the oxidation of reduced riboflavin-5-phosphate. The mean change in absorbance per minute (10 animals) was 0.20 ± 0.07 and the activity was calculated as 34.8 $m\mu$ moles/minute/100 mg tissue. After anaerobic incubation of oyster mantle with 2,3-C^{14} fumarate, the ratio of radioactivity in succinate to that in fumarate was 2.72:1, and after aerobic incubation the ratio was 0.46:1. Anaerobic incubation of mantle homogenate with fumarate, ATP, and NaF indicated no significant removal of free inorganic phosphate, nor did use of a glucose-hexokinase trap demonstrate a coupled phosphorylation. These negative results are not conclusive, and may indicate only a need for better methods. Two experiments in which differential centrifugation was applied to homogenates in 0.20 M TES buffer showed greater activity of both fumarate reductase and succinate dehydrogenase in the soluble than in the particulate fraction. These findings indicate a net reversal of part of the Krebs cycle during anaerobiosis, and explain, in part, the extraordinary tolerance of the oyster to anaerobiosis. (Supported by grant GB-2775 from the NSF.)

12

AURIN M. CHASE, MARTIN CRANE, WILLIAM R. LEAHY, JR., JOHN O. THEOBALD, JR., and SELMA L. LAPEDES, Princeton University.

Protection of kidney mutarotase (aldose-1-epimerase) by D-glucose, 2-deoxy-D-glucose and D-mannose against heat denaturation.

Stabilizing, activating and adverse effects of substrates upon their enzymes are all well known (Grisolia, Physiol. Revs., 1964), and recently Chase and Crane (Amer. Zoologist, 1965, vol. 5, p. 239) reported that the temperature optimum for mutarotase activity shifts from 42°C, when 0.8% glucose is present, to 49°C with 5.0% glucose, indicating protection by substrate.

These experiments have now been extended by studying heat denaturation of mutarotase, alone and also with D-glucose, 2-deoxy-D-glucose or D-mannose present; the last two being inhibitors rather than substrates of this enzyme. These three compounds differ only in C_1 and C_2, so such experiments might be expected to yield information on enzyme-substrate complex formation in this system, and might also ultimately aid in comparing this enzyme with the mutarotases of

P. notatum (Bentley and Bhate, J. Biol. Chem., 1960) and of *E. coli* (Wallenfels and Herrmann, Biochem. Zeitschr., 1965).

The mutarotase was purified from hog kidney by Li's method (Arch. Biochem. Biophys., 1965) and its activity assayed according to Li, Chase, and Lapedes (J. Cell. Comp. Physiol., 1964). Under our experimental conditions inactivation of mutarotase, exposed by itself to a temperature of 50°C, was half complete in 15 minutes. If 3% D-glucose, 2-deoxy-D-glucose or D-mannose was also present during the heat exposure the inactivation rate was significantly less; the deoxy sugar producing the least effect.

These results might be interpreted as indicating that C_1 and C_2 may both be involved in forming the enzyme-substrate complex in this system. (Aided by NSF grants GP-579 and GB-4300.)

13

C. S. HAMMEN, University of Rhode Island.

Carbon dioxide production and fixation in the nematode, *Panagrellus redivivus*. (Introduced by John S. Rankin, Jr.)

The free-living nematode *Panagrellus redivivus*, which is easily cultured on fermenting oatmeal, has been reported to give unusually low respiratory quotients, 0.63 fresh and 0.40 starved, when studied by the paired-flask or Warburg direct method. Several species of plant-parasitic nematodes respire at markedly lower rates when carbon dioxide has been completely removed from the air, and the results above could be due to this effect. We have studied gaseous exchange in *P. redivivus* by means of the newer method of Warburg and Krippahl (1959), which employs a flask with an elevated trough and connecting sidearm, arranged so that alkali can be formed at the end of an experimental period, permitting determination of the oxygen consumption and the carbon dioxide production in each flask. During measurement of net exchange the worms were respiring in an atmosphere with carbon dioxide content of room air gradually increasing to no more than 0.7 per cent. Five experiments with 50 mg of nematodes in each flask gave a respiratory quotient of 0.734 $\pm$ 0.028. Carbon dioxide fixation was measured by incubating worms for 10 minutes at 25° with $NaH^{14}CO_3$ of high specific activity, and counting the radioactivity of dried organic material. The rate was 16.3 micromoles fixed per gram per hour, which was 11.8 per cent of the rate of oxygen consumption measured under equivalent conditions. Addition of this fraction to the carbon dioxide produced gives a respiratory quotient more representative of the diet of this nematode. (Supported by grant GB-2775 from the NSF.)

14

C. M. WINGET, Physiology Branch, Ames Research Center, NASA, Moffett Field, California.

Morphological and biochemical changes associated with a change in photoperiod (*Gallus domesticus*). (Introduced by J. Oyama)

Certain morphological and biochemical changes associated with a change in the photoperiod were observed in 20 female Leghorn chickens. These animals were divided into two groups. The first group was maintained for 168 days in 14 hours of light and 10 hours of darkness (14 L: 10 D); the second group was maintained for 112 days in 14 L: 10 D and 56 days in 0 L: 24 D. The data obtained at time of sacrifice included body, ovary, oviducal, pituitary, diencephalon and pineal gland weights as well as a sample of whole blood. The approximate pineal gland cell size was estimated by counting the number of nuclei per standard field. Sexual maturity was only evidenced in the first group (14 L: 10 D). The weight of the body, ovary, oviduct and pineal gland from the group maintained on light (14 L: 10 D) was significantly greater ($P>0.025$, 0.001, 0.001, 0.05) than those of birds receiving no light (0 L: 24 D). The volume of the pineal gland cells in those birds which were on a 0 L: 24 D schedule was significantly less ($P<0.001$) than the birds receiving light. The birds on 14 L: 10 D displayed a greater enzyme activity in the following enzyme systems evaluated: plasma and pituitary alkaline phosphatase; plasma, pituitary and diencephalon acid phosphatase; plasma and diencephalon cholinesterase.

15

AUSTIN W. PRITCHARD and ROGENE A. KASPAREK, Oregon State University.

The relationship of oxygen tension and metabolism in two mud-dwelling shrimp, *Callianassa californiensis* and *Upogebia pugettensis*.

The blue mud shrimp (*Upogebia pugettensis*) and ghost shrimp (*Callianassa californiensis*) both live in mud flats where the oxygen concentration in the interstitial water approaches zero when the tide is out. The relationship of oxygen tension to metabolic rate was determined for both species, using a Beckman macroelectrode and Model 160 physiological Gas Analyzer. The results showed that both species were good regulators. The critical oxygen tension obtained for *Callianassa*, however, (about 10-20 mmHg) was considerably lower than that for *Upogebia* (about 45-55 mmHg). Heart rates were measured and followed roughly the same pattern as metabolic rate. Water samples taken from *Upogebia* burrows at low tide and analyzed by a Microwinkler procedure, showed an oxygen concentration range of 0.5-1.0 cc/l. *Callianassa* in this area does not construct firm burrows and is probably exposed directly to interstitial water. Analysis of interstitial water for oxygen content showed an almost complete absence of oxygen when the flats had been uncovered for a maximum period. These very preliminary field data suggest that *Upogebia* has somewhat more oxygen available to it during tidal exposure than does *Callianassa*. (Supported by Grant GM-13241-01 from the USPHS.)

16

JOHN L. FREHN, Illinois State University.

Effect of temperature of incubation on liver tissue respiration in cold-exposed rats.

Studies were made of the effect of cold exposure (3-7 weeks at 5°C) on the respiratory activity of both liver mitochondria and whole liver homogenates in rats. Determinations were made polarographically at 5°, 15°, 25°, and 35°C using the

YSI oxygen monitor system and included measurements of the efficiency of oxidative phosphorylation as well as respiration rates in the presence of excess substrate (succinate and beta-hydroxybutyrate), excess substrate plus ADP, and excess substrate plus ATP (homogenates only). The efficiency of oxidative phosphorylation appears to be unaffected in mitochondria from cold-exposed rats at all temperatures using both substrates. The results also indicate that mitochondrial respiration is unaffected by cold exposure at all temperatures. However, the succinate-ADP respiration rate is significantly increased in liver homogenates from cold-exposed rats when measured at 35°C. The significance of these findings is discussed in relation to previous studies which have been carried out at only one temperature. (Supported in part by Research Grant GM 13358-01 from the USPHS.)

17

MUKTI HARIHARAN, Brown University.

Effect of eye-stalk removal on the concentration of carotenoid pigments in the whole crabs (male *Uca pugilator*), digestive glands, hypodermis and shell and isolation, identification, estimation of the various carotenoid pigments. (Introduced by J. P. Green)

Effect of eye-stalk removal on the total concentration of carotenoid pigments in the whole crabs (male *Uca pugilator*), digestive glands, hypodermis and shell was studied and different pigments were isolated, identified and estimated.

Eye-stalks were removed from crabs and after 0, 4, 8, 16, 32 days pigments were extracted with acetone and petroleum ether (b.p. 30-58°C) from the whole crabs, digestive glands, hypodermis and shell. Concentrations of the total pigments were measured from their O.D. using a standard curve.

Amount of carotenoid pigments per gram of wet body weight decreased in the whole crabs after removal of eye-stalks and it was statistically significant after 16 and 32 days post eye-stalk removal.

Eight chromatographically distinct pigments have been isolated from the whole crabs. Five of these fractions have been identified as (1) β-carotene, (2) hydroxyketocarotenoid, (3) flavoxanthin, (4) astacin, (5) a hydroxyketocarotenoid having greater absorptive power than (2). Two of the other new pigments have λ max at 450 mμ and the third one has its λ max at 452 mμ. Total concentrations of the pigments listed above are estimated in various experimental groups.

Pigment concentrations in the digestive glands increased after 4 days and that of hypodermis increased after 16 days post eye-stalk removal. These data indicate that after eye-stalk removal carotenoid pigments from different parts of the body migrate to the digestive gland and are transported to the hypodermis preceding moult.

The experimental data also suggest that the total concentrations of carotenoid pigments in the whole crabs and their distributions in the digestive gland, shell and hypodermis are controlled by hormonal factor(s) present in the eye-stalks. (Supported by the Department of Biology, Brown University.)

18

WILLIAM A. DUNSON and AARON M. TAUB, The Pennsylvania State University.

Salt glands in sea snakes (*Laticauda*).

It is well known that marine birds, turtles and one marine lizard as well as many terrestrial lizards and birds have salt glands. The presence of a salt gland in sea snakes (Hydrophidae) and marine crocodiles (Crocodilia) has not been established. In this study, the response of sea snakes (*Laticauda semifasciata*) to salt-loading has been investigated. Salt-loaded animals were placed in distilled water or 1 Molal sucrose. Urine samples were collected through a glass catheter sewed into the cloacal opening. Extrarenal ion losses were determined by sampling the medium. Studies were also made on salt-loaded snakes kept dry. In these latter animals, a load of 33 mmoles NaCl/kg body weight elicited secretion of a fluid from the mouth between the rostral and mental scales. The Na and K concentration of this fluid was greater than that of sea water.

Snakes placed in distilled water did not demonstrate a capacity for increased extrarenal excretion of NaCl when salt-loaded. They did drink large amounts of water. In 1 Molal sucrose, very little fluid was swallowed, and extrarenal Na loss increased greatly after salt-loading. Equal amounts of Na and Cl were excreted extrarenally; K was lost in much smaller quantities. In 1 Molal sucrose, the extrarenal Na loss was greater than the renal Na loss. The maximum extrarenal rate of Na loss in a salt-loaded snake weighing 290 g was 72 μmoles/100 g hr. Before loading, the rate was 4.4 μmoles/100 g hr. These data demonstrate that the major route of excretion of a Na load in these animals is extrarenal. The salt gland involved in this excretion appears to differ in position and perhaps homology with the glands described previously in reptiles. (Supported in part by NASA Grant Tr. 5994.)

19

LARRY C. OGLESBY, Reed College.

Responses of *Nereis limnicola* to osmotic stress.

Fluxes of chloride and water in the fresh-water nereid polychaete *Nereis limnicola* were studied following the imposition of a sudden osmotic stress of a transfer from one salinity to another. After such a transfer, the chloride concentration of the animal adjusts to that appropriate to the new salinity after about 8 to 12 hours. This adjustment takes a longer period of time in animals initially acclimated to a salinity in which they are capable of osmoregulation, and is due to movements both of chloride and of water down their respective concentration gradients. Movement of chloride, especially, is slowed in animals acclimated to low salinity, even when this movement is in the same direction as the hypothetical active transport of salts. Rates and magnitudes of chloride and water fluxes are much lower than in the less euryhaline *N. succinea* and *N. vexillosa* previously studied (Oglesby, 1965, Comp. Biochem. Physiol. 16:437). These results reinforce the earlier suggestions that active transport of salts from the medium across the body surface is not a major factor in the maintenance of hyperosmotic body fluids in low salinities, and that *N. limnicola* is less permeable to salts when acclimated to low salinities. (Supported by grant GB-4429 from the NSF.)

20

BERTWELL K. WHITTEN and CLARENCE J. GOODNIGHT, Western Michigan University.

Strontium-89 and calcium-45 accumulation in an aquatic oligochaete.

The accumulation, retention and tissue distribution of Sr-89 and Ca-45 in tubificid worms of the genus, *Limnodrilus*, was studied. A non-linear regression analysis program was utilized to analyze the data. The rate of accumulation and loss of strontium was slightly slower than that of calcium. A nearly linear reduction in the accumulation of the radionuclides occurred with an increase in the stable calcium concentration of the medium.

Radioautograms demonstrated that the radionuclides were heavily concentrated on the surface of the epidermis; in the epithelial tissue of the epidermis; and in the ampulla region of the nephredia. The heavy concentration in the epithelial region of the epidermis is probably due to surface adsorption and to the formation of calcium or strontium complexes with the mucoprotein secretions from the epithelial tissues. (Supported by Public Health Service Research Grant RH 00215.)

21

DAVID H. EVANS, Stanford University.

Sodium, chloride and water balance of a euryhaline, intertidal teleost.

The intertidal teleost *Xiphister atropurpureus* is able to withstand salinities ranging from 100% sea water (480 mM Na/kg; 560 mM Cl/kg) to 10% sea water. Renal losses (measured by open cannulation) are .48 mM Na/kg/day, .60 mM Cl/kg/day and 3.5 ml water/kg/day in 100% sea water and .62 mM Na/kg/day, .48 mM Cl/kg/day and 9.5 ml water/kg/day in 10% sea water. Inflow by drinking (measured using C-14 Inulin) is 3.8 mM Na/kg/day, 4.5 mM Cl/kg/day and 8 ml water/kg/day in sea water and .77 mM Na/kg/day, .90 mM Cl/kg/day and 1.6 ml water/kg/day in 10% sea water. The parts played by diffusion, active transport and osmosis in the total balance were measured by the efflux of Na-22, Cl-36 or tritiated water from injected fish. In 100% sea water diffusional influx is 105 mM Na/kg/day and 70 mM Cl/kg/day; diffusional efflux is 36 mM Na/kg/day and 21 mM Cl/kg/day; active extrusion is 72 mM Na/kg/day and 54 mM Cl/kg/day. In 10% sea water diffusional influx is 3.3 mM Na/kg/day, and 4.7 mM Cl/kg/day; active uptake is 6.1 mM Na/kg/day and 6.4 mM Cl/kg/day; diffusional loss is 9.6 mM Na/kg/day and 12 mM Cl/kg/day. Net water flux in sea water (27 ml/kg/day) is 1% of the total flux and approximates the drinking rate. Net water flux in 10% sea water (9.4 ml/kg/day) is 0.4% of the total flux and approximates the urine loss. Thus, *Xiphister* appears to be a relatively impermeable fish that is able to change its permeability to sodium and chloride when transferred to 10% sea water. (Supported by a USPHS Predoctoral Fellowship.)

22

WILLIAM E. BRADSHAW and DAVID G. SHAPIRIO, The University of Michigan.

The role of food photoperiod, and temperature in the termination of larval diapause in *Chaoborus* (Diptera, Culicidae).

When *Chaoborus* larvae are captured from ice-covered ponds in January and maintained in the laboratory at 24°C, on short day photoperiod (8 hours light in 24; 8:16) without food, only 1-2% initiate metamorphosis. This arrested development appears to be a larval diapause in which appropriate combination of temperature, photoperiod, and nutritional stimuli are required for resumption of development. Analysis of the contol of diapause in *Chaoborus* has been initiated, with particular reference to photoperiod and nutrition. Larvae fed but kept on short daylength showed much lower and more erratic termination of diapause than larvae fed and maintained on long daylength (17:7). Moreover, larvae kept on long daylength, but starved, failed to show a high proportion of development. Two days' exposure to food and long daylength was adequate to induce a maximum rate of pupation in about 50% of animals treated, but longer exposure was necessary for pupation in 90% of the animals. The same batch of larvae showed a change in response during eight months' maintenance at 5°C. on 8:16 photoperiod without added food. This change involved a shift in the dominant stimulus for termination of diapause from long daylength to food, when studied at 24°C. This shift to food as the major stimulus may provide a means of discriminating between food as primarily a nutritional requisite for development, and food as a physical, environmental cue, analogous to the role of abdominal distension in *Rhodnius*. (Aided by PHS Grant GM-06101.)

23

SUZANNE GASTON and MICHAEL MENAKER, University of Texas.

Photoperiodic response of hamster testes.

Two experiments were carried out to describe the effect of different photoperiodic regimes on testis weight in hamsters. In one experiment juvenile males were raised to sexual maturity in 8 separate light-tight boxes (6 animals/box) each on a different 24-hour light cycle (400-500 lux) varying from continuous darkness to continuous light. After one month of treatment the right testis was removed and the animals were returned to the photoperiod from which they were taken for another month, at which time the other testis was removed. After the first month, testis weights were different only at the extremes—the average testis weight on 22 hours of light was twice the average of those on 2 hours. However, after the second month, the unilaterally castrated animals showed a more marked response at all photoperiods; average testes weights increasing 4-fold from short to long day lengths. These results suggest that photoperiod had little or no effect on testes during growth.

The purpose of the second experiment was to clarify the photoperiodic response of adult testes. Juveniles were maintained 6 per cage for 6 weeks on LD 13:11, then placed on different light regimes in 11 light-tight boxes for 6 weeks. A photoperiodic response of testicular weight was

obtained, having a break point between 11 and 12½ hours of light. Average testes weights on longer photoperiods showed a 5-fold increase over those on day lengths of less than 11 hours. The response was better defined in the second experiment than for either curve in the first. This can be explained if one assumes compensatory mechanisms are operative during the second month of the first experiment.

It has previously been suggested that caging 2 or more hamsters together would obscure the response to low photoperiods. Such an effect was not observed in these studies. Testicular weight bears no relationship to body weight under any conditions tested, and body weight is not related to photoperiod. (Supported by a grant from the University of Texas Research Institute, NSF Grant GB-3806, NIH Training Grant 5-T1-GM-836-03.)

24

ALAN PRIEST BROCKWAY, The Ohio State University.

The spiracular filters and water loss in pupae of two species of *Hyalophora*.

The spiracular filters covering the spiracular openings impede bulk flow of air. Because of the pore effect, this resistance to bulk flow does not necessitate reduced diffusive exchange. Excised spiracular filters of *Hyalophora cecropia* and *H. gloveri*, mounted on short segments of water-filled polyethylene tubing, were placed over P_2O_5 in still air for two hours. The tubes plus the filters were weighed, replaced over P_2O_5 and weighed two hours later. The rate of water loss over this second two-hour period was calculated. The integrity of the spiracular filters was destroyed by breaking the "teeth," the water level restored and the rate of water loss over the second two-hour period determined. For *H. cecropia* the average rate through intact filters was 0.36 ± 0.04 mg/hr; through the broken filters 0.40 ± 0.06 mg/hr ($P<0.10$). For *H. gloveri* the rates were respectively 0.29 ± 0.08 mg/hr and 0.45 ± 0.08 mg/hr. This difference is significant ($P<0.01$). This relationship was confirmed for intact pupae of *H. gloveri* over P_2O_5 in high P_{CO_2} (to hold the spiracular valves open). The average weight loss over a two-hour period was 0.451 ± 0.127 mg/g/hr when the spiracular filters were intact and 0.697 ± 0.098 mg/g/hr when broken ($P<0.01$). Thus the structure of the spiracular filters can reduce diffusive gas exchange as well as impede bulk flow.

The differences in the structures of the spiracular filters of these two species will be discussed.

25

THOMAS E. MOORE and ROY T. SAWYER, The University of Michigan.

The mechanism of cicada timbal action (Insecta: Homoptera: Cicadidae).

Stroboscopic, audiospectrographic, and oscillographic analyses of disturbance squawks of intact and mutilated males of three species of 17-year cicadas (Tibicininae: *Magicicada*) suggest the following conclusions concerning timbal action in cicadas: 1) timbals operate alternately, the in-pop of one of the pair normally overlapping the out-pop of the other; 2) the greater energy produced during an in-pop obscures the less intense out-pop of the opposing timbal, except at the end of songs; 3) when contralateral in- and out-pops are not synchronized the sound sequence is of short duration; 4) timbals are essentially composed of a membrane supporting a posterior plate (connected dorsally to its muscle by an apodeme) and a series of anterior smaller ribs (from 3 to 13, depending on the species) plus a few even smaller plates near the line of buckling: 5) the plate and ribs have two positions, timbal-muscle contraction causing low amplitude movement of the plate and higher amplitude movement of ribs associated with popping from rest position to the other position while buckling along an anterior-posterior line, timbal-muscle relaxation allowing return to rest position; 6) each buckling rib produces a burst of sound, the rib nearest the plate buckling inward first and outward last, and rib structure is associated with the sort of sound produced; 7) tracheal sacs function as resonant chambers, condensing the band of emphasized frequencies and increasing overall sound-levels by about 5 db at 2 inches. (Supported by NSF grant GB-3850.)

26

B. W. GEER, Knox College.

Choline activity in the reproduction of *Drosophila melanogaster*.

Adult *Drosophila melanogaster* that have been grown on a carnitine supplemented diet fail to reproduce unless choline is included in their diet. The reproductive capacity attained is dependent on the concentration and the length of time that choline is fed. Following the initial feeding of choline, a lag period ensues before the adults begin to reproduce. Matings with choline-raised adults show that carnitine-raised males are sterile, whereas carnitine-raised females have a restricted reproductive capacity. Compared with choline-raised females, carnitine-raised females lay fewer eggs and a lower percentage of the eggs hatch.

By feeding choline substitutes, the activity of choline for the fecundity of carnitine-raised adults has been shown to be very specific. Homocholine stimulates egg production, whereas β-methylcholine, 2-dimethylaminoethanol, sulfocholine, betaine, monoethylcholine, and diethylcholine have no effect on females. None of the choline substitutes promotes male fertility. Feeding adult choline-raised flies a carnitine supplemented diet reduces their reproductive capacity significantly. (Supported by Grant GB-4838 from the NSF.)

27

RICHARD L. MILLER, Oregon State University.

Chemotaxis during fertilization in the hydroids *Tubularia* and *Gonothyrea*. (Motion picture)

Species specific chemotaxis of the sperm of two species of the hydroid genus *Campanularia* has been described (J. Exp. Zool., 162:1-22). This phenomenon has been observed in three more species in two widely separate genera: *Gonothyrea* and *Tubularia*.

The sperm of *Tubularia crocea* and *T. marina* are attracted to the tentacular area of their respective female medusoids (sporosacs). Qualitative cross-reactivity exists between these two species,

The sperm of *Gonothyrea clarkii* are attracted to the base of the tentacles of the female medusoid (meconidium) and possibly to the tentacles as well. Many sperm strike the tentacles and stick to them, twisting about until they come to lie close to the tentacle, parallel to its long axis. They then begin to migrate along the tentacle either proximally or distally. In the best preparations, the sperm that migrate distally turn around by flipping their heads off the tentacles and reversing direction 180°. Sperm migrating in either direction may turn in this way but the most common direction taken up is proximal. The sperm appear to move more rapidly as they near the basal area. They do not respond to hydranth tentacles in any way.

Active alcohol extracts of the gonangia and colonies of *G. clarkii* and of the hydranths of both species of *Tubularia* have been prepared. The biological and chemical equivalence of the two *Tubularia* extracts has not been determined. It is significant, however, that the two species normally occupy different habitats. The extract prepared from *Gonothyrea* tissues attracts *Gonothyrea* sperm weakly, *Campanularia flexuosa* sperm not at all, and *C. calceolifera* sperm strongly. *C. calceolifera* sperm migrate on the medusoid tentacles of *G. clarkii* and the sperm of *G. clarkii* are attracted to the aperture of the female gonangium of *C. calceolifera*. The sperm of *C. flexuosa* do not stick to or migrate on the tentacles of the *G. clarkii* medusoid. Both *Campanularia* species are apparently restricted to the Atlantic while *G. clarkii* is a Pacific form.

Migration of *G. clarkii* and *C. calceolifera* sperm along tentacles appears to be a situation where chemotaxis and contact guidance of a rudimentary type are combined. It also seems likely that the species specificity during chemotaxis does not exist between hydroids that have never competed for each other's sperm. (Supported by an NIH postdoctoral fellowship.)

28

MARIE A. DIBERARDINO, THOMAS J. KING, and LINDA BOHL, Institute for Cancer Research.

Nuclear transplantation of differentiated male germ cells.

Amphibian nuclear transplantation studies have demonstrated that most somatic nuclei undergo progressive developmental restrictions during embryogenesis (see Gurdon, in "Advances in Morphogenesis," vol. 4 Abercrombie and Brachet, eds., Academic Press, 1964; King, in "Methods in Cell Physiology," vol. 2, Prescott, ed., Academic Press, in press). By contrast, primordial germ cell nuclei have been found to be developmentally totipotent (Smith, Proc. Natl. Acad. Sci., U. S. 54:101, 1965). In the present study the developmental capabilities of nuclei from differentiated germ cells of metamorphosed and adult *Rana pipiens* have been tested via transplantation into enucleated host eggs. Donor nuclei were obtained from primary spermatogonia of the metamorphosed frog and in the case of the adult from a mixed population of cells consisting of primary and secondary spermatogonia and primary spermatocytes which were not distinguishable from each other.

Nuclei of metamorphosed and adult testes promoted blastula development in 16% and 10% of the cases, respectively. Most of the complete blastulae obtained from these two sources arrested before completing gastrulation; none developed into normal larvae. By comparison, control transfers of nuclei from undetermined blastula and gastrula cells promoted blastula formation in 77% of the cases and 54% of the complete blastulae developed into normal larvae.

These studies indicate that genetically totipotent nuclei of definitive male germ cells undergo during their differentiative phase a regulatory change which restricts their capacity to promote the normal development of test eggs. The basis of this change will be discussed. (Supported by USPHS grants CA-05755 and CA-06927.)

29

MONA L. RUBIN, DIANE B. AR and GEORGE W. NACE, The University of Michigan.

The virus inhibiting action of a lysozyme present in normal frogs but lacking in tumor frogs and eggs.

An antigen present in normal but absent from kidney adenocarcinoma frogs was identified and subsequently shown to possess bacteriolytic activity. Seasonal changes in this activity, differences between populations with different incidents of this viral tumor, and the suggestion that lysozyme may have antiviral properties led to a new hypothesis concerning the etiology of this tumor (Nace *et al,* Ann. N.Y. Acad. Sci., 126:204, 1965).

On attempting purification this enzyme was found to exist in three forms. Only L-3 was found in *Rana pipiens* eggs. L-3 and L-1 were found in skin. L-2 and L-1 were found in normal kidney, but only traces of L-3 were found in the kidney tumor.

Antiviral activity of these enzymes was tested using FV-3 virus isolated from tumor bearing frogs and obtained from Dr. K. A. Rafferty. This virus forms well defined plaques in monolayer cultures of fathead minnow cells grown in modified Eagle's Basal Medium at 22°C for 5 days. Preincubation of the virus in a mixture of frog lytic substances L-1 and L-2 led to complete inhibition of plaque formation at enzyme concentrations equal to or above normal physiological values. Neither a mixture of frog forms L-1 and L-3 nor chicken egg white lysozyme inhibited plaque formation until extremely high concentrations were reached. Susceptibility of tadpoles to this virus and aspects of the etiology of this tumor become understandable in the light of these results. (Supported by USPHS grants GM 05409 and CA 06929 and American Cancer Society Institutional Grant IN-40.)

30

WILLIAM A. ELMER and LOUIS J. PIERRO, University of Connecticut.

Analysis of the growth inhibitory factor of Creeper embryos.

The presence of a growth-inhibitory substance in embryonic Creeper tissues has been suggested by two reports. Wolff and Kieny (Develop. Biol., 7:324, 1963) reported that growth *in vitro* of tibiotarsi from normal embryos is inhibited by the

presence of Creeper embryo extract in the culture medium. We have confirmed this observation, and found that similar results are obtained when tibiotarsal rudiments from Creeper embryos are cocultured with rudiments from normal embryos in the presence of extract prepared from normal embryos (Amer. Zool. 4:1404, 1964). Continued analysis of these effects have been concerned with (1) obtaining more precise control over the assay system, (2) investigation of the biochemical nature of the assumed inhibitor and (3) study of the incorporation of labeled precursors into macromolecules in normal and in Creeper tibiotarsi. Preliminary data confirm the observation of Kieny (Arch. D'Anat. Micro. et de Morph. Exp., 51:577-586, 1962) that the growth-inhibitor is acetone precipitable. Data obtained from incorporation studies suggest that in the presence of Creeper rudiments, synthesis of protein in normal tibiotarsi is inhibited by the end of 24 hours in culture. This inhibition is reflected in a statistically significant difference in protein content by the end of 72 hours. (Supported by grants GB1940 and GB4714 from the NSF.)

31

LOUIS J. PIERRO and JEAN SPIGGLE, University of Connecticut.

Abnormal separation of the lens vesicle and corneal opacities in C57 Black mice.

Corneal opacities can be detected in a small number of C57 Black mice following the opening of the eyelids. Discrete small opacities are found in eyes of normal size; more generalized opacities are found in eyes which are reduced in size but are large enough to protrude through the eyelids. In eyes with discrete opacities the cornea is characterized by a gap in the stroma and disorganization of the overlying epithelium. Similar anomalies are found in eyes with more generalized opacities, and in addition, the cornea and iris are continuous along their stromal components. Various stages in cataract formation are seen in eyes with either type of corneal defect. Abnormalities observed in eyes of embryonic and neonatal mice include connection of the lens vesicle to the surface ectoderm beyond the stage that separation normally occurs, fusion of the lens to the developing cornea, fusion of the developing iris to the cornea, and various irregularities in the corneal stroma and the lens. These observations suggest that the corneal opacities are related to the delayed and abnormal separation of the lens vesicle from the surface ectoderm and the consequent block in the migration of mesenchymal cells which contribute to the stroma of the cornea. A role of the altered corneal-lens spatial relationship in the initiation of cataract formation also seems likely. (Supported by grant HD 01061 from the USPHS.)

32

KARL S. WITTMAN, The City College of New York.

Effect of genetic background on expressivity and penetrance of the "Brachyury" gene in the mouse. (Introduced by William Etkin)

The effects of different genetic backgrounds on the expressivity and penetrance of the mutation "Brachy" (T/+) locus in mice was tested.

Heterozygotes of the Brachyury (T/+) mutation are characterized by shortened tails.

In the black spot strain where this mutation is originally maintained, the tail shortening is the result of 1) vertebral fusion, 2) fragmentation of tail vertebrae, and 3) reduction in number of tail vertebrae.

Brachy mutants of the black spot strain were outcrossed to C57Bl and DBA/2J strains. On the C57Bl background, the T/+ heterozygotes formed tails whose average length was slightly greater than that of T/+ heterozygotes of the black spot strain. The number of vertebral fusions was the same on the two backgrounds, but reduction in number of tail vertebrae was less severe on the C57Bl background than on the black spot background.

On the DBA/2J background, the "Brachy" gene produced tails with less severe shortening than in the other two strains examined. Vertebral fusion was absent in T/+ DBA heterozygotes and the number of tail vertebrae formed was higher than in heterozygotes of either strain.

Crosses between T/+ heterozygotes obtained from the black spot strain when outcrossed to normal DBA/2J mice led to a ratio of 3 normal to 1 short tailed, instead of the expected 1:1 ratio.

These experiments indicate that the residual genetic background modifies both penetrance and expressivity of the "Brachy" gene. (Supported by Training Grant 5T01 HD00116 awarded to Max Hamburgh.)

33

PATRICIA A. SMITH, Northwestern University.

The origin of tumorous chambers in the ovary of the *fused* mutant of *Drosophila melanogaster.*

The morphology of the normal germarium has been described by Koch and King (J. Morph., in press). The germarium is subdivided into three regions: (1) the mitotically active area where clusters of 16 interconnected cystocytes originate from apical cystoblasts which have undergone four consecutive divisions, (2) the region where the cystocytes are covered by centripetally migrating mesodermal cells, and (3) the region where the germarial cyst is transformed into the first egg chamber in the vitellarium. Females which are homozygous for the sex-linked, recessive, semisterile gene, *fused,* produce ovarian tumors. The ovaries of such females are normal in morphology at eclosion, but tumors form as the fly ages. A three dimensional reconstruction was made from electron micrographs taken of thin sections from different levels in a germarium from a five-day-old *fu/fu* female. Region three of the germarium appeared normal. Region two contained three 16-cell cysts, each enclosed by follicle cells, and two cysts sheathed in a common mesodermal envelope. Thus, in some cases, compound chambers arise in *fu/fu* because different adjacent cysts are not separately enclosed by follicle cells. Region one was abnormal both in the number and in the location of large single cells. In the normal germarium there are only one or two single cells which are located apically. In the *fu/fu* germarium 10 single cells were found, some of which were subapical. Also, invasion by mesodermal cells occurred in region one. The origin of tumorous chambers is

discussed in relation to these findings. (Supported by grant 1-F2-GM-24,082-01 from the USPHS.)

34

WILLIAM S. KLUG and ROBERT C. KING, Northwestern University.

The ovarian pathology of the *suppressor*^{2} of *Hairy-wing* mutant of *Drosophila melanogaster*.

E. B. Lewis demonstrated that the recessive gene, *su*^{2}*-Hw* (3-54.8), when homozygous suppresses various specific alleles of many genes (*Bx, bx, bxd, ci, ct, dm, f, Hw, lz, sc,* and *y*). Flies homozygous for this suppressor show only one striking morphological abnormality—female sterility. To obtain information concerning the ovarian phenotype, light microscopic studies were made of Feulgen-stained whole mounts and azure B-stained, plastic embedded sections of ovaries from mutants of various ages. Vitellogenesis was found to be greatly inhibited. As a result, the oocyte grows very little and becomes enveloped by multiple layers of follicle cells. Normally, the oocyte receives a stream of RNA-rich cytopasm from adjacent nurse cells. However, the concentration of nuclear and cytoplasmic RNA within mutant nurse cells is reduced, and the distribution of DNA is abnormal. During normal oogenesis, the nurse cell chromosomes undergo a series of endomitotic replications resulting in a jumbled mass of Feulgen-positive threads which fills the nucleus. However, the mutant nucleus contains four or five well defined, condensed masses of Feulgen-positive material which increase in size as the nurse cell grows. Between these chromosomal masses are found multiple, small nucleoli. Normally, nucleoli grow and fuse into ribbon-like chains. This female sterile mutation therefore resembles the mutants *fs 2.1, mr,* and *sn*^{36a} which show an abnormal nuclear behavior of nurse cells and a concurrent retardation of vitellogenesis. (Supported by grant 5TIGN903 from the USPHS.)

35

ROBERT ARKING and RALPH HILLMAN, Temple University.

The developmental genetics of the *eyeless-Dominant* locus in *Drosophila melanogaster*.

This study concerns the effects of the mutation *eyeless-Dominant* (*ey*^{D}) upon the development of *Drosophila melanogaster*. The mutation, reported as a lethal addition, is associated with a within the fourth chromosome.

Two types of reciprocal pair matings have been analyzed: (a) the P-1 cross of the balanced lethal, *sv*^{de}*//ey*^{D}, with an inbred *Oregon-R* strain; and (b) the F-1 cross of *ey*^{D}*//+* × *ey*^{D}*//+*. Both of these give offspring which fall into one of three classes: (1) viable flies with normal eyes; (2) viable flies exhibiting a great decrease in the number of facets/eye; and (3) flies that die during the early pupal stages.

Most of the work has involved the lethal progeny. Histological examination of the abnormally developing pupae has revealed, in both types of crosses, a characteristic syndrome of diverse developmental abnormalities which are all related by a common cessation of development during the prepupal period. The survival data in the embryonic, larval and pupal stages of the F-2 progeny have revealed the existence of non-pupating larvae which do not undergo further molts but remain in the third instar for periods as long as 120 hours after their normal sibs have pupated. At this time they either die or undergo an abortive pupation. The phenocritical phase in the *ey*^{D} therefore occurs in the last few hours of the third larval instar.

These data suggest that the ecdyson/juvenile hormone balance is affected by the *ey*^{D} mutation. Experiments are now in progress to elucidate the precise mode of action of this locus. (Supported in part by a NSF Predoctoral Cooperative Fellowship and in part by Grant GM 10480 from the USPHS.)

36

HARRY SENECA and PAT PEER, Columbia University, College of Physicians & Surgeons.

Trypanosoma cruzi lipopolysaccharide.

Chagastoxin or the lipopolysaccharide of *Trypanosoma cruzi* is extracted with 88% phenol from virulent *T. cruzi*. It is a white amorphous precipitate, insoluble in water, saline, alcohol, ether, acetone, acids and alkalis, unstable at room temperature, and phenol saline suspension readily loses its potency even in the ice box. It maintains its potency when lyophilized.

In doses of 25 to 200 μg, it imparts partial protection to mice, but in 500 μg dose times 16, it produces solid immunity.

Massive doses activate latent chronic *T. cruzi* infections in mice, resulting in death, but less than 10 μg has no such effect.

Intradermal injections, in infected and immunized rabbits give rise to skin reaction, but normal animals do not respond.

The lethal dose is 500-1,000 μg given in four successive doses. In such animals, there is focal necrosis in the liver, microscopic hemorrhages and round cell infiltration. The kidneys show tubular necrosis and microscopic hemorrhages, the glomeruli get involved much later. Heart shows muscular degeneration and round cell infiltration. Microscopic hemorrhages are also present in adrenals and spleen.

It induces hyperpyrexia and leucocytosis in rabbits. The sera of immunized sheep and rabbits contain agglutinins, precipitins, immobilizing and protective antibodies.

37

RONALD A. BAYNE, CHARLES A. KALAF, and JOHN F. ROBERTS, North Carolina State University.

Protein compositions of mitochondrial fractions of trypanosomes.

The proetin compositions of mitochondrial fractions from the culture form of *Trypanosoma conorhini* and the kinetoplastic and akinetoplastic forms of *T. equiperdum* were compared by gel filtration elution patterns and disc gel electrophoresis.

Mitochondrial fractions were obtained by differential centrifugation of trypanosomal homogenates by the method of Agosin and von Brand (Exptl. Parasitol., 4:548, 1955). These fractions, containing

flagellar and lipid materials as well as mitochondria and kinetoplasts, were solubilized with sodium dodecyl sulfate (SDS) and chromatographed on a Sephadex G-100 gel column (1.5 × 80 cm) equilibrated and eluted with 0.3% SDS. The initial elutions were concentrated by dialysis and further separated by polyacrylamide column electrophoresis by the method of Takayama, MacLennan, Tzagoloff, and Stoner (Arch. Biochem. Biophys., 114:223, 1964).

Elution and electrophoretic patterns for preparations of the culture form indicated a more complex protein composition than the blood stream forms (6 vs. 3 elution peaks and 13 vs. 5 electrophoretic bands). These findings support the hypothesis that respiratory differences between these forms are due to changes in enzymatic composition. There appears to be no difference in the protein composition of kinetoplastic and akinetoplastic forms of *T. equiperdum*. These results are interpreted in relationship to proposed kinetoplast function.

39

KENNETH GOLD, Osborn Laboratories of Marine Sciences.

The role of ciliates in marine ecology. I. Isolation and cultivation of a member of the Order Tintinnida. (Introduced by R. F. Nigrelli)

A species of *Tintinnopsis,* abundant in the plankton off the jetty adjacent to the New York Aquarium during June, 1966, was micropipetted in large numbers into cold, sterile sea water. This is the first record of the continuous cultivation of a tintinnid *in vitro,* and the results of the treatments used and the requirements for maintenance of the ciliates are reported here.

Cultures of *Tintinnopsis* sp. were maintained in heat sterilized sea water. An antibiotic mixture, used in the original isolation, was added to the sea water periodically to prevent the accumulation of large numbers of bacteria. Additions of the penicillin-streptomycin mixture resulted in a final concentration of 50 μg each per ml.

Cultures can be maintained in the light or dark at temperatures in the range 11-15°C. If cultures are kept in the dark, (the usual practice), feeding must be frequent but sparse. Usually, small numbers of food organisms are added at 2-3 day intervals or when food is completely grazed down. Illuminated cultures are fed less frequently or not at all, depending upon the rate of grazing by the ciliates and/or rate of growth of the prey.

Foods used to grow the tintinnids are yeast, *Saccharomyces cerevisiae,* and the flagellates *Isochrysis galbana, Rhodomonas lens,* and *Platymonas* sp. No attempt has been made to free the tintinnids of bacteria completely. While all of the foods seem to be ingested (and digested) rapidly, it is not certain whether they are all required. Tracer experiments are in progress to clarify food preferences. (Supported by Contract NYO-3658-2 with the U. S. Atomic Energy Commission.)

40

EUGENE C. FISCHER, St. John's University and U.S. Naval Applied Science Laboratory.

Growth of ciliates under high hydrostatic pressure. (Introduced by D. M. Lilly)

Five species of Tetrahymenid protozoans were subjected to increased salinity of the medium and found to be eurhyaline, tolerating full-strength sea water. Osmosensitivity of ciliates was reduced by increased incubation temperature. Increased salinity prolonged the lag and stationary phases of growth, while decreasing population densities. When ciliates were compressed hydrostatically, the immediate morphological responses (sphere formation and loss of ciliary activity) were reduced by the increased osmolarity of the medium. Growth of all organisms tested was possible under compressions to 3000 psi at 19°C. Hydrostatic compression was depressive to the log phase of growth, but this depression was reduced by increased salinity. Oxygen metabolism of pressurized cultures was greater than that of unpressurized control cultures at atmospheric pressure. Distinct levels of oxygen toxicity were found in pressurized cultures, the toxicity of environmental oxygen being increased by hydrostatic compression. Hyperbaric oxygen was highly stimulatory to Tetrahymenid growth except when oxygen concentration in the medium was increased by means of gas pressure according to Henry's Law.

It may be concluded that the methods developed for the culture of protozoans under high hydrostatic pressure will be of value in the study of deep ocean pressures on the metabolisms of planktonic and benthic microorganisms.

41

ROGER H. TRUMBORE and STEPHEN J. KAUFMAN, State University of New York at Binghamton.

Some effects of 8-azaguanine on *Brachiomonas submarina* grown in synchronous culture.

The imposing of a light-dark regimen of either 8 dark and 16 light or 12 dark and 12 light causes a synchrony of cell division in *Brachiomonas submarina* with the cell division occurring only during the dark phase. The high percentage of cells in approximately the same physiological state provides an ideal tool for the effect of metabolic inhibitors on the life cycle of the cell.

Cultures of *Brachiomonas submarina* were grown in defined medium in a cycled light-dark sequence of 12 hours dark-12 hours light at 500 foot-candles and 24°C. The defined medium was supplemented with various concentrations of 8-azaguanine. Final 8-azaguanine concentrations of 1×10^{-3} M to 1×10^{-6} M were inhibitory to cell growth without being toxic. At these concentrations, the characteristics of the cells in the population changed. Larger cells which appeared sluggish and swollen were much more common than in the controls. Cultures were supplemented with 8-azaguanine at various periods during the cycled light-dark regimen to see if there was any time of maximum inhibition by the metabolic analog.

Indications are that the most critical time period of the life cycle for the addition of 8-azaguanine is that stage immediately preceding the normal division of the cells; i.e., during the first few hours in the dark period. Possible mechanisms of action will be discussed.

42

MARVIN H. CANTOR, JOAN BARTHOLOMEW

and WILLIAM THIEMAN, San Fernando Valley State College.

Synchronization of cell division in the flagellate, *Polytomella agilis.*

Synchronization of cell division can be induced in the flagellate, *Polytomella agilis,* by means of a repetitive temperature cycle of 22 hours at 9°C followed by 2 hours at 25°C. No division occurs during the cold period. Doubling of the population begins within 30 minutes following the onset of the warm period and is complete within 30 minutes after transfer to 9°C. This rapid doubling time is to be contrasted with the generation times of exponentially growing cultures, namely 31 hours at 9°C and 5.6 hours at 25°C. The fission index increases from 0.003 at 9°C to 0.012 at 25°C.

Changes in the rates of oxygen consumption were followed over the cell cycle using conventional Warburg manometric techniques. The Qo_2 of cells harvested at the end of the cold period is 18.6 microliters/10^6 cells/hour. During the warm period the Qo_2 drops to 10.1 and remains at this low rate through the early portion of the subsequent cold period. These data suggest a reduced energy requirement during division and a partitioning of the oxidative machinery of the cells among the progeny at the time of division.

Changes in the patterns of DNA, RNA and protein synthesis over the cell cycle have been measured and will be discussed.

43

JOHN E. BYFIELD and OTTO H. SCHERBAUM, University of California, Los Angeles.

mRNA metabolism during induction of division synchrony in *Tetrahymena.*

Protein and RNA metabolism have been further studied in *Tetrahymena.* The kinetics of incorporation of C^{14} uracil and C^{14} amino acids were determined for single temperature shifts and during repeated cycles (29°-34°) leading to synchrony of cytodifferentiation and cell division. Elevation of the culture temperature to levels capable of inducing synchrony reduces incorporation of labeled precursors into both protein and RNA. The suppression of protein synthesis can be related to increased rate of mRNA breakdown. RNA synthesis is similarly reduced when studied by C^{14} uracil incorporation but indirect assays of RNA polymerase activity indicate the reverse, i.e., a slight stimulation during the early heat shocks. This discrepancy appears to result from a reduction in the rate of uptake of labeled precursors into intra-cellular nucleotide pools, presumably expanded by increased mRNA breakdown. By the end of the synchronizing heat-treatment the net cellular mRNA pool has been reduced to 25% of the value present in log cells. When the efficiency of mRNA translation is studied over the range of temperatures permitting growth it can be shown that it is reduced at temperatures above or significantly below the optimum values. The mechanisms of this loss of efficiency appear to be different, however. Since division synchrony can be induced by temperature cycles both above or below the optimum culture temperature, it is concluded that the mechanism of induction of synchrony is intimately related to these induced shifts in messenger metabolism. (Supported by USPHS GM-06461-07.)

44

ARTHUR L. BACON and HAROLD E. FINLEY, Howard University.

Cultivation of a loricated peritrich, *Cothurnia.*

This report describes a method for maintaining stock cultures of *Cothurnia* in non-axenic culture media. Recently, this peritrich was collected from a pond in Kenilworth Aquatic Gardens, D.C., described in previous publications by Finley and McLaughlin, in Progress in Protozoology, 1961.

In the laboratory, filaments of algae bearing many cothurnias were vigorously washed in running tap water to remove unwanted microorganisms. The petitrichs were adapted to laboratory conditions, in Columbia culture dishes, by placing 5 or 6 washed algal filaments in pea broth. The broth was prepared by boiling 1 g pulverized *Pisum sativum* seeds in 1.8 l pond water for 10 min and then bringing the concoction to room temperature. On the 3rd day after inoculation many cothurnias were attached to the dish, others were attached to algal filaments. By that time the broth was heavily bacterized, therefore, it was necessary to remove the algal filaments, wash them in tap water, and inoculate them into another dish of fresh broth; the original dish and its cothurnias were washed in tap water also, and supplied with fresh broth.

After 6 to 12 days of the described handling the peritrichs were cultivated in a dilute pea broth in order to hold the bacterial population at a lower rate of growth. The dilute broth was prepared in tap water instead of pond water, 1 g pea in 3.6 l tap water. After the adaptation period the peritrichs were prolific at pH 7.8, 23°C constant temperature. They tolerated fluctuations of temperature and pH in the range 18°-35°C, and 7.2-8.2°C, respectively. This non-axenic culture method brings *Cothurnia* within range of sophisticated experimentation. (Supported by grant AI 00800-12 from the USPHS.)

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THEODORE J. WALKER, Scripps Institution of Oceanography.

Behavioral signs of benthic feeding by the Calif. Gray Whale in Scammon's Lagoon, Baja, California. (Introduced by H. E. Winn)

The discovery that the Calif. Gray Whale spends many hours a day feeding on the bottom of Scammon's Lagoon eliminates the accepted notion that whale feeds only in the Arctic. Speculation as to why the gray whale spends its winter in the coastal lagoons now seems unnecessary in view of these findings. The feeding behavior is markedly different from that observed in the Arctic. Feeding animals swim against the tidal current, head down, with the tail awash or slightly submerged. The whales feed in groups of four to twenty abreast on a single line of bearing, separated from one another by distances in excess of the visibility (15-20 ft), except for mothers with calves. Alignment must be achieved by acoustic means—possibly by the noise generated as the

whale dredges the bottom. Formation feeding occurs only in the shallower regions of the lagoon, generally 20-35 ft of water. Aerial observations establish that the feeding takes place over beds of eel grass, and that whale contacts the bottom while over on its side. The bottom sediment is not swallowed but flushed out through the filters, as the whale continues to move forward against the current. The water astern of the formation is very turbid, and as the whales surface to breathe, the flushing can be seen clearly, as the mouth is open exposing the baleen. Every few surfacings the whales extend their heads out of the water vertically in an attitude called "spyhopping." The position taken by the whale places the back against the current, and judging from the steadiness and duration of the posture, the whale is believed to be standing on the bottom. More often than not, the eyes do not clear the water. Invariably the mouth is tightly shut, and the floor of the mouth can be seen swallowing. Spyhopping is associated with feeding, and provides a conspicuous sign of feeding. Although there was no opportunity to examine stomach contents, the eel grass becomes scarred and potted by the feeding activity. It is presumed that animal is separating out macroscopic animals of the benthic community including the eel grass and other sea weeds as when uprooted they float to the surface. Eel grass plants do not appear at the surface and are presumed ingested. Baby whales are also believed to be feeding like the mothers, as they surface flushing sediments through the baleen which is already several inches long.

54

BORI L. OLLA, Sandy Hook Marine Laboratory.

Studies on rhythms in a school of adult bluefish, *Pomatomus saltatrix*.

In a previous study, rhythms of activity in a small school of adult bluefish, kept in a large, indoor, experimental tank, were shown to be highly correlated with simulated normal diurnal changes of light, and to keep closely apace with gradual changes in the light regime. Two further series of experiments have shown that when the light regime was shifted abruptly by seven and twelve hours, there was a lag of several days before the rhythm of the fishes came to correspond with that of the shifted light. When the light was held at a constant low level (between five and twenty foot candles), the shifted rhythm that had been established continued for several days. These results indicate that rhythms of activity in bluefish have an endogenous component.

While daily rhythmic patterns of activity measured under simulated normal diurnal changes of light were generally the same throughout the year, there appeared to be a significant seasonal variation in the rate of activity. This was independent of temperature.

55

J. T. ENRIGHT, Scripps Institution of Oceanography, University of California.

Endogenous rhythms in marine organisms.

An endogenous biological rhythm represents a temporally varying physiological substrate which determines the nature and intensity of an animal's behavioral responses to concurrent environmental stimuli. Such rhythms, which are usually synchronized by cyclic environmental factors, may thus play a major role among those factors affecting the spatial distributions of motile marine organisms.

The most striking examples of this phenomenon are to be found in time-compensated celestial orientation, but other sorts of behavioral response are often also involved. Activity rhythms synchronized by the tides have been shown to underlie the tidal migrations of a variety of sand-beach crustaceans. Recent evidence now demonstrates that the daily vertical migrations of several sorts of neritic zooplankters also depend upon endogenous rhythms, which can persist under constant conditions and are synchronized by light-dark cycles. Longer-period endogenous rhythms (fortnightly and monthly) which have previously been shown to underlie reproductive cycles in certain species, can apparently also produce long-period cycles in spontaneous locomotor activity, thus also affecting long-term spatial distributions.

Environmental adaptation requires not only that the organism be in the "right place," but that he be in the "right place" at the "right time." Orientation in space and in time are therefore often intimately related.

56

MICHAEL SALMON, De Paul University.

Courtship behavior and distribution of fiddler crabs in Florida. (Motion picture)

Previous studies (Salmon, M., Zoologica 50:123-149) on the Sand Fiddler crab (*Uca pugilator*) showed that males attracted females during the day with a species-specific "waving" movement of their enlarged cheliped. When a female approached the male, he moved into his burrow and began producing sounds by rapping the claw against the substrate. At night males produced sounds almost continuously. It was hypothesized that the sounds attracted females when visual cues were inoperative.

These studies were extended to three species in Florida, two of which have not been reported and are at present unidentified. Waving display of all species was analyzed from films. Males increased their rate of waving when a female approached, but were aggressive to other approaching males. At night, males of *U. rapax* produced sounds, but not by rapping. The mechanism is not known, but the ambulatories are moved against the substrate when sounds are produced. The sounds resemble a low-pitched "honking." Another species, similar to *U. rapax* in size and coloration, had a different form of wave and produced sounds with distinct frequency and temporal patterns. A second unidentified species produced sounds by rapping. All three species produced more sounds during low tides in the middle of the month than the month's end.

The coast of Florida was surveyed at about 50-mile intervals and the distribution of all species was determined. Species present, in addition to *U. rapax* and the two unidentified forms, were *U. pugilator, U. thayeri, U. pugnax* and *U. minax*. (Supported by Grant GB-3430 from the NSF.)

57

ROBERT A. STEVENSON, JR., and ARTHUR A. MYRBERG, JR., University of Miami.

Behavior of the bicolor damselfish, *Eupomacentrus partitus* in the field and in the aquarium.

General patterns of behavior of *Eupomacentrus partitus,* were observed in the field by the use of underwater television and by diving and were compared with the behavior of individuals of the same species in a 280 gallon aquarium. The amount of time that individual male fish were engaged in feeding and in reproductive activities was measured. Physical and biological factors that were thought to influence this behavior were also measured. These included time of day, current speed and direction, type and location of cover, and the presence of other species of fish. Under field conditions, fish spent the majority of their time in the morning feeding. In the afternoon, they did not feed as much as in the morning, but spent an increased amount of time engaged in activities associated with reproduction. Feeding, as seen in the field, was not observed in the laboratory where reproductive activities occupied most of the fishes' time. (Supported by ONR Contract No. NONR 840 (13), NR 104-495.)

58

DAVID W. WALLER, Bureau of Commercial Fisheries Tuna Resources Laboratory and University of Wisconsin.

Phonations of free-ranging spotted porpoises in the eastern Pacific. (Motion picture)

Sonic activities and concurrent behavior of free-ranging spotted porpoises (*Stenella graffmani*) in the east-central Pacific were recorded under non-experimental conditions and during underwater rebroadcast of their natural phonations. From sound-spectrographic analyses, three classes of phonations were distinguished: clicks, whistles, and grunts. These resemble in general features phonations of related delphinids; it is likely that they also have functional parallels. Thus, most clicks probably represent echolocation. However, some click-series exhibited varying patterns of formants, indicating the possibility of communicatory function for such clicking. Modulation of whistles was relatively simple. The rebroadcast experiments and observations of group activities of this species suggest that certain whistle patterns concern social interactions and conditions, especially maintenance of grouping, rather than other environmental conditions.

59

RONALD J. SCHUSTERMAN, Stanford Research Institute, Menlo Park, California.

Visual orientation and discrimination in seals and sea lions.

Several species of seals and sea lions, including *Zalophus californianus, Phoca vitulina* and *Eumetopias jubatus,* have been tested on an underwater size-discrimination task by the psychophysical method of constant stimuli. All species were capable of discriminating size-difference ratios as small as 1.06:1 with little apparent difference in proficiency between species. This suggests that most if not all pinnepeds have good underwater visual acuity.

Investigation of underwater discrimination and transfer by *Zalophus* has revealed remarkably efficient discrimination of stimulus patterns differing only with regard to shape. Most of these discriminations were maintained near or at a perfect level of accuracy even when the shapes were re-oriented 45°, 90° or 180°. Moreover, preliminary data suggest that at least one California sea lion is capable of solving a wide range of visual discrimination problems after a single information trial. In line with Hobson's recent observation regarding the tendency of seals and sea lions to silhouette their prey against the ambient surface light, all species thus far tested approached stimulus targets from a position considerably below the lower edge of the targets. From results such as these, it is expected that many species of pinnepeds rely strangely on visual cues for the detection and discrimination of objects under water.

Finally, preliminary data will be presented on the ability of *Zalophus* to use a vocal signal to transmit information to an experimenter about its differential sensitivity to selected aspects of its environment.

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W. E. EVANS and B. A. POWELL, Michelson Laboratories, U. S. Naval Ordnance Test Station, China Lake, California.

Dolphin sonar discrimination; a motion picture technical report.

Echolocating delphinids display extraordinary capabilities in detecting echoes, and determining the location and nature of the echo source. In order to accomplish this they must be able to discriminate subtle differences in intensity, frequency, and combinations of these acoustic parameters.

In an attempt to quantify the contribution of one of these parameters (echo intensity) to sonar discrimination in delphinids, a blindfolded Atlantic bottlenose dolphin (*Tursiops truncatus*), was conditioned to always select a 30 cm diameter, 0.22 cm thick copper disc when presented, paired with other discs identical in all respects except thickness. Knowing the acoustic impedance of the seawater, and the thickness and acoustic impedance of copper discs, it was possible by computation to select a series of targets, which differed in acoustic reflectivity by a known amount from the standard target.

At a comparison of 0.22 cm copper versus 0.27 cm copper the animal displayed random behavior, selecting the standard (0.22 cm thick) target correctly in 60% of the trials. However, at a comparison of 0.22 cm copper versus 0.32 cm copper, the responses were correct in 75% of the trials.

Using a combination of electro-optical recording equipment and a broad-band magnetic tape recording system it was possible to measure in detail some of the relationships between the sonic emission and scanning behavior associated with the previously described sonar discrimination.

62

ROBERT R. CARDELL, JR., Harvard University.

Observations on the fine structure and distribution of cell type #5 (stellate cell) in the pars distalis of the salamander (*Diemyctylus viridescens viridescens* Rafinesque) pituitary gland.

Salamander pituitary glands were fixed in 3% glutaraldehyde, post-fixed in osmium tetroxide, dehydrated, and embedded in Epon. Ultrathin sections were cut on an ultramicrotome and the sections studied with an electron microscope.

The fine structure of five distinct cell types in the pars distalis of the salamander pituitary gland has been described by Cardell (J. of Ultrastructure Res. 10:317-333, 1964), and apparently four of these are involved in the synthesis and secretion of the pituitary hormones. The ultrastructure of the remaining cell type (#5) does not suggest, however, a secretory function.

This latter cell type occurs throughout the pars distalis and its long cytoplasmic projections penetrate the intercellular spaces between adjacent secretory cells. Frequently these thin cytoplasmic processes are located between the secretory cell and the outer basement membrane of the pericapillary space. Cell type #5 occurs at the periphery of the pars distalis and the processes from these cells interdigitate in such a manner to form a sheath around the surface of the pars distalis. Collagen fibers occur between the cytoplasmic processes of these peripherally-located cells. Desmosomes provide regions of attachments between cell type #5 and the secretory cells. Thus the form and distribution of cell type #5 is such that it, in combination with other cells of this type, extends from the peripheral surface of the pars distalis to the interior of the gland and it appears likely that each secretory cell has an intimate association with this cell.

The function of cell type #5 is obscure but these observations show that cells of this type form a continuous network throughout the pars distalis. It is suggested that these cells either provide support, perform a regulatory role in the pituitary, or are involved in the release of the hormones from the secretory cells. (Supported by Grant GM 06637 from the USPHS.)

63

JOHN M. MALLETTE and SYLVIA L. EUBANKS, Tennessee A & I State University.

The cytological effects of mestranol and norethindrone on the pituitary gland of fourteen day chick embryos.

It has been known for quite some time that progestins and estrogens inhibit pituitary gonadotropin secretions; however, in the present investigation an attempt was made to find out if there might be a difference in the cellular components of the pituitary gland by 14 day chicks that had been injected with oral contraceptives containing mestranol and norethindrone. A total of three hundred and fifty eggs was used in the experiment. The optimum concentration of both drugs was found to be .01 mg/500 ml distilled water. The controls were injected with the solvent only. The results obtained in this study were: (1) no apparent differences in the number of cells occupied by the pituitary, (2) the percentage of development in the experimental groups was very low as compared to the controls. Excessive hemorrhaging was noted in the brain area of the experimentals. (Supported by grant GY-182 from the NSF.)

64

ROBERT C. THOMMES and CHARLES F. McCARTER, De Paul University.

Adenohypophyseal control of water balance in the developing chick embryo.

Embryos were hypophysectomized at 36-40 hours of incubation by the "partial decapitation" method of Fugo (1940) and were sacrificed at 24-hour intervals from day 10.5 through 18.5 of incubation. Upon sacrifice, all head structures of both normal and pituoprivic chick embryos were removed by cutting between the 5th and 6th cervical vertebrae. Hypophysectomized animals are characterized by a lack of given head structures. Both wet and dry weights were conventionally determined.

Although hypophysectomy produced no significant change in total body water content from normal values of 10.5 (11.071 g water/g dry body weight) and 11.5-(11.331)-day-old embryos, by day 12.5 a statistically significant increase in water content of pituoprivic animals had occurred. This statistically significant increase persisted through the 18th day of incubation although water content of both normal and experimental animals declined from the 12th through 18th day.

Pituitaries from 10-day donor chick embryos transplanted to the chorioallantois of 9.5-day hypophysectomized embryos returned water balance to normal as evidenced by the water content of 18.5-day hypophysectomized animals plus transplants. (Supported by grant HD 01475 from USPHS.)

65

MARTIN P. SCHREIBMAN and KLAUS D. KALLMAN, Brooklyn College and The New York Aquarium.

Freshwater survival after hypophysectomy in teleosts.

In several species of freshwater fishes belonging to the families Cyprinodontidae and Poeciliidae (Atheriniformes) the ability to survive in fresh water is under hormonal control (Gen. Comp. Endocrinol. 6:144, 1966). Hypophysectomy of these teleosts leads to death in several days but they can survive indefinitely in dilute sea water (1.2%). These investigations have now been extended to five additional species of this order to determine the distribution of this freshwater survival mechanism. Essentially similar results have been obtained. Fish were treated as previously described. All completely hypophysectomized fish that were returned to fresh water died or were in severe shock while those that were retained as controls in dilute sea water survived. The mean survival time (in days) for the first investigated are: *Poecilia sphenops*, 2.6 $\pm$ 0.3; *P. vittata*, 2.3 $\pm$ 0.4; *Aplocheilus* sp., 9.0 $\pm$ 4.4; *Nothobranchius guentheri*, 8.6 $\pm$ 2.7; and *Xiphophorus couchianus gordoni*, 12.3 $\pm$ 2.9. Fish that were removed in severe shock were returned to dilute sea water. Those that recovered showed similar responses to fresh water when retested several weeks later. Based

on the data available it appears that all species of Atheriniformes require their pituitary gland to survive in fresh water but significant differences exist between genera in the length of survival. The short survival time of *P. sphenops* and *P. vittata* is consistent with reports for two other *Poecilia* species. The longer survival time of *X. c. gordoni* compares favorably with other species of *Xiphophorus*. (Supported by grants GB-3843 from the NSF and CA-06665 from the USPHS.)

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DAMODHAR V. NAIK, Dartmouth Medical School.

Pituitary-adrenal relationships in mice with hereditary nephrogenic diabetes insipidus, with special emphasis on the pars intermedia. (Introduced by Hilda W. Sokol)

The hypophysis and supraoptic and paraventricular nuclei of mice with hereditary nephrogenic diabetes insipidus (DI) have been compared with those of normal mice of the same strain. The hypothalamic neurosecretory cells exhibit greater activity in the DI mice. Neurosecretory material (NSM) fills the pars nervosa of both DI and normal mice, the density of NSM being slightly less in the former.

In addition to the larger size of the hypophysis in DI mice, the relative proportions of its component parts differ from the normal. The enlargement of the pars intermedia (p.i.) is especially conspicuous: whereas the ratio, volume of pars nervosa/volume of pars intermedia, is 1:1.5 in normal mice, it is 1:3 in DI mice. Closer cytological examination revealed hypertrophy of many p.i. cells with nuclei averaging 8.7 micra in diameter in DI mice versus 7.0 micra in normal mice. There appears to be a greater penetration of pars nervosa tissue laden with NSM in the p.i. of mice with diabetes insipidus.

Another distinguishing characteristic of the DI pituitary gland is the presence of aldehyde fuchsin positive granules which are dispersed among the peripheral cells of the pars distalis bordering the hypophysial cleft. Accumulation of such granules is greatest in regions directly opposite neurohypophysial strands which invade the pars intermedia.

Cytological changes in these specific regions of the hypophysis were effected by dehydration, formalin stress, and adrenalectomy. These results, coupled with the finding of enlarged adrenal glands in DI mice, suggest but do not prove a functional relationship between neurosecretion, pars intermedia, and adrenal cortex. (Supported by USPHS grant AM 08469, the United Health Foundations, the Life Insurance Medical Research Fund, and Merck Sharp and Dohme.)

67

RONALD J. BARFIELD, Rutgers University.

Stimulation of gonadotropin secretion in the female ring dove by limited exposure to a mate.

The female ring dove comes into full reproductive condition and ovulates as a result of 7 days exposure to a male, even if separated from him by a glass partition (Erickson and Lehrman, J. comp. physiol. Psychol., 58:164, 1964). The objective here was to investigate the relationship between the quantity of stimulation presented to a female and the resulting amount of gonadotropin secretion as reflected by development of her reproductive tract.

Experimental females were caged adjacent to mates but separated from them by movable opaque partitions. A limited daily visual exposure to the male was effected by substituting a glass plate for the partition. 48 females were divided equally among 4 experimental groups and correspondingly saw courting males for either 15, 30, 60, or 120 minutes per day for 7 days. As controls, 12 females received no exposure and 12 received continuous visual exposure to mates. At the end of 7 days the development of the oviduct and follicles was ascertained.

Mean oviduct weights in milligrams for the 6 groups were as follows: No male, 983; 15′, 1110; 30′ 1334; 60′, 2069; 120′, 2072; continuous, 3617. The degree of follicular development paralleled oviduct weight, and the numbers of females ovulating in each group were 0, 1, 0, 3, 5, and 9, respectively. In terms of the measures employed, it appears that the day-to-day release of gonadotropin by the female ring dove is continuously graded in accordance with the quantity of external stimulation received. (Supported by grant GM-1135 from the USPHS to Dr. D. S. Lehrman.)

68

ROBERT ORTMAN, City College of New York.

The Paradise Whydah in the Weaver Finch test.

The abdominal and subtail feathers of the Paradise Whydah (*Steganura paradisea*) have been employed in the Weaver Finch test for luteinizing hormone (LH) and human chorionic gonadotropin (HCG) by Witschi (1955, 1959). Segal (1957) reported that the Napoleon Weaver finch (*Euplectes afra*) is sensitive to 0.005 mg of LH or 30 IU of HCG (depending on commercial source). He believes these birds are about equally sensitive to LH (personal communication).

We have used (variably) neck, breast, abdominal and subtail feathers. Birds were fed *ad libitum* Hartz Mountain Finch Seed or a mixed seed diet and maintained at room temperature under natural lighting conditions (northern exposure). Between 0.005 and 1.0 mg of LH (equine or bovine) or 60 and 150 IU of HCG were administered in two, daily injections (intramuscular) to 26 birds after a post-plucking interval of 4 to 10 days. Only one bird gave a positive response (melanized bar in the regenerating feather). This was shown in feathers from all four regions.

To exclude the possibility that a critical period was missed 15 birds were deplumed and promptly injected. Animals received 10 daily injections for a total of either 0.25 or 2.5 mg of LH (equine) or 1,000.0 IU of HCG. No positive responses were obtained.

Thirty control birds, which were uninjected, Ringer's fluid-injected or water-injected, gave negative responses.

The Paradise Whydah appears unreliable and unsatisfactory in the Weaver Finch test. The totality of conditions surrounding their successful use has not been effectively disclosed by Witschi. (Supported by USPHS grant No. AM 07204-03.)

69

ALBERT H. MEIER, KENNETH B. DAVIS, and RICHARD LEE, Louisiana State University.

The diurnal rhythm of the fattening response to prolactin in the Golden Topminnow and the White-throated Sparrow.

Increases in body weight in response to injections of prolactin have been reported in mammals, birds and amphibians. Our studies indicate that the time of prolactin injection with respect to the photoperiod is of fundamental importance in eliciting a body weight response. Injections during the middle of a 16-hour photoperiod are stimulatory to body weight increases in both the Golden Topminnow, *Fundulus chrysotus,* and in the White-throated Sparrow, *Zonotrichia albicollis.* However, injections given at the beginning or at the end of the photoperiod suppress the body weight. The changes in the body weights are due primarily to changes in the lipid component. Carcasses of the control birds averaged 6% extractable lipid, whereas those of the prolactin-treated groups were 3% and 13% for the early and mid-day injections, respectively. A similar relationship was found in the Golden Topminnow. In addition, a combination of follicle-stimulating hormone and luteinizing hormone promotes fattening responses in the Topminnow which are indistinguishable from those produced by prolactin. In the White-throated Sparrow, the gonadotropins augment the fattening produced by prolactin but have no effect, alone. These studies suggest that there is a diurnal period of sensitivity in many vertebrates during which responses to fat-inducing hormones may occur. (Supported by Grant GE-3679 from the NSF and by the Endorinology Study Section of the USPHS.)

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N. S. MILBURN, Tufts University.

Changes in the fine structure of cockroach endosymbiotic bacteroids correlated with the development of the cockroach.

Endosymbiotic bacteroids have been described in a wide variety of insects which live on specialized diets such as blood, tissue fluids, or plant juices. They also occur in the tissues of a few omnivorous insects such as the cockroach. There is evidence that these bacteroids produce nutritional factors necessary to the cockroach (Brooks, 1963). This study is concerned with changes in the fine structure of bacteroids in the fat bodies of the false ovoviviparous cockroaches, *Byrsotria fumigata* and *Nauphoeta cinerea.*

Large numbers of rod-shaped bacteroids are found within the fat bodies of adult reproductive females. The volume of the fat body decreases sharply during the period of egg development and continues to do so after oviposition when the egg cases are in the female's brood sac. These roaches eat little or no food during this gestation period.

As the stored glycogen, lipid and protein disappears from the fat body cells, similar changes occur in the cytoplasm of the mycetocytes in which the bacteroids are living. Bacteroids in depleted mycetocytes lose their bacterial cell walls, enlarge and become spherical protoplasts. Their nuclear regions are surrounded by regular concentric layers of fibrous material which form a shell about the bacteroid. Eventually such metamorphosed bacteroids are extruded from the mycetocyte. The possible control of the bacteroid metamorphosis by neurohormones and its relation to the amounts of stored metabolites in the fat body will be discussed.

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HELEN GHIRADELLA, JAMES CRONSHAW, and JAMES CASE, University of California, Santa Barbara.

The fine structure of the aesthetasc hairs of *Pagurus samuelis.*

Light microscopic studies on the thin-walled aesthetasc hairs of decapod crustaceans have revealed that each hair consists of a sheath of cuticle surrounding the dendrites of many bipolar neurons. The neuron cell bodies are grouped in a spindle beneath the base of the hair. Fine structural studies on the antennules of a small hermit crab, *Pagurus samuelis,* have revealed that the dendrites are ciliary in structure along part of their length; the basal portion of each shows well developed rootlets terminating in basal bodies, usually two per cell. More distally, each dendrite divides into cylindrical branches, each containing a longitudinally oriented microtubule which appears continuous with one of the tubules of the basal body. The microtubule-containing branches make up most of the length of the dendrites within the hair, and apparently terminate within the lumen of the hair with no special end-structures or connections to the outside.

Dye penetration studies have revealed that the aesthetascs are extremely permeable; this fact, together with the presence of a large number of dendrites within, suggests a chemosensory function for these organs. We are now attempting to elucidate the means by which chemicals can penetrate the cuticle and reach the dendrites. Cuticular pores have been observed near the bases of the hairs, but at the present time, none has been found in the more apical regions. We have not yet obtained views of the extreme tip. Fine fibrils are visible in the cuticle, and these appear to have a more dispersed arrangement in the apical region of the hairs than in the basal region, suggesting that the cuticular structure itself might be more permeable in the distal region of the hairs than in the proximal. (Supported by University of California Faculty Grants 289 and 380, and U. S. Public Health Service grant USPHS-NB04372.)

73

THEODORE J. O'TANYI, JR. and SAUL B. BARBER, Lehigh University.

Stretch receptors in *Limulus* limbs.

A proprioceptor organ connected to the apodeme of the dactyl opener muscle has been found in *Limulus* walking legs and chelicerae. Serial sections show that 40 or more neurons are situated in a branch of the small leg nerve near the point where the nerve ends and anchors to the apodeme. More proximally the nerve is held by connective tissue to the posterior surface of the tarsal wall so that the more distal part of the nerve is stretched, and released from stretch, when the dactyl is passively closed and opened, respectively. With the tarsal and dactyl structures intact only closure of the dactyl stimulates the receptor units.

When the apodeme receptor nerve is freed from its cuticular attachment, the adequate stimulus is the stretch of the nerve trunk. Similar kinds of units, including both phasic and tonic types, are excited by either method of stimulation.

The apodeme organ is similar both histologically and functionally to the femur-patella proprioceptor organ previously described (Pringle, J. W. S., J. Exp. Biol., 33:658, 1956; Barber, S. B., J. Exp. Zool., 143:283). In both organs the receptor neurons are located in a ganglion-like swelling of the nerve and the units are normally excited by stretch. However, different structures are used in the two organs to provide a reference length upon which the stretch stimulus is superimposed. The receptors of both organs should be considered as stretch receptors. (Supported by Grant NB05953-01, NIH, USPHS.)

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WILBUR F. HAYES and SAUL B. BARBER, Yale University and Lehigh University.

Structure and properties of the *Limulus* tendon receptor organ.

Each of the walking legs of *Limulus polyphemus* (L.) has a tendon receptor organ (Barber and Hayes, Comp. Biochem. Physiol., 11:193-198, 1964) that is structurally unlike all the other proprioceptor organs in the leg. The tendon is a cylinder of dense connective tissue with fixed ends in the trochanter. Embedded in the anterior end of the tendon are about 30 large multipolar neurons supplied by a small sensory nerve. Two accessory muscles originating in the coxa are connected to the tendon close to the region where the sensory cells are located. One of these muscles is also connected indirectly to the femur. Thus, the structure of the organ is suited for monitoring the position and movements of both the trochanter and femur relative to the coxa.

Unidirectional movement units have been recorded from the tendon organ nerve when the trochanter or femur was forcibly extended. Position units in general display maximal responses at full extension and minimal responses at full flexion of these segments. During, and immediately following, extension the discharge frequency increases and then drops to a new equilibrium frequency. Segment flexion, however, causes a temporary cessation of position unit responses, followed by a rise to the new equilibrium frequency. These responses are probably mediated by the effects of the movements on tension changes in the accessory muscles. In particular the "inhibition" of tonic units by segment flexion is probably due to the resultant transient loss of accessory muscle tension. (Supported by grants from the NINDB, NIH.)

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ROD SUTHERLAND, Clark University.

Fine structure of the crayfish thoracic cord and leg nerves. (Introduced by R. F. Nunnemacher)

Ultrathin cross sections of the main nerve going into each leg and the ventral thoracic cord of *Orconectes virilis* were examined with an electron microscope to determine the number and diameter of the fibers. The nerves were prepared by standard methods. Micrographs of cross sections of each nerve and the ventral cord were made. The numbers of fibers of varying sizes within each nerve were determined and compared. Observations show that the smallest fibers are under 0.15 μ in diameter and are found in all of the nerves examined. The fine fibers (0.15 to 1.0 μ) are most numerous in the second walking leg (14,811). The fiber of largest diameter (27.4 μ) is found in the first walking leg. The fourth walking leg has the greatest number of fibers (20,075). The ventral cord at the level of the third thoracic connective contains 5,293 fibers. Of these 2,967 are fine fibers. There are four large fibers in the third connective with an average diameter of 88.8 μ, the largest of which is 92.4 μ. A comparison of the third thoracic connective with the fifth abdominal connective shows the latter to contain about twice the number of fibers (9,393), but with fewer large fibers. (Supported by grant NB03989 from the USPHS, NIH.)

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ZIEDONIS SKOBE, Clark University.

Nerve fiber distribution of the circumesophageal nerve connective in Decapod Crustaceans. (Introduced by R. F. Nunnemacher)

Cross sections of circumesophageal nerve connectives of several Decapod Crustaceans were examined with an electron microscope to determine the number and diameter of fibers within the connective. Fixation, dehydration, and embedding were accomplished using standard techniques. Overlapping pictures were taken and assembled into montages. Individual fibers were counted according to predetermined groupings.

Uca, Carcinus, Libinia, and *Cancer* have an average of 2,600 fibers, with a variation of 6%. The diameters of these vary from 0.12 μ to 30 μ. The largest number of fibers is in the 1-3 μ range, and may constitute up to 50% of all fibers. *Callinectes sapidus* differs by having no fibers smaller than 1.5 μ, the greatest number is in the 6-9 μ range, and its largest fiber has a diameter of about 48 μ.

Both *Gecarcinus lateralis* and *Grapsus grapsus* show a larger than average number of fine fibers, bringing their totals to 3,000 and 4,000 respectively. *Grapsus* also has an increase over the average number of large fibers, fourteen of which were between 60 μ and 80 μ. The large fibers are unusual in crabs since they are commonly associated with Decapods such as *Orconectes* and *Crangon* having the tail flipping reflex.

The crabs *Pagurus longicarpus* and *P. pollicaris* have a bundle of about 5,500 fibers measuring less than 0.5 μ. This bundle of fine fibers elevates the total for these crabs to 8,300 and 9,600, respectively. (Supported by grant NB03989 from USPHS, NIH.)

77

RUTH R. BENNETT, University of Massachusetts, Boston.

Spectral sensitivities of locust retinula cells.

Light flashes elicit generator potentials from visual cells in the eye of the locust (*Locusta migratoria*) similar to those reported for most other insect visual cells, when conventional intracellular electrode recording techniques are used. In an

initial study of twenty locust visual cells, the reciprocals of the relative numbers of quanta required to evoke a certain generator potential (dynamic phase) amplitude were deduced from response energy curves; the latter were found by using various intensities of monochromatic lights of 350 to 600 mμ; and spectral sensitivity curves for each cell were plotted. All cells showed a high sensitivity in the blue (maximum 430 mμ) region of the spectrum. In addition, all cells showed some green sensitivity (maximum approximately 515 mμ) but this varied, in different cells, from 15% to 100% of the blue maximum for the cell. The results are consistent with the hypothesis that two "Dartnall-type" visual pigments are contributing, in various ratios, to the spectral sensitivity of each cell.

Since locust visual cells are sensitive to the plane of polarization of incident light (Shaw, Nature 211:92, 1966), tests with polarized monochromatic light were carried out in an attempt to separate possible interacting blue-sensitive and green-sensitive receptors. Although a small amount of interaction between cells seems to occur in certain cases, possibly due to electrode placement, it is probable that all visual cells studied contain the two visual pigments hypothesized above. (G. A. Horridge made the resources of the Gatty Marine Lab., St. Andrews, Scotland, available for this study and contributed invaluable advice; initial phase of project performed in collaboration with John Tunstall.)

78

ARNOLD ESKIN and MICHAEL MENAKER, University of Texas.

Sound stimuli as entraining agents of circadian rhythms in sparrows.

Thus far only light and temperature pulses have been adequately established as entraining agents for circadian rhythms although there are several reports indicative of the existence of other entraining agents. Daily sound (bird song and white noise) was tested for its ability to entrain the circadian locomotor rhythm of the sparrow, *Passer domesticus*. Several birds produced activity records which satisfy all the criteria for the rigorous demonstration of entrainment. The signal controlled the phase and period of the rhythm and masking was excluded by having free runs before and after application of the signal. Also several birds showed good initial phase and period control for several days, but subsequently broke away from the signal and assumed circadian periods in the presence of the signal. In a few cases the bird song had no influence on the phase or period of the locomotor rhythm. In all cases in which the signal failed to entrain, the free running period of the rhythm in constant conditions was outside the range 23 hr 35 min to 24 hr 45 min. No sex differences were noted in the ability to be entrained by the signal.

Mutual interaction of the rhythms of birds was also studied. Birds were able to entrain one another if their individual free running periods were not very different. (Supported by a grant from the University of Texas Research Institute, NSF Grant GB-3806, NIH Training Grant 5-T1-GM-836-03, and NIH Fellowship Award 1-F1-GM-32,240-01.)

79

FRED T. ERSKINE, D. W. HOWE, and B. C. WEED, Worcester Polytechnic Institute.

The discharge period of the weakly electric fish *Sternarchus albifrons*. (Introduced by L. P. Granath)

The discharge period (reciprocal of frequency) of the weakly electric (a-c) fish *Sternarchus albifrons* was studied to establish limits of stability for the signal from the normal fish, and to find those factors which may affect the discharge signal under normal conditions.

For different specimens of healthy fish the discharge period may vary from about 1.5×10^{-3} to 1.0×10^{-3} sec (650 to 1000 Hz) at room temperature (23°C). The discharge period is very dependent on temperature with a slope of approximately -0.05×10^{-3} sec/C° (+40 Hz/C°) and a Q_{10} of 1.5.

It appears that the "normal" instability of the fish discharge period is of the order ±1 part in 10^4 (±0.01%). Superimposed on this are two types of variations. First is a slow repetitive deviation from the normal period of ±1 part in 10^3 (±0.1%) having a random duration of from 1 to 3 minutes. Second there is a rapid deviation from the normal period in the direction of decreasing period followed by an exponential recovery to nearly the original period. These "spikes" represent a variation of approximately 2 parts in 10^2 (2%) with a duration of from 0.1 to 100 sec and occur at about 10- to 15-minute intervals.

Other factors which have been found to affect the discharge period of the healthy fish are exertion (i.e., causing the fish to swim rapidly in a forced current of water) and "chasing" (i.e., stimulation by an external a-c electric field. The largest observed change in period due to exertion is about a 0.3% decrease. The "chasing" experiment was carried out using a battery powered variable frequency oscillator for external a-c stimulation and the largest observed change is a 6% decrease in period. (Supported by PHS Grant 1 RO1-NB06659-01.)

80

DONALD W. HOWE, JR., FRED T. ERSKINE, and L. P. GRANATH, Worcester Polytechnic Institute and Clark University.

Threshold sensitivity of *Sternarchus albifrons* to electric fields.

The fish were trained to go to a feeding trough following electric stimulation. As training progressed, the uniform field was reduced progressively to the minimum field which would cause the fish to exhibit the typical rapid forward and backward swimming in its hiding tube preparatory to feeding. After conditioning, obvious responses were obtained using sinusoidal signals from 30 Hz to 3000 Hz, and sensitivity curves were determined. A minimum threshold (minimum stimulus) of 0.2 microvolt/cm was obtained for all individuals (at the fish frequency, 700 Hz), with a secondary minimum of 0.3 to 0.5 microvolt/cm at the second harmonic for some speci-

mens. No significant difference in the sensitivity curve was noted when non-uniform fields from point sources were used, or when the fish was trained with signals of frequencies other than his own.

The conditioned response method of determining sensitivity results in a lower threshold than the method of observing the change in electrical output frequency of the fish induced by carefully selected frequencies of stimulation. This "chasing" threshold has been found to be 15 microvolts/cm r.m.s.

The potential distribution about the fish was measured, and from this the distance at which the field was reduced to 0.2 microvolt/cm was calculated. We conclude that one *Sternarchus albifrons* can detect another at a distance of three meters. (Supported by NONR contract 3694 (02) and PHS Grant 1 RO1-NB06659-01.)

81

RICHARD J. TASCA and NINA W. HILLMAN, Temple University.

Studies on ribonucleic acid synthesis in preimplantation stage mouse embryos.

Two-cell embryos were collected from superovulated, random-bred, albino mice. These embryos were treated in one of two ways. They were either frozen immediately or they were placed into culture and allowed to develop until they reached the desired preimplantation stage (Brinster, J. Exp. Zool., 158:49, 1965). At specific times these latter embryos were removed from culture and frozen for biochemical studies.

In addition to measuring the total RNA content at various cleavage stages, ribonucleotide ratios were determined. For this, the nucleic acid was extracted and hydrolyzed by a modified Schmidt-Thannhauser technique (Gebicki and Freed, Anal. Biochem., 14:253, 1966). The nucleotides were then separated on ion-exchange thin-layer chromatograms, eluted, and amounts of ribonucleotide-3', 2'-phosphates were measured (Randerath and Randerath, Anal. Biochem., 12:83, 1965). Up to this point, our results have shown that these preimplantation stages have a high uridylic and guanylic acid content, and low amounts of adenylic and cytidylic nucleotides.

RNA synthesis has been studied through the use of the following techniques: Embryos were incubated and pulse-labelled with radioactive precursors. The RNA was then extracted and separated into nucleotides by the procedure shown above. The rate of synthesis was calculated by determining the amount of labelled compound which was incorporated into the nucleotides in a defined period of time. Data from these and related experiments will be presented. (Supported by NIH Research Grant HD-00827 and NIH Training Grant 1T1-HD-138-01.)

82

ELERI A. JAMES, University of Washington.

Nucleic acid metabolism in regenerating and dividing *Stentor coeruleus*: the effects of microinjected and externally applied actinomycin D and puromycin. (Introduced by A. H. Whiteley)

If the oral structures are removed from a stentor, a regeneration primordium will form. Successive stages in development of the anlage, which resembles a division primordium, have been defined by Tartar (1961, *The Biology of Stentor*, Pergamon Press), the whole process taking 10-12 hours. Initiation and maintenance of primordia depends on the nucleus. In the present study the role of DNA dependent RNA synthesis and polysomal function during regeneration and division have been examined by means of actinomycin D and puromycin inhibition. The drugs were externally applied and micro-injected, the latter method ensuring an immediate effect and permitting an exploration of time and duration of action of each drug, presumably reflecting specific periods of RNA and protein synthesis.

Primordia were inhibited by a 48-hour pretreatment with 50 μg/ml actinomycin. When micro-injected at 0 hours to a cytoplasmic concentration of 3-6 μg/ml regeneration was immediately blocked. After 5 hours regeneration was insensitive to this treatment, although a second primordium could not be induced. External pretreatment for 2 hours in 150 μg/ml puromycin inhibited regeneration and division. Sensitivity of regeneration to the injected drug (about 10 μg/ml cytoplasmic concentration) was greatest between 2 and 5 hours, after which it was independent of puromycin inhibition.

These observations indicate that removal of oral structures initiates DNA templated RNA synthesis, required during the early stages of regeneration for primordium development. Puromycin-sensitive protein synthesis appears to occur during intermediate stages of regeneration, but not at the start or termination of the process.

83

M. C. NIU and A. LEIKOLA, Temple University.

Induction of gut formation by liver RNA.

Previous experiments have shown that liver-RNA treated segment of the posterior primitive streak is capable of inducing neural formation and self-differentiating into organized but unidentified tissue. In order to get the self-differentiating potentiality fully expressed, the liver-RNA treated segment was implanted into coelomic cavity of the 60-hour chick embryos.

Posterior third of the primitive streak was excised from the 14-16 hours chick blastoderm and treated in Locke solution with (experimental) and without (control) liver-RNA (absorbancy units$_{260m\mu}$ 80/ml) in cold room (2-4°C) for 14-18 hours. Prior to implantation, each segment was washed once with Locke solution. All implants were allowed to develop 7-11 days. About 15% of the control, including the serum albumin, ribonuclease and boiled liver-RNA treated series, and 44% of the experimental were recovered. The remainder were apparently absorbed.

Growth and differentiation of the control were generally poorer than the experimental and seldom extended beyond epithelial linings of epidermal or endodermal type. The occurrence of gut-like structure was rare (2%). In striking contrast, approximately half of the recovered experimental had well defined gut.

Liver-RNA was incubated with crystalline ribonuclease. About 85% became acid soluble. This loss is accompanied by a significant reduction in rate of the liver-RNA initiated differentiation. It is still to be seen, however, that this effect is produced specifically by liver-RNA. Besides, the active fraction of this RNA would have to be defined and physico-chemically characterized. (Supported by NSF and the Population Council.)

84

ROBERT C. FITZSIMMONS and N. W. KLEIN, Marquette University.

Utilization of proteins by explanted chick embryos grown on horse serum.

Previous studies have shown that a mixture of ovalbumin and horse serum will replace egg yolk as a nutrient medium for explanted chick embryos (Klein, in preparation). Ovalbumin specifically supports the growth and development of the embryo proper and horse serum specifically supports the yolk-sac membrane.

To evaluate the components of horse serum responsible for this stimulation, serum was fractionated with ammonium sulfate at 50% saturation. The precipitate containing primarily α, β, and γ-globulin was found to stimulate maximum protein nitrogen accumulation in the membrane. In contrast, the supernatant containing mainly albumins was ineffective. Sub-fractions of horse serum were then prepared by the salting out method of Cohn, *et al.* (J. Biol. Chem., 62:3386, 1940). Subsequent growth studies indicated the membrane stimulatory effect was due to the α and/or β-globulin fractions of horse serum.

Attempts were then made to determine if the enhanced protein nitrogen accumulation in the yolk-sac was due to a stimulation of protein synthesis by the horse serum or increased protein uptake. Embryos cultured *in vitro* for 48 hours on ovalbumin or horse serum plus ovalbumin media, each in the presence and absence of puromycin were given a terminal two hour pulse with ^{14}C-leucine. Accumulation of labeled leucine into membrane protein nitrogen was markedly reduced in the presence of puromycin in both ovalbumin and horse serum-ovalbumin media. Total membrane nitrogen accumulation was greater in the presence of serum and the specific activity was lower. These results suggest that horse serum stimulated intact protein uptake by the yolk-sac membrane. (Supported by Grant 5TI/HD-27 from USPHS and by NSF Grant No. GB 2388 to N. W. Klein.)

85

GAIRDNER B. MOMENT, Goucher College.

An electrophoretic analysis of proteins from regions of differing growth potentials in earthworms.

Previous work has shown that the number of segments regenerated in the posterior direction declines in a linear manner as the level of amputation is moved posteriorly in earthworms (Moment, J. Morph., 86:59, 1950) and that the "information" specifying the number of new segments to be formed is localized within each level (Moment, J. Exper. Zool., 117:1, 1951 and Crowell, Biol. Bull., 115:321, 1958).

Proteins were extracted by the usual methods, i.e., homogenization in distilled water, 0.14 M NaCl, Weber's solution, etc., from the body wall, gut, nervous system, and other tissues of the common barnyard earthworm, *Eisenia foetida*. Comparisons were made between proteins of different tissues and proteins from the same tissue taken from segments 40-50, 50-60, 60-70, 70-80, and 80-90 which is virtually the terminus of proliferative growth in this species. Electrophoresis was carried out at 5°C in acrylamide gel using both the column disc and the broad slab methods. Amido black and Ponceau red were the stains.

Each tissue showed a characteristic pattern of protein bands which in the case of the body wall was composed of at least 16 lines. The same tissue from different anatomical levels showed differences in banding. Histones have not, as yet, shown such differences. Whether or not any of the differences so far observed are causally related to growth potential remains undetermined. (Supported by Grant GM-04774 from the NIH.)

86

BURTON C. STAUGAARD, University of New Hampshire.

Arginase activity in developing mouse liver.

Previous investigators have shown that, in the rat, arginase activity is detectable in the liver of early embryonic stages and that the enzyme activity increases markedly at birth, and that further increases occur coincidentally with weaning.

This investigation determined the arginase activity of prenatal and postnatal mice, and followed the changes in activity level to the adult condition. Aqueous homogenates of liver tissue were made and the supernatant fluid was assayed for arginase activity by determining the amount of ornithine produced by the splitting-off of urea from arginine.

A low level of arginase activity was detectable in the livers of mice during embryonic development. Following birth, the arginase activity was markedly higher and maintained a relatively constant level for about 10 days. Further increases in activity can be correlated with dietary change following eruption of teeth and opening of eyes, till adult levels are attained. (Supported by grant CURF-217 of the University of New Hampshire.)

87

JOSEPH W. VANABLE, JR., Purdue University.

Biochemistry of *Xenopus laevis* skin metamorphosis: Tryptophan hydroxylation.

It has been found previously (Vanable, Develop. Biol. 10:331, 1964) that during the metamorphosis of *Xenopus* larvae, the ability of the skin to decarboxylate 5-hydroxytryptophan (5-HTP) increases over that of the pre-climax skin, thus making more possible the production of 5-hydroxytryptamine (5-HT), which accumulates in the subepidermal granular glands maturing at this time. Evidence is now available that pertains to the ability of the pre- and post-metamorphic climax skin to carry out the first step in this pathway, the hydroxylation of tryptophan. Skin fragments of pre- and post-climax animals were cultured as previously described (Vanable, Am. Zool. 5:663, 1965), except that the culture medium also con-

tained: Eagle's MEM vitamins instead of Trowell's; Iproniazid (a monoamine oxidase inhibitor), 1 mg/ml; NADPH, 0.1 mg/ml; 6,7-dimethyl, 5,6,7,8-tetrahydropterine, 0.16 mg/ml; and labeled DL-tryptophan (indolylalanine-3-^{14}C), 20.5 mc/mm, 0.5 μc/ml. The radioactivity of 5 mm segments of thin layer chromatograms of formic acid-acetone extracts of the cultured skin was assayed by scintillation counting. In the post-climax skin, counts were found at an R_f corresponding to 5-HT, suggesting strongly that the tryptophan-^{14}C had been hydroxylated and then decarboxylated by this tissue under these conditions. There were no significant counts above background either at the R_f for 5-HTP or for 5-HT in the pre-climax skin, indicating that the hydroxylation of tryptophan by this tissue was somewhat less than that of post-climax skin under these conditions, if not altogether absent. (Supported by Grant GB-361 from the NSF.)

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HECTOR TIMOURIAN, Bio-Medical Research Division, Lawrence Radiation Laboratory, University of California, Livermore.

A comparison of subcellular fractions from unfertilized and fertilized sea urchin eggs.

The ribosomal fraction obtained from unfertilized eggs was always inactive when tested in combination with unfertilized or fertilized egg supernatant or with "total" fertilized egg homogenate. The ribosomal fraction from fertilized eggs was always active when tested with either unfertilized or fertilized egg supernatant or with unfertilized egg "total" homogenate. However, the amount of incorporation by fertilized egg ribosomes was always higher in combination with the fertilized egg supernatant than with the unfertilized egg supernatant. When the fertilized egg ribosomal fraction was further fractionated by centrifugation through a sucrose density gradient, and each fraction tested with unfertilized egg supernatant, almost all activity was found in the fractions containing polysomes.

The unavailability of s-RNA in the unfertilized egg and a lower activity of amino acid-RNA synthetase may account for the lower activity of the unfertilized egg supernatant. The amount of amino acid incorporated into the s-RNA fraction by fertilized egg ribosomes in the presence of supernatant from fertilized eggs was higher than the amount incorporated in the presence of supernatant from unfertilized eggs. The incorporation of amino acid into protein by unfertilized egg total homogenates was enhanced by the addition of yeast s-RNA; however, the activity never reached that of the fertilized egg homogenates. Yeast s-RNA did not enhance protein synthesis by fertilized egg homogenates. (Supported in part under USPHS fellowship No. EPD-9044-C2 and in part under the auspices of the Division of Biology and Medicine, USAEC.)

89

ROBIN I. GOODFELLOW, Western Reserve University.

Evidence for the functional specialization of mitochondria for lipid metabolism. (Introduced by M. Locke)

In *Rhodnius* fat body each lipid droplet has a single, localized "cap" of esterase (Wigglesworth, Quart. J. Micro. Sci., 99:441, 1958) also containing the dehydrogenases of isocitrate, α-ketoglutarate, succinate, malate, glutamate, β-hydroxybutyrate and lactate (Wigglesworth, Nature, 210:759, 1966). Such precise localization suggests the presence of an equally localized organelle. The problem is to find methods for esterase demonstration giving products suitable for electron microscopy.

Fifth instar larvae of *Calpodes ethlius* were fixed after ecdysis when lipid droplet size is minimal. Tissues were treated for esterase by the Tween method (Gomori, Microscopic Histochemistry, 203-206, 1953) and by an azo dye procedure (Holt and Hicks, J. Cell Biol., 29:361, 1966). To demonstrate free fatty acids, tissues were incubated with calcium ions followed by lead substitutions, also a control for the Tween method.

Light microscopy of the Tween material showed localized deposits in the lipid droplets. Electron microscopy revealed dense deposits on the mitochondria. The calcium-lead incubated material gave similar results, indicating that these deposits may be lead soaps derived from free fatty acids. The proximity of mitochondria and small lipid droplets, both containing lead deposits, suggests the mitochondria are the source of the lipid.

Electron microscopy of the azo dye preparations showed the reaction products always associated with lipid droplets. In material with large droplets, the product obscured subcellular structure; with small droplets, the product was found with lipid droplets forming adjacent to mitochondria.

This evidence and the presence of other mitochondrial enzymes suggests that the structure associated with the esterase cap is the mitochondrion. The presence of more mitochondria than lipid droplets indicates that there may be functional specialization of mitochondria. (Supported by grant GM 09960 from the United States Public Health Service and by the NSF.)

90

NORMAN B. RUSHFORTH, Western Reserve University.

An analysis of spontaneous contraction pulse patterns in *Hydra*.

Hydra spontaneously produces electrical potentials which are associated with contractions of the body column (Passano and McCullough, J. Exp. Biol. 41:643, 1964). These were originally termed contraction-burst potentials since Passano and McCullough noted that the potentials usually occurred in bursts. By using methods which allow long-term recording from essentially unrestrained animals we have observed that many animals give such potentials as single, widely spaced events rather than in bursts. Thus, we term such potentials contraction pulses (CP's).

Records taken for a continuous period of 12 hours from 10 hydra of each of five species have been analyzed. Inter-pulse intervals (the time in seconds between consecutive CP's) were computed for successive pairs of CP's. Each interval was arbitrarily classified as a long interval, or as a short interval if it was greater or smaller than 10 seconds, respectively. A burst was defined as two or more consecutive short intervals comprised

of three or more CP's. Statistical techniques were employed to test the stationarity and species specificity of several parameters characterizing pulse patterns.

For all five species it was found that 50 per cent or more of all intervals were long intervals and, therefore, not in bursts. The species, however, differed in their degree of "burst type" activity as characterized by the inter burst interval. The mean and (standard error) was found to be: *H. pirardi* 166 sec (20), *H. viridis* 441 (44), *H. fusca* 1136 (273), *H. littoralis* 1552 (554) and *H. pseudoligactis* 1871 (567). However, within a burst the mean interval was longer for *H. pirardi* 7.3 (0.3) and *H. viridis* 7.5 (0.2) than *H. pseudoligactis* 4.2 (0.6), *H. littoralis* 22 (0.4) and *H. fusca* 2.4 (0.5). (Supported in part by grants from the NSF and NIH.)

91

DONALD S. BURKE and NORMAN B. RUSHFORTH, Western Reserve University.

Inhibition of pacemaker activity in *Hydra*. (Introduced by A. L. Burnett)

Hydra possess several pacemaker systems (Passano and McCullough, Nature 199:1174, 1963). Spontaneously produced electrical potentials associated with contractions of the body column (C.P.'s see Rushforth Am. Zool. abstract) are inhibited when *Hydra* feed on live *Artemia salina* nauplii. The clear supernatant of centrifuged diluted artemia homogenates and reduced glutathione (GSH) also block spontaneous CP's. Endogenous potentials recorded from excised tentacles associated with tentacular contractions (TCP's) are also inhibited by these stimuli.

Hydra pirardi and *Hydra pseudoligactis* give different responses to a regime of two minutes of bright light followed by a control period of two minutes of ambient light. The frequency of CP's in *H. pirardi* is greatly enhanced during exposure to light and reduced during control periods. Conversely CP activity in *H. pseudoligactis* is inhibited in periods of direct illumination and increased during control periods. The frequency of TCP's originating from isolated tentacles of both species is increased by light.

Feeding *Hydra* live *Artemia salina* nauplii inhibits light driven (*H. pirardi*) and "dark driven" (*H. pseudoligactis*) CP activity. Artemia homogenates and GSH (10^{-8} M) have similar effects. The duration of inhibition by GSH of light induced CP's in *H. pirardi* is approximately linearly proportional to the log of the concentration of GSH over the range 10^{-8} to 10^{-5} M GSH. Inhibition by 10^{-5} M GSH lasts 38 $\pm$ 7 minutes.

These results suggest that the contraction pulse (CP) and tentacle contraction pulse (TCP) pacemakers are under complex photic and chemical control. (Supported in part by grants from the NSF and NIH.)

92

JAMES CASE and JAMES MORIN, University of California, Santa Barbara and Harvard University.

Glutamate suppression of neuroeffector processes in coelenterate.

In an investigation of the role of amino acids in feeding behavior of the pennatulid, *Renilla kollikeri*, suppression of luminescence and neuromuscular activity by l-glutamic acid was observed. The rarity of agents active upon coelenterate neuroeffector systems prompted further investigation of this phenomenon.

While local application of low concentrations of many amino acids to single anthocodia may induce typical acceptance or rejection responses, depending on the nature and concentration of the stimulant, exposure of the entire colony only to l-glutamate or several related compounds rapidly induces a general suppression of neuroeffector processes characterized by: probable transmission failure in the nerve-net, reversal in sign of feeding responses, extreme extension of anthocodia, along with continued maintenance of hydrostatic pressure. Luminescence in response to electrical stimulation fails within 1 to 2 minutes of exposure to 0.025 M l-glutamate although luminescence may still be induced by isotonic KCl. All of these effects are rapidly reversible by washing in sea water.

Homocysteic acid and cysteine were the only other substances with exactly similar suppressive effects. Aspartic acid, cysteic acid, glutamine and even D-glutamate were without effect, as were glutamate derivatives with substitutions on the alpha carbon or amino group. However, gamma benzyl glutamic acid induced a remarkable post-excitatory intensification of luminescence.

These observations will be discussed in terms of inhibitory neural processes in the coelenterates. (Supported by USPHS Grant NB-04372.)

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WILLIAM H. EVOY, University of Miami, DONALD KENNEDY, Stanford University and BENJAMIN DANE, Tufts University.

Central nervous control of posture in the crayfish abdomen. (Motion picture)

The angles between segments of the crayfish abdomen are adjusted by antagonistic tonic flexor and extensor muscles. Coordinated control of output to these two muscle groups is supplied by repetitive activity in interneurons which make connections in several ganglia with different degrees of effectiveness. In some preparations, it has been possible to isolate more than 6 such central command elements; each of these exerts its maximal influence at a different point in the abdomen. Recording of ganglionic output, combined with analysis of motion pictures of the corresponding movements, has shown that a given command produces repeatable changes in conformation of the abdomen, and that different commands produce characteristic final maintained postures. Other command fibers control sequential movements of the segments, seen as a wave of excitation which affects ganglionic outputs in a metachronous fashion.

In the intermediate segments, output changes evoked by command fibers in either half of the abdominal nerve cord are bilaterally symmetrical. However, many of these command fibers evoke bilaterally asymmetrical responses in other motor systems, e.g., lateral movements of the first abdominal segment on the thorax, "steering" movements of the uropods and tonic movements of the swim-

merets. Behavioral observations indicate a relationship between metachronous beating of the swimmerets and of the walking legs also under the control of command fibers. Thus activity in single central neurons which make appropriate connections to a wide variety of output channels throughout the animal can initiate complex movements. (Supported by Grant OSR-334-66 from the U.S. Air Force and Grant NB-02944-06 from the U.S. Public Health Service.)

94

JON W. JACKLET and MELVIN J. COHEN, University of Oregon.

Neuronal regeneration: electrophysiological, histological and behavioral correlates.

The metathoracic peripheral nerves, 3 through 6, were severed or crushed on one side in the adult male cockroach *Periplaneta americana*.

Between 3 and 5 days after the operation, the miniature end plate potentials of the denervated coxal muscles disappear. There is a 20% decline in the resting potential of the affected muscles and this is correlated with an inability to excite the muscles by neural stimulation. There is a maximal concentration of RNA in the perinuclear cytoplasm of the injured neurons and the appearance of axon sprouts from the regenerating proximal nerve stump. About 2 weeks after injury the proximal stump axons have crossed the 1-2 mm gap and are well established in the distal stump. The nuclei of the regenerating neurons begin to assume an eccentric position in the soma and the perinuclear RNA ring has disappeared.

Complete functional recovery of the limb movements can occur at 45 days but may take up to 100 days. The other correlates of muscle re-innervation are (1) return of the muscle fiber resting potential to normal, (2) return of miniature end plate potentials, (3) return of evoked electrical activity in the muscles and (4) shift of the neuronal nuclei back to a central positon.

Innervation is necessary for the trophic maintenance of the muscle. The perinuclear RNA concentration is correlated with initiation of axon sprouting. The eccentric position of the neuron nucleus seems associated with increased synthesis required for the maturation of the regenerating neuron and the re-establishment of neuromuscular transmission. (Supported by PHS research grant NB-01624 to M.J. Cohen and PHS physiology training grant 667-2T1GM336-06.)

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DONALD M. MAYNARD and W. BURKE, The University of Michigan and University of Sydney.

Electrotonic junctions and negative feedback in the stomatogastric ganglion of the mud crab, *Scylla serrata*.

The stomatogastric ganglion of the mud crab contains 30-35 neurons. One of its characteristic patterns of activity involves rhythmical discharges in motor axons traveling to stomach muscles in the *N. ventriculi dorsalis*. The rhythm occurs in both intact and isolated ganglia, and one of its prominant features is the alternating discharge, in bursts, of two groups of fibers. The first consists of two large fibers (A_1) and at least two small fibers (A_2); the second group consists of at least one large fiber (B).

Intracellular recordings were obtained from the neuron somata of the above three groups. Simultaneous penetrations of the two A_1 neurons indicate that they are electrotonically coupled and are reciprocally excitatory to one another. A_2 neurons also appear coupled with one another and with the A_1 elements, forming a multiunit positive feedback system. *B* neurons are remarkable in that they receive a continuous barrage of hyperpolarizing IPSP. During the burst of *A* neurons a larger slow wave of hyperpolarization develops. The subsequent repolarization of *B* cells frequently leads to spike discharges, presumably by rebound excitation. The *B*-cell impulses in turn produce hyperpolarizing IPSP in many of the *A* neurons. Accordingly, *A* and *B* neurons appear to be linked in a negative feedback system that insures alternation. (Experiments were performed aboard R/V ALPHA HELIX and supported in part by USPHS grant NB-06017-01.)

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INGRID WALDRON, University of California, Berkeley.

Mechanisms for the production of impulse bursts in the nervous system of the flying locust. (Introduced by D. M. Wilson)

In normal flying locusts, bursts of activity in depressor motoneurons alternate with similar bursts in elevator motoneurons. However, the production of periodic bursts in the motoneurons does not depend upon this alternation. When sensory input depresses elevator activity, periodic bursts of depressor activity continue even after all elevator activity has apparently ceased. This observation suggests that, if the flight pattern is generated by motoneurons, the production of rhythmic bursts depends upon positive coupling among synergistic units. When impulse activity starts in a group of positively coupled neurons, it tends to spread and to reach high frequency. Accumulated refractoriness then causes a frequency decrease and diminished feedback may result in total cessation of activity until the units are rested and a new burst starts. Further evidence for this positive feedback mechanism for burst production is that there is a decrease in successive intervals in flight motoneuron bursts. Another possible explanation for both observed phenomena is that the flight rhythm is generated in a layer of interneurons which drives the motoneurons, though we have no evidence for this yet. Modeling experiments show that a variety of mechanisms can generate the flight pattern, and synergism of several such mechanisms may contribute to greater stability of pattern production in the locust as compared to the model systems. (Supported by NIH Grant NB 03927 to D. M. Wilson and an NSF Predoctoral Fellowship to the author.)

97

JAMES L. BAIRD, Jr. and ALAN R. MELTON, Lafayette College.

Neural control of maneuvering in the flight of the flesh fly *Sarcophaga bullata*.

Flies are tethered to a movable platform in a small, variable flow wind tunnel. The platform

can be maneuvered in the roll, pitch and yaw axes with respect to the moving airstream. Electrolytically tapered silver wire electrodes are inserted in the left and right dorsoventral indirect flight muscles by means of a pair of semi-micro manipulators, the tether serving as a reference electrode. Flight movements are induced by the moving airstream and the neuromuscular electrical activity patterns of each side of the animal are fed through paired high gain A.C. amplifiers to a stereo tape recorder.

The tether platform is adjusted to provide a roll, pitch, or yaw angle with respect to the airstream, simulating similar free flight maneuvers. Electrical records indicate the neuromuscular activity patterns which would be necessary to return the flies to straight and level flight.

Comparisons between "normal" (i.e., straight-and-level) flight and induced roll or yaw indicate differences in the electrical activity occurring in the indirect flight muscles of the left and right sides. As has often been reported, there is no synchrony between neuromuscular activity and wing beat frequency in the flight of dipterans. However, records indicate that activity patterns in the indirect muscles of either side are synchronous during straight flight. In roll or yaw maneuvers, changes in amplitude and/or phasing of neuromuscular activity patterns between sides is noted. A hypothesis to explain these differences and their effect on the flight performance of the flies will be proposed.

98

JOAN KENDIG, University of California, Berkeley.

Interactions between motoneurons in the locust *Schistocerca gregaria.*

In a flying locust bilaterally homologous muscles contract simultaneously. This synchrony may be due to a common "driver" neuron or to coupling between the motoneurons themselves. The motoneurons of the wing depressor muscles can be fired antidromically by electrodes implanted in the muscles. When a burst of stimuli occurs late enough in the firing cycle to delay or abolish the following orthodromic spike in one of the stimulated motoneurons, the spike in the corresponding heterolateral muscle occurs up to 3 msec earlier than in the preceding normal cycle. An antidromic impulse in one motoneuron therefore has an excitatory effect on another.

Intracellular recording from flight muscle motoneurons in thoracic ganglia shows that a stimulus to the depressor motoneurons of one side is followed 7 msec later by a 2 mV depolarization in the impaled heterolateral motoneuron. During random stimulation of the anterior nerve cord in a non-flying preparation, impulses in related muscles tend to be synchronous; there is also a pronounced latency dependence at about 7 msec (Wilson, in prep.). The latter is probably the result of the intracellularly observed depolarization summing with the excitatory input from the nerve cord. No intracellular event which might cause motoneuron synchrony has as yet been observed. (Supported by NIH Grant NB 03927 to Donald M. Wilson and an NSF Postdoctoral Fellowship to the author.)

99

ROGER E. CARPENTER and JEFFREY B. GRAHAM, San Diego State College.

Thermoregulation in the long-nosed bat, *Leptonycteris sanborni.*

Daily or seasonal hypothermia is characteristic of most Microchiroptera which have been studied. An exception to this general pattern is the long-nosed bat, *Leptonycteris sanborni,* which, like *Macrotus californicus* (Leitner and Ray, Amer. Zool., 4:295, 1964), maintains a high body temperature. The uniqueness of homeothermy in this suborder makes the physiological response to temperature in *L. sanborni* of interest.

Adult *L. sanborni* weigh between 19 and 26 grams, and feed on fruit and nectar. They occur from southern Arizona to southern Mexico.

This species maintains its body temperature (T_B) at a mean of 36.1°C at ambient temperatures (T_A) between 5° and 35°C. At low T_A, T_B is more variable (30-39°C). Only a few bats are unable to maintain T_B for four hours at T_A of 5°-10°C; these animals survived T_B as low as 17°C, but died if T_B dropped much lower.

The basal metabolic rate of *L. sanborni* is 1.66 cc O_2/g/hr, in a thermal neutral zone between 36° and 39°C. The lower critical temperature is indistinct, but lies between 33° and 36°C. At T_A between 5° and 25°C, thermal conductance is constant, and metabolism increases with decreasing temperature according to the formula: $M = 9.22 - 0.252\ T_A$. This curve extrapolates to normal T_B on the ordinate.

Heart rates have a minimal value of 400 beats/minute at $T_A = 41$°C. Heart rate is related to T_A as follows:

Beats/minute $= 994.1 - 12.07\ T_A$.

Although the performances of *Leptonycteris* and *Macrotus* are in sharp contrast to the apparent general pattern of thermoregulation among Microchiroptera, the small diversity of groups studied, their generally temperate zone distribution, and insectivorous diet must be considered. Hibernation and daily torpor may be instead specific adaptations required by the relatively few bats living in areas of seasonal lack of food or cold climate. (Supported by Grant GB 4570 from the NSF.)

100

PHILIP LEITNER and PAUL LIGHT, St. Mary's College of California and University of California, Berkeley.

Thermoregulatory behavior at high temperatures in three species of California bats.

Myotis yumanensis, Antrozous pallidus and *Tadarida brasiliensis mexicana,* in the loft of a large barn, were exposed to frequent heat stress during the summer. However, while temperatures near 50°C commonly occurred in the roost during the day, thermal gradients were steep, and the full impact of the heat stress was avoided by slight movements.

Most bats began to move when ambient temperatures approached 40°C. Congregations of bats on beams or in crevices near the ceiling gradually dispersed downward while increasing the spacing between individuals. On very hot days, bats usually abandoned positions near the ceiling and sought

refuge in cooler parts of the loft. However, there appeared to be a compromise between avoidance of high temperatures and preference for high or sheltered roosts. Consequently, bats were voluntarily exposed to relatively high temperatures (40°C or above) for many hours during the day, despite the availability of cooler temperatures in the loft. *Tadarida* and *Antrozous* tended to face higher temperatures than *Myotis* because of their greater reluctance to use the cooler, exposed roosting sites.

Interspecific differences in the avoidance of high temperatures were also evident in laboratory thermal gradients, *Tadarida* being most tolerant to heat and *Antrozous* the least. (Supported by grant GB-2885 from the NSF to Paul Light.)

101

STEPHEN L. KIMZEY, IRMGARD CHAPMAN, and JOHN S. WILLIS, University of Illinois.

Maintenance of K ion concentration by erythrocytes during hibernation.

It has been known since the 1930's that during cold storage human erythrocytes exhibit a net loss of K. The red blood cells of such deeply hibernating mammals as hamsters (*Mesocricetus auratus*) and ground squirrels (*Citellus tridecemlineatus*) are regularly exposed to low temperatures for extended periods of time. We have therefore investigated retention of K ion by the red cells of these species at a temperature of 5-7°C both *in vivo* during hibernation and *in vitro* during cold storage.

The mean K concentration of the erythrocytes of 8 hibernating hamsters (101 $\pm$ 3 μeq/ml cells) was not significantly lower than that of 14 awake hamsters (106 $\pm$ 3). Preliminary results with a small sample of hibernating ground squirrels indicate that the erythrocytes of this species also maintain a high K concentration at low temperatures. These findings differ from those of Andrus, *et al.* (J. Cell. Comp. Physiol. 65:415-418, 1965) who found that in the red cells of hibernating pocket mice *(Perognathus fallax)* with a body temperature of 18°C, potassium concentration declined markedly.

The red blood cells of hamsters and ground squirrels also exhibit a cold resistance *in vitro*. After ten days of storage at 5°C the K content of their cells was 85% of the initial concentrations. By comparison guinea pig erythrocytes under the same conditions retained 60% of the initial K, while only 50% remains in stored human blood. These results are in accord with those of other studies demonstrating the ability of various tissues of hibernators to maintain ionic gradients at low temperature. (Supported by Grant GM 11494 from the USPHS.)

102

DAVID E. DAVIS, Pennsylvania State University.

The induction of torpidity in woodchucks *(Marmota monax)*.

Laboratory experiments, based on knowledge of the natural environment, have been conducted to determine the role of various factors in the entrance into torpidity. Woodchucks, captured in the wild, were kept in rooms at several temperatures or in coolers at 6 C. They were fed or deprived according to the experimental program. Experiments showed that woodchucks deprived of food and kept at 6 C became torpid but woodchucks fed at 6 C did not. Some woodchucks deprived of food at 20 C became lethargic in winter. Minor disturbances in the laboratory do not arouse woodchucks. Woodchucks exposed to very low temperatures (—20 C) aroused promptly. Woodchucks that eat when aroused do not become torpid again unless again deprived. Thin woodchucks become torpid as readily as fat ones. The latency of torpidity varies with season, being short (a week) in December and in March and long (a month) in summer. The woodchuck has bouts of torpor that are short (4-5 days) at first but become longer (10-12 days). The arousal bouts last 2-3 days. Hibernation in nature lasts 3-4 months although presumably the woodchuck is torpid for only 2 months. In the laboratory woodchucks may remain in hibernation (alternating bouts of torpor and arousal) up to 8 months.

103

BRUCE L. WELCH and ANNEMARIE S. WELCH, University of Tennessee.

Apparent reduction in central sympathetic neurotransmitter activity in cold-adapted mice.

Brain noradrenaline and dopamine accumulate more slowly after inhibition of monoamine oxidase (an intraneuronal catabolic enzyme) in cold-adapted mice than in mice maintained at 27°C. These amines are depleted more slowly after inhibition of tyrosine hydroxylase (the rate-limiting enzyme for their synthesis) in the cold-adapted mice. These observations suggest that central sympathetic activity may be lower in cold-adapted mice than in mice living at temperatures near thermoneutrality.

104

FRED J. BRENNER, Thiel College, Greenville, Pennsylvania.

The role of fat deposition in hibernation and reproduction of amphibians.

Field and laboratory studies were undertaken on the relationship between fat deposition and the various phases of life histories of different species of frogs. There was a significant relationship between the amount of body fat and the percentage of the total body fat in the fat bodies and the reproductive condition of the animals. The reproductive conditions of the animals were determined by histological and histochemical methods. A study was also undertaken on the role of the various factors in the environment on fat deposition as well as their relationship to the different phases of the life histories of the different species. (Supported by Grant #602-Johnson Fund from the American Philosophical Society.)

105

PETER J. BENTLEY, C. F. HERREID, and K. SCHMIDT-NIELSEN, Duke University.

Respiration of a monotreme, the echidna *Tachyglossus aculeatus*.

The respiration of the echidna is of considerable interest since little information is available for non-placental mammals. Also, the echidna has a body temperature of 31°C with half the oxygen consumption expected for a placental mammal of

the same size. Furthermore, the burrowing habit of the echidna may be associated with respiratory specializations. End tidal (alveolar) concentrations of CO_2 (5-6%) and oxygen (14-15%) were similar to those of placental mammals. In laboratory pens under crushed corn cobs, echidnas voluntarily tolerated concentrations of 5% CO_2. Inhalation of various mixtures of CO_2 and air showed that minute volume increased as the per cent CO_2 was increased. The increase was due to greater tidal volume rather than higher respiratory frequency. In contrast, when minute volume increased in echidnas breathing room air, the increase was due to higher respiratory frequency. The sensitivity of echidnas to CO_2 seems low, but definite conclusions cannot be drawn until results on more species are available. (Supported by NIH grant HE-02228 and NIH Research Career Award 1-K6-GM-21,522 (KSN).)

106

DONA J. FOWLER and CLARENCE J. GOODNIGHT, Western Michigan University.

Daily rhythms in the arachnid, *Leiobunum longipes*.

In previous papers (Science, 152:1078, 1966; Amer. Soc. of Zool., 6:187, 1966; Trans. Amer. Micro. Soc., 85:378, 1966) the daily rhythm of locomotion and 5-hydroxytryptamine secretion in *Leiobunum longipes* from West Central Indiana was described. The rhythms of both locomotion and hormonal secretion were shown to have a unimodal pattern of peak activity, which appeared during the same hours at night. This paper is a report of the locomotor activity of a population of the same species located in Southwestern Michigan. The behavior data as well as preliminary work on secretion of 5-HT suggest that the Indiana population and the Michigan population are separate physiological races.

107

JOHN CARRUTHERS FERGUSON, Florida Presbyterian College.

Insensitivity of starfish digestive glands to metabolic inhibitors and drugs.

Isolated digestive glands from the starfish, *Echinaster spinulosus,* are currently being used for experiments in echinoderm nutrition and nutrient transport. Three physiological parameters have so far been studied in these preparations. These are their (1) tonic state, (2) uptake of amino acids and sugars from incubative media, and (3) consumption of oxygen. While on the basis of Q_{10} and other properties these processes all appear to be energy dependent, each has demonstrated a remarkable insensitivity to many drugs noted for their ability to inhibit or modify metabolic processes in other organisms. Among the drugs that have been tested are the following: sodium iodoacetate, potassium cyanide, sodium fluoride, dinitrophenol, acetylcholine bromide, epinephrine bitartrate, dihydroxyphenylalanine (DOPA), serotonin (not on respiration), and phloridzin and sodium azide (only on respiration). These have been used at various concentrations, in some cases as high as 20 mM. Each substance fails to modify the normal responses except that acetylcholine initiates prolonged contractions of the organs, and this contracted state may be inhibited by extended exposure (30 to 75 min) to cyanide. Stronger poisons, such as trichloroacetic acid, have the more pronounced effects one would expect. While impermeability of the tissues to the drugs, or alternative energy pathways, may be suggested as reasons for the lack of sensitivity of the organs, no completely satisfactory explanation for the phenomenon can yet be offered. (Supported by NSF grant GB-4994.)

108

S. K. AGGARWAL and R. C. KING, Northwestern University.

The ultrastructure of the thoracic endocrine system of female *Drosophila melanogaster*.

The morphogenesis of the female ring gland during the larval and prepupal stages is described. The mature organ contains tissues of four types: (1) corpus allatum cells, (2) prothoracic gland cells, (3) corpus cardiacum cells, and (4) tracheal cells. Axons bearing spherical neurosecretory granules pass from the corpus cardiacum to the corpus allatum. Ecdysone and juvenile hormones are thought to be manufactured by smooth surfaced endoplasmic reticulum consisting of filaments and tubules contained in the prothoracic gland cell and the corpus allatum cell, respectively. Between the first instar and pupation the cells of the prothoracic portion of the ring gland increase in volume by 100 times, while their nuclei increase 10 times. The increases in nuclear and cytoplasmic volumes are but 6 times and 9 times for corpus allatum cells. The prothoracic gland breaks down during metamorphosis. The anatomy of the corpus allatum-corpus cardiacum complex is described for adult females varying in age between 0 and 24 days. The corpus allatum is known to produce a hormone influencing vitellogenesis. The ultrastructure of the gland suggests that this hormone is also synthesized within smooth surfaced endoplasmic reticulum. The corpus cardiacum consists of a central bundle of axons surrounded by cortical cells. Many of the axons are thought to originate as projections from specialized, secretory neurons residing in the pars intercerebralis. Neurosecretory spheres are carried posteriorly in these axons by traveling bulges. The cortical cells contain myriads of neurosecretory spheres and an extensive rough endoplasmic reticulum consisting of interconnected bloated cisternae which arise from the outer membrane of the nucleus. (Supported by Grant GB 4891 from the NSF.)

109

ELIZABETH A. KOCH, Northwestern University.

The division and differentiation of *Drosophila* cystocytes.

Light and electron microscopical studies allow a descriptive account to be given of the morphogenesis of the egg chamber of *Drosophila melanogaster*. The study demonstrates that the mitotic products of a single cystoblast generate a branching chain of 16 interconnected cystocytes. Two specific cystocytes enter meiotic prophase, while the rest become nurse cells. The two pro-oocytes form synaptinemal complexes in their nuclei. However, one of the two cells later switches back into the nurse cell developmental pathway. The elongation of the

synaptinemal complexes is described, and estimates are made of the time involved in their formation. These complexes continue to be synthesized long after the DNA replication which gives the oocyte its 4C DNA content. This finding implies that at least some genetic crossing over follows DNA replication. Suggestions are given as to the mechanisms by which (1) the future cleavage planes of cystocytes are programmed, (2) the pro-oocytes are differentiated from nurse cells, and (3) the oocyte is chosen from the twin pro-oocytes. The contrasting behaviors of the oocyte and nurse cell nucleoli are described. During oogenesis nucleolar synthesis of ribosomal RNAs is suppressed in the oocyte and concurrently stimulated in the nurse cells. It follows that the nurse nuclei are the major sources of the prodigious quantities of ribosomes found in the ooplasm of the mature oocyte. (Supported by grant GB-4891 from the NSF.)

110

J. LAI-FOOK, Western Reserve University and University of Toronto.

The ultrastructure of muscle insertions in insects. (Introduced by M. Goldsmith)

Insect muscles have their insertions in the cuticular exoskeleton. The discontinuous growth imposed by this type of skeleton necessitates the renewal and reestablishment of muscle insertions. This study describes the fine structure of the myoepithelial junction, the epitheliocuticular connexions and the continuity in these relationships during moulting in larvae of *Calpodes ethlius* (Hesperiidae).

The myo-epithelial junctions may be described as extensive, interdigitating regions of intermediate desmosomes or 'zonulae adhaerens.' Myofilaments extend from these desmosomes in the muscle while microtubules traverse the epidermis from the desmosome to the plasma membrane adjoining the cuticle. The epidermis is anchored to the cuticle by tonofibrilles.

The muscle insertions are continuous from the 4th to the 5th larval instar. The epicuticle and endocuticle are deposited around the tonofibrilles which remain attached to the old cuticle. In the region of the insertion the cuticle appears to be dissolved at a reduced rate. The almost complete immobility of the larvae prior to ecdysis may coincide with the detachment of the tonofibrilles from the old cuticle. (Supported by Grant GM 09960 from the United States Public Health Service.)

111

WILLIAM B. MUCHMORE, University of Rochester.

The so-called somite growth center of salamander embryos.

In recent years several investigators have considered that the cells of the dorsomesial section of the developing somite in the salamander embryo constitute a "somite growth center." The somite growth center has been defined as a group of cells which remain undifferentiated and by continued division supply cells to the more ventral areas of the somite. Contrary to this idea, study of colchicine-treated *Ambystoma* embryos of various ages reveals no unusual mitotic activity in the dorsomesial cells of the somites. Indeed, observation shows that a majority of these cells are actually muscle cells which are, however, much smaller than the usual myotomal cells and possess a smaller complement of myofibrils. They develop in a manner comparable to that of the main portion of the myotome, but form separate muscles lying between the spines of adjacent vertebrae. Cells which may be added to the myotome after its initial establishment and cells which form vertebral cartilages appear to be supplied by dividing interestitial and peripheral cells. The extensive results of Holtzer and Detwiler (J.E.Z., 123:335-370, 1953) can be explained more satisfactorily in these terms than on the basis of a discrete somite growth center. (Supported in part by Grant GB 1006 from NSF.)

112

JEANNE A. POWELL, Bryn Mawr College.

Effects of amputation on denervated and partially innervated regenerate limbs of *Triturus viridescens*. (Introduced by Jane M. Oppenheimer)

Morphogenesis was studied 10 and 18 days following bilateral hand amputation and concomitant unilateral denervation of 30, 40 and 50 day regenerate forelimbs of *Triturus*. The level of amputation was confirmed by histological examination of the hands. The younger the regenerate, the more precocious was hand regeneration in the innervated limb, and the further advanced was the regression of tissue in the denervated limb. The younger regenerates of both limbs exhibited greater cartilage matrix breakdown and cell release than older ones. The released cells of the innervated limb contributed to the growing blastema, which may account for the precociousness of differentiation in the younger regenerates. In nerveless limbs the cell release was accompanied by cell loss, which accounted for the observed regression.

Regenerates of the same age which were allowed to proceed through morphogenesis without nerves were studied similarly. Denervation was performed at stages after the regenerate becomes independent of nerve supply. Muscle differentiation proceeded in the absence of nerves after primary denervation, but after hand amputation and redenervation. regeneration was blocked, muscle degenerated and cartilage regressed. In some cases no second denervation was performed; reinnervation occurred and muscle tissue was maintained. In a few such cases hand regeneration was initiated. Its incidence suggests that the younger the regenerate, the smaller the nerve supply needed to initiate renewed regeneration. (Supported by a U.S. Steel Foundation Fellowship.)

113

JOHN W. SECHRIST and ARTHUR LAVELLE, University of Illinois.

Neurofilaments and initial neuroblast differentiation.

Classical neurologists based their descriptions of neurocytogenesis largely on silver impregnation of neurofibrils. Recent investigators using autoradiography and electron microscopy have suggested that the neural epithelium consists of a morphologically homogeneous population in which cell types cannot be differentiated. Our preliminary

investigations (Anat. Rec., 154:420, 1966) using a combined silver and autoradiographic technique indicated that a distinct neurofibrillar network could be observed in cells adjacent to the lumen during final mitosis as well as in paired daughter cells undergoing apolar and bipolar stages before reaching the mantle zone.

In order to determine what this neurofibrillar network represented at the ultra-structural level, the hindbrains, forebrains, and retinas of three-day chick embryos were prepared for high resolution electron microscopy. If embryonic neurofibrils represent clumps of neurofilaments as they presumably do in the adult, one should be able to observe these filaments in a small percentage of mitotic and post-mitotic cells in the epithelial zone as well as in the mantle zone. Lyser (Dev. Biol., 10:433, 1964) has given an excellent description of filaments in early motor neuroblasts of the chick, but she has questioned whether these may be equated with neurofibrils of light microscopy. Our investigations with high resolution electron microscopy indicate that embryonic neurofibrils are most likely clumps of neurofilaments. The appearance of filaments or neurofibrils soon after the final DNA synthesis would suggest that they are the earliest criteria for recognizing a neuroblast and that they play an important role in the outgrowth of processes. (Supported by grants B-415 and 1T1-MH-8396 from the USPHS.)

114

DORIS J. BURDA, Stanford University School of Medicine.

Characteristics of endoneural vascularity in embryonic brains exhibiting overgrowth.

A study has been made of the manner by which blood vessels penetrate avian neural tissue under abnormal conditions of overgrowth. Brain defects were induced experimentally by the removal of the anterior rhombomere of the chick embryo at approximately stage 11-12 (Hamburger-Hamilton). The telencephalic and mesencephalic regions responded with the formation of convoluted, tumor-like masses resulting from an increase in mitotic activity along the ventricular border. The embryos were sacrificed at daily intervals, embedded in paraffin, sectioned at a thickness of 5 μ, and stained with hematoxylin and eosin. Comparisons were made between the endoneural vascular pattern of defective brains and that of normal brains at a comparable age.

The overgrown portions characteristically show large hemorrhagic areas which develop from the surface vessels and from the penetrating capillaries in regions where there are marked convolutions in the neural wall. Enlarged sprouts from the surface vessels create deep indentations along the border of the marginal layer. As in normal brain tissue, penetration occurs at right angles to the surface of the brain, and the penetrating vessels then send branches cephalad and caudad to form longitudinal endoneural sinuses. In the defective brains, these sinuses are tortuous and of greater diameter than is the case under normal conditions of development. Although the overgrown tissue shows an increase in mitotic activity along the ventricular border, vessels seldom invade this region of the ependymal layer. (Supported by Grant 1 RO1 HD 01679-01A1 from the USPHS.)

115

CHARLES W. GIBLEY, JR. and JEFFREY P. CHANG, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

Electron microscopic observations on the functional mesonephros of the 8-day chick embryo.

Morphological, histochemical, and physiological studies from this laboratory and elsewhere suggest the pronephros, mesonephros, and metanephros of vertebrates should be adjudged not as separate kidneys but as intergrading regions of a holonephros. Information on the ultrastructural level is lacking.

Proximal tubule cells: Nuclei are spherical and basally oriented. Mitochondria are round or elongate with clear cut cristae. Intramitochondrial granules occur sporadically. The Golgi, lying adjacent to the nucleus in apical cytoplasm, consist of flattened lamellae and associated secretion droplets. The cytoplasm is filled with ribosomes; occasionally, spiral in arrangement. Lipid droplets are scattered throughout the cytoplasm. Characteristic microvilli project from the apical end of cells. Basal ends of tubules are bounded by a homogeneous basement layer. Adjacent epithelial cells are separated at their base and exhibit interdigitating processes between cells. At their apices cells are joined by desmosomes.

Distal tubule cells: Nuclei are round and centrally located. Microvilli are predominantly absent; when present, they are short and blunt. Cells are closely allied at their base and joined tightly at their apices. Interdigitating processes are not as prevalent as in proximal tubules. Infoldings of the plasma membrane are prominent and compartmentalize mitochondria.

Glomerulus: Endothelial cells are elongate bordering the capillary lumen and their membranes contain indefinite pores. Epithelial pedicels extend from the cell body interdigitating with each other and resting on the basement membrane. The latter consists of three layers resembling those in adults.

The results of this investigation furnish additional evidence for the holonephric theory of vertebrate kidney evolution. (Supported by NSF Institutional Grant GU-1499 and grant CA-05312 from USPHS.)

116

BETTINA H. HARRISON and DONALD I. PATT, Boston University.

Lymphopoiesis in palatine tonsil of the fetal rabbit.

Tonsils of fetal rabbits from 17 days *post coitum* to birth have been studied by light and electron microscopy. The basal cells of the epithelium of the tonsil anlage are slightly smaller, rounder, and more basophilic than the more superficial cells. Light microscopy has revealed the progressive development of buds of tonsil epithelium which push into the underlying mesenchyme. Associated with the basal cells of these buds are small numbers of small, round, basophilic cells which appear in the mesenchyme on the 19th day. They increase in number progressively with age and can be identified as lymphocytes by the 27th day.

By electron microscopy the round basophilic cells in the mesenchyme show large nuclei, prominent nucleoli, relatively little cytoplasm, little rough-surfaced endoplasmic reticulum, free ribosomes in the cytoplasm, and large oval mitochondria. These cells resemble lymphocytes of the 19-day fetal thymus. Micrographs of the epithelium show that the basal cells also have large nuclei, very scanty rough endoplasmic reticulum, and large oval mitochondria. They also have desmosomes between cells and half-desmosomes at the basement membrane. Mesenchymal cells, on the other hand, have copious cytoplasm with rough endoplasmic reticulum, pale-staining oval nuclei, and no free ribosomes.

An epithelial origin has been proposed for lymphocytes in the hamster thymus (Weakley, Patt, & Shepro: 1964) and the bursa of Fabricius (Ackermann & Knouff: 1959). The present study seems to indicate a similar origin of lymphocytes in the rabbit tonsil by migration and differentiation of basal cells from epithelial buds of the tonsil anlage. (Supported in part by grant HE-06214, USPHS.)

117

JOSEPH S. BALSANO, PETER ABRAMOFF, and REZNEAT M. DARNELL, Marquette University.

Serological evidence of hybridization in Mexican poeciliid fishes.

Serum albumin is evolutionarily conservative, and the literature supports the view that each vertebrate species is characterized by a single serum albumin as determined by electrophoretic mobility. Presence of two serum albumin bands within a single individual is taken to be evidence of interspecific hybridization.

In the present study of the evolutionary history of poeciliid fishes of eastern Mexico it has been found that typically each species possesses a single serum albumin of distinct and characteristic mobility. On the other hand, numerous individual fish have been found to possess more than one albumin band. Such double albumin electropherograms can be exactly duplicated by mixing isoaliquots of serum from the presumed parental species. Furthermore, by mixing isoaliquots of serum from the hybrid and one of the parental species it is possible to demonstrate electrophoretic identity of one of the albumin bands of the hybrid. By the same procedure the other band may also be identified.

Employing this technique it has been demonstrated that one of the species (*Poecilia formosa*) is of hybrid origin since all of the individuals tested have two albumin bands. These represent the combination of single bands of two distinct species occurring naturally in the area. In addition, it has been found that among the single-albumin species of eastern Mexico extensive hybridization has taken place in areas of sympatry. Mixing techniques have demonstrated the parental species of the hybrids. Validity of these conclusions is being tested by a breeding program. (Supported by grants GB-3313, GB-4712 and fellowship 65064 from the NSF.)

118

BROTHER LAWRENCE CORY, St. Mary's College, California.

A morphologically unique frog population in an ecologically rigorous environment.

Studies to the present indicate that frogs of the *Rana boylei* complex in higher altitudes of the Sierra Nevada Mountains in California (*Rana mucosa* according to Zweifel, R., Univ. Calif. Publ. Zool., 54:207-292, 1955) display a unique pattern of geographic variation (Cory, Am. Zool., 2 (3):401 and 2 (4): 515, 1962). This involves a major assemblage of characteristics distinctive of the animals from a major drainage system, with a unique pattern of more trivial variation superimposed on this general pattern among animals from populations within this major drainage system, but more of less isolated from each other. General altitudinal distribution limits from north to south in the mountains have also been determined. One population found at the altitudinal limit for its latitude and at the base of a high north-facing exposure, thus being in an extremely cold environment, does not conform to this geographic pattern. Adults show more intrapopulation variation than is typical for these frogs and they do not conform to the morphological pattern characteristic of their drainage system. In spite of this, the young, newly-metamorphosed animals mostly do conform, though the types of variants found as adults do occur among them. A tentative explanation is offered, based on the concept of genetic load of variability.

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GLADYS S. KING, Trinity College, Washington, D.C.

Studies of non-tumor kidneys of Vermont *Rana pipiens* from three seasons during storage at hibernation and laboratory temperatures.

A biochemical lesion stage characterized by increasing P^{32} uptake and distinctive cellular structures in non-tumor kidneys of second season frogs stored at 25°C has been previously described by the author (Ann N.Y. Acad. Sci. 126:222-236, 1965). The study has been extended to include first and third season frogs maintained at 25°C as well as in hibernation at 5°C. The paper to be presented will consider the results of this research with non-tumor kidneys in relation to later tumor incidence.

The cellular structures which accompany changes in P^{32} incorporation were originally designated as: film blackening inclusions (FBI), and *phospholipid*-containing nuclei (PCN). The former have now been identified as RNA and additional histochemical tests have verified the lipid character of the latter. PCN are presently recognized as markers for cells undergoing necrosis.

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MARSHALL J. YOUNGBLUTH, University of Hawaii.

Aspects of the ecology of the cleaning wrasse, *Labroides phthirophagus* Randall. (Introduced by E. S. Reese) (Motion picture)

Cleaning symbiosis consists of cleaning fish approaching displaying host fish and removing ectoparasites from body surfaces and gill chambers. This relationship is widespread among neritic fish in tropical seas.

The ecology of the endemic Hawaiian cleaning

wrasse was studied in the field. Cleaning activity is diurnal and confined to a particular area called a "cleaning station." Cleaning stations, characterized by a "shelter" substratum and an abundance of reef fish, appear to be stable habitats but the resident cleaners at certain stations vary in number and size. In the evening the wrasse encapsulates itself within a mucous envelope.

Labroides phthirophagus, an obligate cleaning fish, is nospecific in the fish it cleans. The diet consists primarily of caligoid copepods and larval grathiid isopods. The number of host fish cleaned per day appears to be constant. The removal of *L. phthirophagus* from selected patch reefs did not result in a change in the number of host fish cleaned or in a decrease in the reef's fish population.

Comparative studies were made at Eniwetok Atoll, Marshall Islands, on *L. dimidiatus* and *L. bicolor* which occupy the same ecological niche. (Supported in part by Grant GB-3651 from the NSF and by the Eniwetok Marine Biological Laboratory.)

121

JOHN A. MORRISON and EMIL W. MENZEL, JR., Laboratory of Perinatal Physiology, Puerto Rico, and Delta Regional Primate Research Center, Covington, La.

Adaptation of a rhesus monkey group to artificial group fission and transplantation to a new environment. (Motion picture)

In June, 1966, H group of Cayo Santiago, Puerto Rico, consisted of 82 semi-wild *Macaca mulatta* of known lineage and stable social organization. Then, 56 members were trapped, transported to the uninhabited 350 acre island of Desecheo, and released. They were studied continuously for the first week and periodically thereafter.

(a) Even with abundant space and absence of pressure from other groups, they maintained a cohesive organization. Only two (both males, one an ex-solitary) deserted. Two parties that had been released a day apart shortly reunited.

(b) Only a fraction of the new island was explored, and within a few days travel was at a low level. The group was found using the same trails one month later.

(c) Excitement was low throughout. Flight distance from observers remained low. A majority of the time on the new island (where the animals were no longer provisioned) was spent in foraging and resting. Except perhaps for grooming, social interactions decreased. Play disappeared. Coloration of the female sex skin, and the breeding season, were retarded.

The members of the group who remained on Cayo Santiago were also studied. Except for one male, who deserted during the breeding season, they too remained an intact band. They maintained H group's old dominance over 3 other groups (now numerically superior) for about 6 weeks. In the near future these animals will be taken to the new island, so that range, travel patterns, social cohesive factors in the entire group (or splitting along family lines), and general adaptation can be further analyzed.

122

NEAL R. FOSTER, Academy of Natural Sciences of Philadelphia.

Major biological changes in the evolution of killifishes.

Comparative behavioral and life history studies on 40 species of 21 genera, together with morphological studies on these and about 60 other species and 13 other genera, have led to the following hypotheses regarding the evolution of killifishes (Pisces, Cyprinodontidae and Oryziatidae). Probably derived from some euryhaline Eocene atherinoid inhabiting shore areas of the Tethys Sea, the evolutionary precursor of killifishes was a small, terete, pelagic, surface-swimming fish which fed primarily on plankton. (Morphologically, it possessed metapterygoid and parietal bones, long epiotic processes, poorly protractile or nonprotractile premaxillaries, three ossified basibranchials, a well-developed hemal funnel receiving the end of the elongated swim bladder, free pseudobranchiae, two epurals, and a trifid median hypural.) The protocyprinodont displayed only a slight degree of sexual dichromatism, a trait correlated with its strongly developed schooling behavior. After a brief courtship, the male and female clasped against one another in open water, not in contact with any substrate, and 20 to 100 fertilized eggs immediately scattered downward onto the bottom.

As killifishes exploited niches in clearer, more ephemeral and thickly vegetated shallow water areas, major evolutionary changes occurred. There was an increased degree of sexual dichromatism and a greater utilization of visual displays in the reproductive behavior. The spawning clasp was now in direct physical contact with mud, sand, or fibrous plant substrata, and eggs were released singly or a few at a time. Territorial behavior of males became centered less around the females and more around specific areas associated with suitable spawning substrata. (Supported by Grant G-23395 from the NSF.)

123

KLAUS D. KALLMAN, Genetics Laboratory, New York Aquarium.

Permanent survival of fin transplants among wild-caught fish of the genus *Xiphophorus.*

In vertebrates skin transplants survive usually only among members of inbred strains or between monozygous twins. An apparent exception was reported several years ago (Copeia, 3:513-522, 1964) when we discovered that a high percentage of fin (skin) grafts exchanged among siblings of wild-caught *X. c. couchianus* survived permanently. Similar experiments with *X. hellerii* (323 grafts, 3 pedigrees), *X. milleri* (857 grafts, 3 ped.) and *X. maculatus* (271 grafts, 8 ped.) that were obtained from large natural populations, were unsuccessful. Only one *milleri* graft survived permanently (one year minimum). However, 2.8% of intra-sib grafts (18 of 622) among the offspring of 5 matings of *X. maculatus* that were taken from a small peripheral population, were successful. Two natural populations of *X. variatus xiphidium* were tested. Seven females and three males were collected in the Rio Santa Engracia where *xiphidium* is abundant, and bred in the laboratory. Out

of a total of 378 intra-sib grafts 29 (7.5%) survived, but the hosts with "takes" were not uniformly distributed among the seven pedigrees. It ranged from zero in one pedigree to 22% (12 out of 52) in another. In the San Carlos Mountains only seven *xiphidium* (3 ♀ ♀, 4 ♂ ♂) were found in a small spring that does not run for more than 150 feet in the dry season. It is separated from the nearest permanent river by more than 30 miles of dry stream bed. Survival of intra-sib grafts in the three pedigrees were 50% (36 of 74), 57% (21 of 37) and 96% (67 of 70). These results demonstrate a relationship between population size and percentage of successful intra-sib fin grafts. Small isolated populations have achieved a high degree of genetic uniformity and may be naturally inbred. The seven per cent "takes" in the fish of the Santa Engracia population may indicate that the *effective* population size is smaller than appears. (Supported by Grant CA 06665 from the National Cancer Institute.)

123a

N. S. HARRIS and P. J. VAN ALTEN, University of Illinois.

The role of sensitized donor tissue in allogeneic transplants.

The allogeneic transplant of mammalian tissue exhibits two phenomena, rejection or tolerance. One of the unbending principles of transplantation immunology is that the fate of the mammalian graft depends solely upon the immune response elicited by the host.

It was the intent of this study to use skin allografts in adult mice as the experimental model in which to investigate the present concept concerning the role of donor tissue in transplantation.

Recent work in our laboratory indicates that prior sensitization of an adult mouse skin graft donor, by intraperitoneal injections of a skin cell suspension, results in an accelerated graft rejection when later transplanted to an unsensitized allogeneic recipient. This was a more rapid rejection than when either just the host or both host and donor were sensitized prior to transplant. Furthermore, histological sections of the spleens from the injected animals indicate a graft versus host (GVH) reaction. Since a GVH reaction or a runting syndrome depends upon the reaction of immunologically competent cells, this may indicate that under certain conditions mammalian skin is an immunologically competent tissue, and has the capacity to develop an immune reaction. (Supported by grant 5T1 HD-16-05 from the USPHS and NSF grant GB2874.)

132

R. G. B. BROWN, Dalhousie University.

The behaviour of courted female *Drosophila pseudoobscura*.

The courted female *Drosophila pseudoobscura* has no acceptance response; instead, she merely desists from displaying one of several rejection responses. She does so most readily between her second and twelfth days of life, provided she is still a virgin; re-matings of fertilised females also occur, but they are relatively rare.

There are two main rejection responses: a downward curling of the abdomen, shown by 1-day-old, subadult females; and an upward raising of the abdomen tip combined with extrusion of the genitalia, shown by older females. Both displays effectively prevent the male from making genital contact. The shift from curling to raising/extrusion is controlled only by age, and occurs whether or not the female has been fertilised. In fact, raising/extrusion is so exaggerated in the older virgins that copulations with females over two weeks old are very rare, at least within the 5-minute observation period.

Apart from fertilising the eggs, copulation has only two effects on the female. For a short time afterwards she becomes very repellant to males; this has nothing to do with any overt behaviour on her part—it is probably an olfactory by-product of the act of fertilisation. Virgin females start to lay when they are 7-10 days old, so copulation does not stimulate egg-laying. However, it advances the start of laying, and increases the numbers of eggs laid. (Supported by a grant from the National Research Council of Canada.)

133

LOWELL R. FRITTS, University of Wisconsin.

Social facilitation in *Drosophila melanogaster*.

Courtship behavior in *Drosophila melanogaster* appears to begin sooner and to be carried out more effectively when large numbers are present. A possible advantage of this might be that the fittest members of the population would be induced to mate sooner and thereby produce offspring at a time when the food source is more abundant.

Observations were made in tape-lined chambers with a volume ratio of 1:10:30. The numbers of virgin pairs observed during a given "run" were kept in this same ratio. Virgin flies were separated from their culture jars twice daily and were aged, males and females separately, in shell vials with food. After two to six days the flies were anesthetized and placed in the observation chambers. As a general rule the larger the group the sooner the beginning of courtship and copulation, and the higher the percentage of copulations. (Supported through the University of Wisconsin Research Participation Program.)

134

VICTOR R. JOHNSON, JR., University of Hawaii.

Pair formation in the banded shrimp, *Stenopus hispidus*. (Introduced by E. S. Reese) (Motion picture)

Field observations of *Stenopus hispidus*, an incidental cleaning shrimp, indicate that it is normally found on the reef in male and female pairs. Pairs were taken to the laboratory, and individuals of approximately the same size were repaired both with the same and opposite sexes. Strange shrimps of the same sex are highly aggressive and usually fight to the death. Within aquaria there appears to be no submissive or appeasement behavior. Strange shrimps of the opposite sex tend to show initial fighting, followed by courtship and the formation of a breeding pair. Experiments were done to determine the major factors involved in

pair formation and hence mate recognition. Pairs of shrimp allowed visual and chemical contact prior to being placed together show quantitatively less fighting and more courtship than pairs placed immediately together. The relative importance of vision and smell, and of controlled amounts of tactile contact are presently being studied. Transfer to new surroundings seems to increase the overall level of aggression. The establishment of pairs and the behavior to other species of shrimps indicates that *Stenopus* is capable of species as well as individual recognition. (Supported in part by Grant GB-3651 from the NSF and by the Eniwetok Marine Biological Laboratory.)

135

MICHAEL H. SMITH, University of Georgia.

Mating behavior in the old-field mouse.

Mating behavior in the old-field mouse, *Peromyscus polionotus,* was studied in the laboratory. Six phases were evident in the mating pattern: 1) initiation of courtship by the female; 2) female running back and forth in front of male: 3) posturing by the female; 4) mounting by the male; 5) thrust-intromission by the male; 6) dismounting by the male. This sequence was repeated many times during the early part of the night and was most frequently observed during the past-partum heat. The male gradually took the initiative as the number of copulations increased. Mating ceased when the female became aggressive and drove the male out of the nest and into one corner of the cage.

The six phases of mating behavior were approximately the same as those described by Tamsitt for the *Peromyscus truei* species group (Amer. Midl. Natur. 65:501-507). However, one major difference was noted. Female old-field mice tend to vocalize during the behavior leading up to copulation; this may be a species specific characteristic. (Supported by AEC grant AT(38-1)-310, a grant in aid from Sigma Xi, and an NIH predoctoral fellowship.)

136

ROBERT E. KUEHN and IRVING ZUCKER, Oregon Regional Primate Research Center.

Mating behavior of *Meriones unguiculatus.* (Introduced by K. Nelson) (Motion picture)

Six male-female pairs of mongolian gerbils were each observed in three mating tests at two-week intervals. Females were spayed and brought into heat with subcutaneous injections of 6 μg of estradiol benzoate and .4 mg of progesterone given 36 hours apart. Testing began 7 hours after progesterone administration. This treatment produced uniformly intense receptivity in all females.

Up to three ejaculations were recorded in tests lasting one hour or less. Typically, the first ejaculation followed 41 intromissions spaced at an average interval of 24 seconds. The second ejaculation was preceded by 27 intromissions at 20-second intervals, and the third, by 22 intromissions 18 seconds apart. The average post-ejaculatory refractory periods following the first three ejaculations lasted 3.6, 4.2, and 4.8 minutes, in that order. In the first ejaculatory series, the average interval between intromissions was twice as long during the first fifth of that series as in the remaining four-fifths. Intromission interval remained constant over the course of the second and third ejaculatory series.

Thumping, a staccato pounding of the rear feet against the substrate in brief, repetitive bursts, was found to be sexually dimorphic and related to sexual performance. Males thumped nearly 20 times as often as females. In the male, the rate of thumping was higher during the first ejaculation than in either of the later series recorded, and it approached a common value near the time of ejaculation in all three series. (Supported by Grants MH 08634 and FR 00163.)

137

F. H. BRONSON, The Jackson Laboratory.

A sex attractant function for mouse preputial glands.

Odor preferences for various urines or tissue homogenates were tested in 40 C57Bl/6J females during 7 successive trials. Trials 1-3 were conducted in small (12×24×8-inch) test chambers and documented the attractant qualities of either male urine containing preputial secretion or of homogenates of the preputial glands themselves (compared with various other types of male or female urine). Trials 4-7 tested preputial homogenates against urine from female or preputialectomized males or against a no odor condition in a much larger test apparatus (4×4 feet) and verified the above finding. The phenomenon was stable across all 4 trials. Dependence of the phenomenon on the stage of the female's estrous cycle and upon the dominance status of the male is under investigation.

138

KEITH NELSON, University of Maryland.

A short-term periodicity in nest-rebuilding of the male three-spined stickleback (*Gasterosteus aculeatus* L.).

The male stickleback fashions a nest entrance by boring into the mat of material he has collected, which eventually results in creeping through the nest, forming a tunnel. Boring is often followed by a short bout of fanning, which during the parental phase serves to ventilate the eggs.

If the male's nest is removed, he will rebuild; rebuilding resembles building of the first nest but is more compressed in time. The latter half of rebuilding is usually statistically stationary. In most records, computation of time-series statistics revealed a clear rhythm in probability of occurrence for boring and fanning, but for no other activities (such as gluing, bringing materials, digging, etc.). In different individuals and at different times the period of the rhythm ranged from about 0.8 to 1.8 minutes; it was remarkably stable over sessions of up to several hours. (Supported by an NIMH Postdoctoral Fellowship and by NSF Grant GB-4881.)

139

JOHN C. MERTZ, University of Illinois.

The organization of the parental fanning of the cichlid fish, *Cichlasoma nigrofasciatum* (Günther). (Introduced by Edwin M. Banks)

Nineteen pairs of *C. nigrofasciatum* were allowed to spawn in a standardized environment. After spawning was completed, the male was separated from the female by a screen partition. The female was left to care for her brood. Quantitative records of fanning and other behavior patterns performed by the female during the period from completion of spawning through hatching (3 days at 26°C) were made with the aid of an Esterline-Angus event recorder activated by a manual keyboard. A total of six parameters of fanning was studied. Their quantitative relationships to intervals of no fanning and to the frequencies of other behaviors were explored.

Of the fanning parameters, the total time spent fanning per observation period (15 minutes) proved to vary most consistently during the course of the fanning cycles of all females. The amount of time spent fanning was highest immediately after spawning, declined sharply during the first day of the cycle, and remained fairly constant during the remaining two days of the cycle. A very similar temporal pattern was followed by the total number of beats of fanning per observation period. Similar patterns were usually (but not in all cases) expressed by the average duration of fanning bouts and the average number of fanning beats per bout. (Supported by Predoctoral Fellowship 1-F1-GM-20, 430-01 from the USPHS to the author, and grant G 24016 to George W. Barlow.)

140

JUDITH M. STERN, Rutgers Institute of Animal Behavior.

The role of the testis in progesterone-induced incubation behavior in ring-doves, *Streptopelia risoria*. (Introduced by D. S. Lehrman)

Progesterone induces incubation behavior in intact ring-doves of both sexes (Riddle & Lahr, Endocrinol. 35:255, 1944). Simultaneously, progesterone inhibits the male's androgen-dependent bowcooing (Erikson *et al.*, in press). In view of this progesterone-androgen relationship, the present investigation was concerned with the possible influence of testicular hormones in bringing about readiness to incubate.

The subjects were male ring-doves with previous breeding experience. Intact and castrated Ss were subdivided equally into two treatment groups (four groups of 15 Ss each): experimental Ss received seven daily intramuscular injections of progesterone (0.1 mg/day); controls received an equal volume of the vehicle alone (0.1 ml of sesame oil/day). A test for incubation behavior was initiated 24 hours after the last injection.

None of the 30 males in the sesame oil control groups showed any signs of incubating in the 48-hour test period. Of the 15 progesterone-treated intact Ss, 13 incubated whereas only 5 out of the 15 similarly treated castrates incubated ($p<.01$). Furthermore, of those birds which incubated, latency was significantly shorter in the intact group.

The presence of the testis provides a suitable milieu for the elicitation of incubation behavior by exogenous progesterone. It is suggested that a gonadal hormone, presumably an androgen, facilitates the incubation-inducing action of progesterone in male ring-doves. (Supported by NIH predoctoral fellowship 1-F1-MH-25, 856-01 [PS].)

141

GORDON M. BURGHARDT, University of Chicago.

Chemical cues in feeding behavior of newborn garter snakes. (Motion picture)

The response of a neonate *Thamnophis sirtalis* toward its first food object, an earthworm, will be seen. The importance of the chemical senses to the initiation of this behavior (the prey-attack-response) is seen in the response elicited in naive snakes by cotton swabs dipped in a water extract of worm mucus. The extract is prepared by placing a worm in warm water for one minute and then removing it. Additional procedural details are outlined elsewhere (Psychon. Sci., 4:37, 1966). The difference between the above responses and defensive behavior which appears at the age of approximately two weeks will also be seen.

Results of parametric and comparative studies involving the chemical control of the prey-attack-response in snakes will also be briefly noted. (Supported by Grant M-776 from the USPHS awarded to Eckhard H. Hess.)

142

MAHLON W. WAGNER and J. TIMOTHY ROWNTREE, Valparaiso University.

Comparative sugar preference in various rodents.

It has been shown that many animals prefer sugar solutions (Carpenter, J. Comp. Physiol. Psychol., 49:139, 1956). Wagner and Rowntree (J. Psychol., 62, in press, 1966) and Wagner, Green and Manley (Science, 148:1473, 1965) have found differing results with various methods of presenting taste stimuli with a paired-comparison method giving more clear-cut preferences.

Relative sugar preference was examined in the following rodents: five species of deer mice, *Peromyscus maniculatus bairdii, Peromyscus leucopus, floridanus, polionotus* and *eremicus;* two species of pocket mice, *Perognathus baileyi* and *penicillatus;* Kangaroo rats, *Dipodomys spectabilis*; Northern grasshopper mice, *Onychomys leucogaster;* gerbils, *Meriones unguiculatus;* and Wistar albino rats and Long-Evans hooded rats. Because of different native habitats and past experiences it would be expected that these various rodents would exhibit different preferences for sugars.

All subjects were exposed to three glucose solutions (10%, 25% and 37.5%) presented randomly in pairs with five replications of each pair. Laboratory rats received 30-minute trials and all other species were exposed for two hours.

Laboratory rats generally showed no strain differences, preferring the sweeter of the pair. All *Peromyscus* species preferred the sweeter solution. There were also species differences in intake and preference with *floridanus* drinking most and *bairdii* drinking least. Grasshopper mice exhibited similar patterns of sugar intake. Kangaroo rats preferred the less sweet solutions. Pocket mice and gerbils generally drank too little to be measured or preferred the sweeter solution. (Supported by NSF institutional grant to Valparaiso University.)

143

H. R. BEHRMAN, O. MALLER, and MORLEY R. KARE, North Carolina State University.

Appetite for protein and magnesium in the wild and domesticated rat.

Protein deficient wild and domesticated rats selected a protein adequate diet in a two-choice situation where the alternative was a palatable sucrose low protein diet. During the choice period the wild rats maintained an uniform intake of protein whereas the dosestimated rats were more variable in their intake of the adequate protein diet and after 5 days consumed more of the low protein sucrose diet. Explanations for the difference in corrective behavior between the wild and domesticated rat will be offered. Protein deprivation resulted in an increase in food curiosity and open field activity in the domesticated rat. Both wild and domesticated rats failed to correct for magnesium deficiency in a two choice situation. Previous experience in correcting a protein deficiency was of no consequence in correcting a subsequent magnesium deficiency. (Supported by grant NB 03896 from the NIH.)

144

THOMAS R. SCOTT, JR. and BARTLEY G. HOEBEL, Princeton University.

Effect of sex hormones on lateral hypothalamic self-stimulation and feeding.

Feeding and rate of self-stimulation in the lateral hypothalamus were experimentally manipulated by estrogen administration. Female rats bearing lateral hypothalamic stimulating electrodes were tested daily for rate of self-stimulation and amount of food ingested. They were also tested with proven male copulators to determine the degree of sexual excitement, and vaginal smears were taken to identfy the phase of their estrous cycle. The experiment consisted of three parts: 1) some subjects were permitted to proceed through their natural estrous cycles, 2) others were artificially put into heat by injecting sex hormones subcutaneously, and 3) a third group was placed into chronic estrous by subcutaneous implantations of estrogen-laden tubes. In each case the results were similar: estrous and high degrees of sexual excitement were accompanied by high rates of self-stimulation. Conversely feeding was inhibited during natural and induced estrous and, in the case of chronic estrous, remained depressed for more than a week. (Supported by grants MH 08493 from USPHS and GE 6289 from the NSF.)

145

DAVID E. DAVIS, Pennsylvania State University.

An annual rhythm of food consumption by woodchucks (*Marmola monax*).

The woodchuck stores internally large quantities of fat which are used during hibernation. The present study was designed to determine what external factors control the annual cycle. The woodchucks were obtained in the wild and kept in rooms or coolers where the temperature and photoperiod could be controlled. They were fed lab chow.

In nature woodchucks emerge from hibernation with considerable fat, lose weight till April, and gain rapidly till September. A variety of experimental manipulations showed that woodchucks followed the natural rhythm of gain in constant (16 hour) or changing photoperiod and at either 6 or 20 C. The quantity of food eaten increased from about 35 g per day in December and January to about 170 g per day in summer and then declined. No experimental manipulation of temparature, photoperiod or availability of food altered the rhythm of food consumption.

146

M. ASHTON BARFIELD, Mount Holyoke College, FRANCES L. FITZ-GERALD, and PATRICIA A. GRUBBS, Yerkes Regional Primate Research Center of Emory University.

Food preferences in lowland gorillas.

Fourteen gorillas were tested in an experiment designed to investigate specific preferences for fifteen different foods commonly available in primate laboratories. After an adaptation phase, formal sessions began in which the foods were presented by pairs. The animal was allowed to select and eat one member of each pair. Fifteen pairs were presented per session to each gorilla until every food had been presented once with every other food. These sessions were replicated once. Grapes, bananas, apples, and oranges appeared to be selected consistently, while white potatoes, laboratory chow, peas, and raw beef were rarely preferred. In general, the food preferences of lowland gorillas are similar to those demonstrated by chimpanzees, gibbons, and rhesus monkey in other studies. (Supported in part through National Institutes of Health Grant FR-00165.)

147

MAHLON E. KRIEBEL, University of Washington.

The role of cell nexuses in the spread of excitation in the tunicate myocardium. (Introduced by A. Kohn)

The tunicate heart is composed of a single layer of cells, circumjacently attached around their apex border to neighboring cells by nexuses. There are no other specialized cell contacts. By measuring the attenuation and time course of rectangular pulses of current applied across the opened and flattened myocardium the specific transverse electrical resistance of hearts of *Ciona* was found to be 230 Ωcm^2 and the capacitance 1.3 μF per cm^2. The specific transverse resistance is so high that even if all the current that was passed had flowed between the cells, the width of the gap surrounding each cell could not exceed 0.1 Å. The nexuses can therefore be considered impermeable to ion flow in the direction perpendicular to the surface of the myocardium. Thus the transverse resistance of the heart must be equal to the series resistances of the membrane resistance of the apical and luminal cell surfaces. The specific membrane resistance of *Ciona* heart cells was calculated to be about 190 Ωcm^2 and the membrane capacitance about 1.6 μF per cm^2. When current is passed through a strip of myocardium in a sucrose gap it can be demonstrated that the myocardial cells are in electrical continuity with each other through the nexuses. A specific nexus resistance of about 0.2 Ωcm^2 was calculated. It is concluded that the spread of excitation in the tunicate heart is by local current flow through the low resistance pathways provided by cell nexuses. (Supported by PHS grants numbered 1 T1 GM 1194 and 5 T1 GM 1194.)

148

ROBERT K. JOSEPHSON, Western Reserve University.

Conduction and contraction in the column of *Hydra.*

Electrical potentials from the conducting system controlling column contraction in *Hydra littoralis* were recorded with a pair of suction electrodes, one attached to the basal disc and the other to the hypostome. Conduction velocity was determined by stimulating through the basal electrode while recording from base and hypostome or by recording from base and hypostome while the column was stimulated at different levels with another electrode. Measured conduction velocities ranged from 3.4 to 8.1 cm/sec (19-23°C). Generally the conduction velocity is not the same for basal and hypostomal conduction but the direction of faster conduction varies from animal to animal. In some experiments a transducer was attached to one of the electrodes to measure column contraction. The onset of contraction was first detected 0.1-0.15 second after the onset of an electrical potential. A single column contraction occurs with two distinct phases: an initial rapid increase in tension reaching a peak in about one second and a subsequent slow increase in tension reaching a broad maximum 5-10 seconds after the onset of contraction. Closely-spaced contractions sum and they smoothly fuse if the electrical potentials from the conducting system are separated by less than about 0.5 second. (Supported by Grant NB06054 from the USPHS.)

149

PHILIP RUCK, University of Wisconsin.

Receptor classes in the eye of the backswimmer, *Notonecta irrorata.*

With optomotor experiments Rokohl (Z. vergl. Physiol., 29:638, 1942) showed that the dorsal-posterior region of the eye of *N. glauca* has color vision. She found no evidence of color vision for the anterior-ventral region of the eye. Her work prompted this study of spectral sensitivities of the same eye regions in *N. irrorata.* Local electroretinograms were evoked by monochromatic stimuli (300-600 mμ) in the dark-adapted state and in the presence of monochromatic fatiguing lights. Spectral sensitivity was calculated as the reciprocal of the number of quanta required to evoke a constant cornea-negative on-wave whose amplitude fell in the range, 160-200 μV. In the dark-adapted state the dorsal-posterior region of the eye has a major peak of sensitivity in the green and a minor peak in the ultraviolet. With monochromatic fatiguing lights the spectral sensitivity function of the dark-adapted eye has been fractionated into three components: (1) a (UV) class with maximum sensitivity at 370 mμ and no peak at longer wavelengths; (2) a (blue) class with a major peak at about 480 mμ and a minor peak at 370 mμ; (3) a (green) class with a major peak at about 530 mμ and a minor peak at 370 mμ. Thus the dorsal-posterior region has the minimum retinal prerequisites for trichromatic color vision. The allegedly color-blind anterior-ventral region of the eye appears to have the same three classes although the blue class is harder to demonstrate. The hypothesis that evidence for three receptor classes includes an artifact attributable to selective filtering action of shielding pigments was tested experimentally and rejected. (Supported by NSF Grants GB-4256 and GB-3390.)

150

T. H. GOLDSMITH and H. R. FERNANDEZ, Yale University.

Sensitivity of compound eyes to ultraviolet light.

The eyes of many insects have prominent peaks of sensitivity at about 340 nm. One purpose of the present work was to extend measurements of spectral sensitivity further into the ultraviolet than had heretofore been attempted. In order to minimize light absorption by accessory pigments, a white eye mutant of the housefly *(Musca domestica)* was employed. The stimulating system consisted of a xenon lamp and a double monochromator. The relative numbers of photons required to elicit equal retinal action potentials in response to one-second flashes were recorded to 250 nm. Sensitivity is maximal at 340 nm and declines at shorter wavelengths.

Measured in glycerine, the cornea of *Sarcophaga* has a single sharp absorption band at 280 nm with a peak absorbance of about 0.7 and negligible absorption in the near ultraviolet. Correction of the spectral sensitivity measurements for the filtering action of the cornea leaves the position of maximum sensitivity unchanged, but the decline in sensitivity of the receptor pigment at wavelengths shorter than 310 nm is less steep than for the intact eye.

Spectral sensitivities of several crustaceans have been measured from 300-650 nm. Wavelengths of maximum sensitivity: blue crab *(Callinectes sapidus),* 507 nm; northern crayfish *(Orconectes sloani, O. punctimanus),* 565 nm; an isopod, the sow bug *(Porcellio scaber),* 515 nm. In each case, selective adaptation by red light left the shape of the spectral sensitivity function unaltered and provided no evidence for the presence of ultraviolet receptors. (Supported by Grant NB 03333 from the USPHS.)

151

LAWRENCE R. MORRIS, HOWARD L. GILLARY and MYRON L. WOLBARSHT, Naval Medical Research Institute.

The effect of light on electrically evoked spike activity in the optic nerve of a land snail.

Illumination of the eye of the land snail, *Otala lactea* Müller, evokes a cornea-negative ERG and concomitant spike activity in the optic nerve (Wolbarsht and Gillary, Physiologist 9: 322, 1966). Electrical stimulation of the isolated eye-nerve preparation elicited a typical compound action potential which was conducted along the nerve with a velocity of approximately 0.1 m/sec. Above threshold, the amplitude of the action potential increased with the intensity of the electric current and saturated to a maximum at high intensities. When the current was near threshold, the amplitude was markedly increased by illuminating the eye. This effect was greatest when the stimulating electrodes were nearest the eye. The time course, spectral sensitivity, dependence on light intensity, and adaptation of the light facilitation were all similar to those of the ERG. This effect seems to be

due to the summation of the effects of electrical stimulation and light-evoked generator processes in the eye. One would not expect to see this facilitation during maximal electrical stimulation and, in fact, at currents where the amplitude of the action potential was saturated, illumination decreased the amplitude. This decrease may be from the refractory period following the light-evoked impulses. (From the Bureau of Medicine and Surgery, Navy Department, Research Task MR 005.04-0013.)

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MARK ATWOOD and PHILIP RUCK, University of Wisconsin.

The effect of CO_2 and metabolic drugs on the ERG of the ocellus of the cockroach *Blaberus crantifer*.

Several components of the ocellar ERG have been identified and allocated to sites of origin in the retinula cells and postsynaptic units of the ocellar nerve (Ruck, J. Gen. Physiol., 1961). Effects of test solutions on the components are studied by building a wax cup around the ocellus, dissecting away sections of the cornea, adding the solution to the cup whence it diffuses into the ocellar tissue. Different concentrations of CO_2 were applied using a flow meter and a chamber built around the animal. When 100% CO_2 is applied to the animal the ERG is abolished within several minutes. At lower concentrations of CO_2 certain components are abolished selectively. The postsynaptic events are the most sensitive and the generator potential the most resistant. Varying concentrations of the drugs sodium azide and potassium cyanide in a standard saline have been tested. Sodium azide at a concentration of 5×10^{-4} g/ml abolishes the ERG in most cases. Some components were gone within a few minutes but the generator potential was more resistant. Potassium cyanide (3 mM) abolishes the entire response within a few minutes. In all these experiments the retinula cell axon response and the postsynaptic responses were lost before the generator potential. It can be seen from these experiments that the ocellus is quite sensitive to high CO_2 or to metabolic drugs and that different components have different sensitivities or at least different time courses of reaction to these experimental procedures. (Supported by predoctoral fellowship 5-F1-GM-24, 402-02 from NIH and NSF Grant GB-4256.)

153

MARTIN MENDELSON, New York University Medical School and the Marine Biological Laboratory.

Mechanical and electrical properties of a sound-producing muscle in the lobster *Homarus americanus*.

The lateral adductor muscle of the basal segment of the second antenna of the lobster is functionally divided into two distinct parts. The larger part produces extremely rapid twitches. The smaller slip functions in the typically slow, graded manner of most crustacean muscle. The fast part of the muscle shows twitches of ca. 10 msec duration at 20°C. The twitches do not fuse into a tetanus but follow stimuli above 100 shocks/sec. The slow slip produces smoothly fused tetani at low stimulus frequencies and reaches peak tensions considerably higher than those achieved by the fast portion. Intracellular recording combined with splitting of the motor bundle revealed that the fast portion is innervated by two motor axons. One produces small, slow, summating PSPs though mechanical fusion is absent. The other motor fiber evokes spike-like potentials (20°C) that often overshoot and have durations of ca. 3 msec. Both the amplitude and duration of these potentials are markedly temperature dependent. These fast potentials often behave in a graded manner although clearly arising from PSPs. Intracellular stimulation sometimes produces what appear to be regenerative responses. The fast potentials are reduced in amplitude by reduction of extracellular concentrations of both Ca^{++} and Na^{+} although the effect of low Ca^{++} at the neuromuscular junction complicates the relationships. Clearly this muscle is highly specialized to produce rapid oscillations of the antenna and is responsible for the faint buzz that lobsters make when disturbed.

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BERTRAM PERETZ, University of Iowa.

The response in a peripheral nerve to electrical stimulation of varying loci on the optic lobes in the frog. (Introduced by H. W. Beams)

It is generally agreed that the optic lobes are the structures integrating sensory inflow with the motor columns in the brain stem and spinal cord in the amphibian brain. Electrophysiological studies have shown that each retinal area has representation on the surface of the tectum via the nerve fibers from ganglion cells. This study was attempted to determine organization in the tectum with respect to peripheral nerve activity.

Experiments were carried out in which frog optic lobes were stimulated electrically and the response to the stimulus was recorded in a contralateral peripheral nerve, (sciatic). The stimulating electrodes were placed in well over 100 sites on the tectum and corresponding sciatic activity was always elicited. Further, the latency of the response recorded in the peripheral nerve showed a marked decrease as the stimulating electrodes were moved in an anterior-posterior direction. Decreases of latency between the two end positions, greater than 30%, are not accounted for by the decreasing distance between stimulating and recording electrodes, less than 2%. When the homolateral tectum was stimulated (recording from the same peripheral nerve as above) the same results were observed.

Both tecta have equal capability for eliciting sciatic activity. In addition, since there is sciatic activity in response to all tectal sites stimulated, this may mean that tectal regions do not possess the degree of specific motor functions suggested by Abbie and Adey (J. Comp. Neurol. 92:241, 1950). Though all tectal loci appear able to give rise to sciatic activity, the more posterior tectal sites seem to have facilitated pathways which are manifested by shorter latencies. (Supported by a special fellowship, 1-F3*GM-32,521-01 from the USPHS and University of Iowa Graduate College.)

155

MICHAEL MENAKER, The University of Texas.

Entrainment of circadian rhythms by light cycles in the blinded house sparrow.

A technique has been developed for the bilateral enucleation and subsequent long term maintenance of *Passer domesticus*. Enucleated sparrows, living free in activity cages, easily learn the location of food and water and of perches which activate microswitches connected to an event recorder. Continuous, long term records of perch-hopping activity can thus be obtained from blinded birds. When maintained either in constant darkness or in constant dim light, enucleated sparrows have activity rhythms with circadian periods which are indistinguishable from those of normal birds held under the same conditions. Artificial light cycles (LD 12:12, approximately 400 lux) will, when imposed on blinded birds, entrain their activity rhythms to a period of 24 hours. As in normal birds, the phase of the daily onset of activity of blinded birds is within an hour of the dark to light transition regardless of the position of the transition in real time. Under light cycles of longer or shorter photoperiod both the daily duration of perchhopping activity and the phase of the activity onset relative to the dark to light transition are affected. The small (1-2°C) temperature rise associated with the light fraction of the LD cycle will not entrain the activity rhythms when applied alone to blinded birds held in constant darkness. The data give strong support to the hypothesis that the brain of the sparrow contains an extra-retinal photoreceptor the output of which is coupled to the entrainment mechanism of the "clock" controlling the circadian activity rhythm. (Supported by Grant GB 3806 from the NSF.)

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ROBERT W. COMPTON, University of Wisconsin.

The skeletal musculature innervated by the facial nerve in the coati *(Nasua)*. (Introduced by W. I. Welker) (Motion picture)

The peripheral facial neuromuscular system of the coati *(Nasua)* has been examined in detail in order to determine: a) the origin and insertion, size, and shape of each skeletal muscle innervated by the facial nerve; and b) the course of the facial nerve and its branches, from the stylomastoid foramen to these muscles. The specialized rostral surface of the rhinarium of the coati is densely innervated by sensory receptors. Rhinarial contact with stimuli is made more intimate and varied by the action of the muscles of the proboscis. Anatomic techniques included gross dissection of embalmed heads and microscopic examination of stained serial sections from the proboscis. Physiologic verification of the innervation of a muscle group by a particular nerve branch was accomplished by monopolar electrical nerve stimulation and visualization of the contracting muscle. Concurrently, the action of each muscle on the various structures of the head, e.g., rhinarium, nasal cartilages, vibrissae, etc., was determined. Photographs of the muscles were taken at appropriate stages of the dissections, and detailed line drawings were made of each muscle from several standard views. Results of nerve stimulation were recorded on cinematographic film. This project was preliminary to a study of the musculotopic organization of the facial motor nucleus in the medulla oblongata. (Supported by grants NB 5326, NB06225 from USPHS.)

157

DAVID B. ROYE, University of Florida.

Primary visual pathways in the frog. (Introduced by K. E. Chernetski)

The projections of primary visual fibers in *Rana pipiens* have been determined using a modified Nauta-Gygax technique designed for staining degenerating fibers in serial paraffin sections, as outlined by Guillery, *et al.* (Stain Tech., 36:9, 1962). After cutting a window in the floor of the cranium the left optic nerve was transected at a distance of approximately 1.5 mm from the chiasma. Specimens operated on in this manner were then allowed to survive for periods of 2, 4, 6, 8, 10, 12 and 14 days. Horizontal transverse and sagittal serial sections were then prepared from brains representing each degeneration time. These sections were then compared with sections made from the brains of sham-operated control animals.

Degenerating fibers of passage were best seen in four day brains. Four contralateral and two ipsilateral visual pathways were noted, confirming the results of Knapp, *et al.* (Acta Neurol. Scan., 41: 325, 1965). The contralateral tracts observed were the retino-tegmental, retino-thalamic, retino-pretectal and retino-tectal tracts. Ipsilateral pathways were the retino-thalamic and retino-preoptic tracts. In addition, a third root not previously described was traced caudally through the intact optic tract to the dorsolateral hypothalamus. This pathway was termed the posterior retino-hypothalamic tract.

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GEORGE C. DEWEY, LEO L. LEVERIDGE and HANS ELIAS, Chicago Medical School.

Blood flow in the renal glomerulus of the frog *Rana pipiens*. (Motion picture)

The blood channels within the glomerulus are surrounded by a common basement membrane, all together. They are not individually wrapped by their own basement membranes. The foot processes of the podocytes are evenly distributed over the entire basement membrane. They cover places under which blood channels are located and places where the endenchyma (specialized endothelium) is solid. Assuming that the foot processes subserve a function in filtration, their presence over solid endenchyma (i.e., far away from blood channels) can be explained by the assumption that the blood channels have no permanent location, but that they shift their positions.

Transillumination studies (Elias, Hossmann, Barth and Solmor, J. Urol. 83, 1960) had shown that, indeed, glomerular blood channels do shift transversely during water diuresis.

This visual observation is now confirmed by microscopic motion pictures. Transverse shifting of blood channels is possible in glomeruli in which a mesangium is not highly developed (such as in man and in the frog). Glomeruli with a highly developed mesangium (e.g., muskrat, desert terrapin) remain to be studied. (Supported by a Grant from Smith, Kline and French Laboratories.)

159

E. W. PFEIFFER, University of Montana.

Comparative anatomical observations of the mammalian renal pelvis and medulla.

Certain anatomical features of the renal pelvis and medulla in several species of mammals are described. Two types of pelves are found among these species. Type I pelvis is an uncomplicated, slightly expanded ureteral ending, while Type II pelvis is an extensive chamber whose wall is thrown into elaborate folds that reach deep into the medulla. In species with Type I pelvis, the medulla has no inner zone, and the outer zone lacks special projections into the pelvis called secondary pyramids.

Species with Type I pelvis differ in certain renal functions from those with Type II pelvis, and the possible relationship of the pelvic anatomy to these differences is discussed. (Supported by grant AM-09975-01 to B. Schmidt-Nielsen from the USPHS.)

160

ROBERT F. ACKERMANN and DOUGLAS B. WEBSTER, New York University.

The central auditory system of the kangaroo rat.

Frozen and paraffin brain sections of eleven adult *Dipodomys merriami* were studied with cell and fiber stains.

There are no apparent subdivisions of the dorsal cochlear nucleus; however, the ventral cochlear nucleus is readily divisible into five portions. The trapezoid body contains thick fibers ventrally with thin and medium fibers dorsally.

The hypertrophied medial superior olivary nucleus extends ventrally into the medial preolivary nucleus. Dorsally, it impinges upon the medial concavity of the lateral superior olivary nucleus, reducing the usual "s" shape of that nucleus to a "u" which has a small "hook" medially. The medial superior olivary nucleus is characterized by triangular cells having three processes, two long dendrites directed laterally in opposite directions and an axon projecting anteriorly. The lateral lemniscus, also hypertrophied, contains a prominent dorsal nucleus. Furthermore, a group of large, multipolar cells lies between the dorsal nucleus of the lateral lemniscus and the inferior colliculus.

The inferior colliculus is composed primarily of small spherical cells containing little cytoplasm. Occasional larger cells are more numerous laterally. Large, multipolar cells are found among fibers of the peripheral capsule of the inferior colliculus. A prominent commissure connects the inferior colliculi.

The pars principalis of the medial geniculate body is characterized by the uniform distribution of its small, spherical cells. Larger cells of the pars magnocellularis lie medial and slightly ventral to the pars principalis. (Supported by Grant NB 05800 from NINDB.)

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PO-CHUEN CHAN, L. IONE JOHNSON, FRANCIS C. MONETTE, JOSEPH LOBUE and ALBERT S. GORDON, New York University.

Deoxyribonucleic acid synthesizing cells in the peripheral blood of normal and splenectomized rats.

Male rats (Long-Evans strain; 250 g) were either splenectomized or sham-operated under ether anaesthesia. Three animals from each group were sacrificed on day 0, 1, 2, 3, 4, 7, and 10 post-operation. Total and differential leukocyte counts were performed on tail blood prior to sacrifice. The blood of each animal was collected by aortic puncture with a heparinized-siliconized syringe and transferred to siliconized test tubes to which tritiated thymidine (2 μc per ml of blood) was added and then incubated for 1 hour at 37°C with constant agitation. After incubation, the blood was centrifuged at 400 rpm for 5 minutes and following this the supernatant recentrifuged at 1,200 rpm for 5 minutes. The leukocyte button thus obtained was resuspended in a small volume of rat serum and smears were made and processed for autoradiography. Labeling indices were estimated by scoring 5,000 mononucleated cells from each animal and the number of labeled mononuclear cells per ml of blood was calculated. It was found that the labeling indices as well as the absolute number of labeled cells per ml of blood were significantly higher in the splenectomized rats than in the sham-operated rats from the second post-operative day on. In addition, mitotic figures were observed in some of the leukocytes cultured from splenectomized rats. (Supported by research Grant HE03357-10 and training Grant HE5645-02 from the National Heart Institute of the U.S. Public Health Service.)

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WILLIAM R. GOODGE, University of Missouri.

Histology and histochemistry of woodpecker salivary glands.

Salivary glands from four species of woodpeckers were fixed in phosphate-buffered formalin or Zenker-formalin, sectioned and stained by a variety of methods to study general morphology and demonstrate secretion products.

The large mandibular gland (glandula picorum) of the Yellow-shafted Flicker *(Colaptes auratus)* and Downy Woodpecker *(Dendrocopos pubescens)*, like that of previously described species is divisible into whitish posterior and darker anterior portions. Mucopolysaccharides were demonstrated in the secretory cells of the posterior, and peripheral cells of the anterior portion. In addition, the secretory cells of the posterior portion contain secretion granules which were stained by acid fuchsin, iron hematoxylin, neutral gentian and phosphotungstic acid hematoxylin. Large droplets with similar staining properties occur within the lumen of many gland ducts. Although Antony (Zool. Jahrb. Abt. Anat. u. Ontog. Tiere, 41:547-660, 1920) noted similar granules, they were then believed to be precursers to the mucous secretion. These granules have the staining properties of typical zymogen granules and, although there is yet no supporting experimental evidence, they may contain digestive enzymes or possibly substances toxic to insect prey. The mandibular glands of the Red-bellied Woodpecker (*Centurus carolinus*) and Red-headed Woodpecker (*Melanerpes erythrocephalus*) are not divisible into two portions, and the entire gland is similar to the anterior portion of that of the other two species. Secretory granules occur, but are much less numerous.

The well developed maxillary gland, opening

near the anterior end of the palate, has an expanded posterior portion which extends into the orbit lateral to the sphenoid rostrum.

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LEWIS B. GOODLEY and ROBERT L. BOORD, University of Delaware.

Quantitative analysis of the hair cells of the auditory papilla of the pigeon.

The auditory papilla was isolated by dissection from 5 membranous labyrinths and mounted *in toto* for direct analysis and measurement. Hair cells were systematically counted by the ocular grid method at a magnification of 43×. By this technique errors inherent in making cell counts from tissue sections are avoided; therefore, correction factors are unnecessary.

The auditory papilla is 0.40 mm wide at its broadest point and has an average length of 3.74 mm. Average surface area is 0.889 sq mm with a range of 0.789 to 1.021 sq mm. The number of hair cells of the auditory papilla ranges from 8,732 to 11,330 with an average of 10,400.

This study of the normal population of auditory hair cells is intended to serve as a basis for investigating the relations among hair cell population, innervation pattern and auditory function in the pigeon. (Supported by Grant NB 05056-02 from the USPHS.)

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V. S. HODGSON and E. S. HODGSON, Columbia University and Lerner Marine Laboratory.

Habituation and discrimination in sea anemones. (Motion picture)

The classic observations on elementary "learning" in sea anemones have been extended by two types of observations on a species especially favorable for study, *Condylactis gigantea*. Populations of this species, living in diverse habitats, exhibit selective types of habituation to stimuli, depending upon those normally present in the environment. Acquisition, loss, and "spread" of these modified behavioral patterns within the nerve net can be demonstrated in the laboratory. Whole animals or isolated tentacles respond differently to a variety of pure chemical stimuli, especially amino acids. Pairing particular foods with electric shocks can result in modifications of behavior which resemble conditioning. Such effects apparently are not the results of sensitization, fatigue, or other general, non-specific mechanisms. The results are discussed in terms of theories of simple learning.

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GEORGE A. PINCKNEY, State University of New York College at Brockport.

An analysis of free operant avoidance behavior in *Carassius auratus*.

The free operant avoidance behavior of *Carassius auratus* was observed in an aquatic shuttlebox. Seventy-two Ss were given a single six hour session with a constant response to shock (R-S) interval of 20 seconds, with one-sixth of the Ss studied with a shock to shock (S-S) interval of 2.5, 5, 7.5, 10 and 20 seconds, respectively. To switch the program from the S-S cycle to the R-S cycle, S was required to swim, through a small tunnel, from one compartment of the shuttlebox to the other. Ss' responses were monitored by a pair of photocells, and the data collected included the number of responses emitted, as well as the number of shocks received, from each 20 minute block of the six hour session.

Analysis of the data revealed that the number of avoidance responses made by S was a U-shaped function of the length of the S-S interval, with the highest performance occuring in the 5 to 7.5 second range. With S-S intervals longer than 7.5 seconds, performance dropped sharply, while Ss trained with a 2.5 second S-S interval developed escape behavior, and tended not to improve beyond that stage. This relationship between S-S intervals was borne out by both the total number of avoidance responses made by S over the six hour session, and the terminal performance of S, as reflected by the number of avoidances made during the final hour of the session. (Supported by Grant 22-11-A from the Research Foundation of State University of New York.)

170

R. E. DAVIS, University of Michigan.

Environmental trigger in the formation of memory in goldfish.

Previous investigations in our laboratory have indicated that long-term memory of shock-avoidance responding in goldfish can be blocked by an intracranial injection of puromycin. When fish are trained immediately following an injection of puromycin they exhibit normal acquisition and memory of avoidance responding during the session but within several hours they show a memory loss. Memory is also obliterated when puromycin is injected immediately following the training session. When the injection is delayed several hours or more after training, however, the fish exhibit normal long-term memory. These results indicate that during the training session a short-term memory is formed which is resistant to puromycin, and that following training memory becomes fixed, or consolidated into a long-term form. The resistance of short-term memory to puromycin suggests that puromycin interferes specifically with the perseverative fixation of long-term memory. The result that puromycin injected immediately following training obliterates long-term memory indicates that the stage of memory fixation which is blocked by the drug begins after the last trial. We have reported evidence that the onset of memory fixation is suppressed by stimuli in the training environment (Davis and Agranoff, Proc. Natl. Acad. Sci., US, 55:555, 1956). Further experiments are to be discussed in which fish were kept in the training environment for hours to days following training. The results confirm that memory fixation is triggered by the changes in the external environment which the fish experience when they are removed from the training tank. Also, extended post-training periods in the training environment appear to extinguish the avoidance response. It is possible that the onset of memory fixation is coupled to levels of arousal or of neural activity which could be manipulated by conditions in the external environment. (Supported by grants GB5125X from the NSF and NIMH 12506-01 from the NIH.)

171

LINDA E. ANDERSON, State University of New York College at Brockport.

Some species differences in the avoidance learning of fish. (Sponsored by George A. Pinckney)

Five species of fish were compared in a shuttlebox avoidance learning situation. The species studied were *Carassius auratus, Xiphophorus helleri, Trichogaster trichopterus, Lebistes reticulatus,* and *Mollienesia sphenops.*

Each fish was given twenty conditioning trials per day for five days. The avoidance response consisted of swimming through a plexiglass doorway which divided the shuttlebox into two compartments. The conditioned stimulus (CS) was the onset of a light in the end of the compartment occupied by the fish and the unconditioned stimulus (US) was an electric shock passed through the water. A 10-second CS-US interval, a 5-second shock, and a 2-minute intertrial interval were used throughout the study. The number of avoidances made on each day as well as latencies to the nearest hundredth of a second were recorded.

An analysis of the response data revealed that Carassius was by far the best performer averaging nearly 18 responses out of 20 trials on the last three days of conditioning. The performance level of Trichogaster and Mollienesia, which averaged 8-10 responses on the last day, was lower than Carassius but higher than Lebistes and Xiphophorus, which averaged only 4 responses on the same day.

The results seem consistent with previous studies in which one of two species were studied, primarily in respect to the rapid learning of Carassius. It was suggested that differences in performance may be attributable to differences in emotionality and natural responses to fear provoking stimuli. (Supported by grant 22-11-A from the Research Foundation of State University of New York to G. A. Pinckney.)

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VELMA J. VANCE and ANN M. RICHARDSON, California State College at Los Angeles.

Reversal learning in Desert Iguanas, *Dipsosaurus dorsalis.*

Current interest in ages of experimental animals prompted a study of reversal learning in large ($M = 44.6$ g) and small ($M = 9.9$ g) Desert Iguanas. Differences in learning are apparent between the two groups which may be of significance both in the design of experiments and in our understanding of the processes of learning. Brightness and position clues were provided for the original discrimination and 5 successive reversals. Experiments were conducted at body temperatures of 34 ± 2°C. Positive reinforcement consisted of 3 minutes on the heated goal (42 ± 2°C) and a meal worm. Trials required to reach the criterion of 4/5 correct responses for 3 consecutive days averaged 35 for small and 57 for large lizards on the original discrimination. Usually more trials were required on the first reversal than the original discrimination but the reversal index (Rajalakshmi and Jeeves, Animal Behavior 13:203, 1965) varied from 0.41 to 10.0.

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K. E. CHERNETSKI, University of Florida.

Modification of responsiveness by passive limb movement in curarized rats. (Motion picture)

White rats were curarized (0.3 mg/kg tubocurarine chloride, Lilly, IM) and maintained under artificial respiration via a tracheal cannula, with 0.5% procain HCl liberally infused into the incision. A 70 msec, 20-30 V shock was applied to the left front paw, followed by a forced movement, a 200 msec, 1 cm adduction, of the right hind foot via an attached lever. 60 such pairings applied every 105 sec lead to the rat's initiating this movement in response to a shock upon emerging from curarization. Consistently positive results have been obtained with a group of 5 rats when the forced adduction is begun during the shock. An interval of 10 sec between the shock and movement gives uniformly negative results after 200 such pairings. A 1.5 sec sounding of a buzzer immediately preceding the shock does not become an effective stimulus in initiating the movement, even after 200 sound-shock-movement stimulus combinations applied to curarized rats; although they do become conditioned to the buzz, as indicated by quivering and galvanic skin responses recorded from the feet. The sound alone occasionally elicits the movement if sound-shock stimuli are presented in rapid succession (e.g., every 2 sec) 10-15 times, followed immediately by the sound alone, as these rats emerge from curarization. These results suggest that proprioceptive input plays a role in establishing the form of response a rat will typically make following a noxious stimulus, such as an electric shock.

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MICHAEL F. HALASZ, The University of Manitoba.

Stability of conditioned delay behavior computed from transient response to perturbation of reward contingency.

Homeostatic mechanisms that buttress conditioned behavior against disturbance remain inaccessible under maintained stimulus conditions. Formal stability properties underlying conditioning may, however, be inferred from transient response to shocklike perturbation of contingency according to systems theory concepts (Macmillan, R. H., *Theory of Control.* Cambridge: University Press, 1951). We employed transient analysis to characterize stability of a labile conditioned state, inhibition of delay (Pavlov), in rats lever responding to auditory CS for water reinforcement. Delay behavior was obtained as prolongation of response latency by temporal displacement of reward availability relative to CS. After latency stabilization on an initial value of this parameter, behavior was perturbed by stepwise incrementation (input) of time between CS onset and reward availability onset. Resultant adjustment of response latency (output) was formulated as a "dimensionless" output/input ratio, $P = (\text{New delay of reward} - \text{Response latency})/(\text{New delay of reward} - \text{Former delay of reward})$. Positive P's indicate incomplete adjustment to new contingency, negatives show overcompensation. P plotted against a "pure number" time scale (time/scaling factor) constitutes

transient response. Observed transients in our rats approximated damped (or overdamped) oscillations predicted by second-degree governing equations. Stability (damping) coefficients were computed from maximum "overshoot" by P. Steady state errors were estimated. Quantitated formal terms (damping, steady error, etc.) were identified with behavioral components, e.g., response chaining, perseveration, that serve as homeostats in this delay paradigm. (Supported by National Research Council of Canada Grant APA-189 and University of Manitoba Graduate School grant.)

175

ROBERT L. GOSSETTE, Hofstra University.

Comparison of successive discrimination reversal performances across 14 different avian and mammalian species.

While a variety of experimental procedures has been employed in the comparison of learning across wide ranges of vertebrates, only successive discrimination reversal (SDR) had been moderately successful in revealing consistent and meaningful inter-species differences. To explore more fully the sensitivity of this method a preliminary study of SDR performances of 14 different avian and mammalian species was undertaken.

Nine species of birds, representing 7 families across 2 orders, and 5 species of mammals, representing 3 families across 2 orders, were selected for study. S's were trained in a formboard box to dislodge stimulus blocks to reveal foodwells for a food reward. A spatial habit was established and then successively reversed for a minimum of 20 reversal problems. Training was continued with each problem until a minimum of 2 or fewer errors was achieved on each problem. Daily sessions of 20 non-correction trials were administered. The principal response measure obtained for analysis was the number of errors per problem.

Measures of errors significantly separated remotely and closely related species in a way consistent with their taxonomic position, with Passeriforme species displaying the fewest errors, Galliforme species producing the greatest number of errors, and intermediate perfomance shown by a Psittacine and 2 Columbiforme species. While 2 species of New World monkeys displayed performance differences consistent with their taxonomic ranking, 3 species of Procyonidae and 1 species of the Mustelidae showed performance that overlapped that of the primates. However, it appears that SDR shows considerable sensitivity to inter-species performance differences. (Supported by NIMH grants MH-08389-01 and MH-11138-01.)

176

LEONARD FRIEDMAN, System Development Corporation.

A theory of instinctive behavioral mechanisms and its use in the interpretation of neurophysiological experiments. (Motion picture)

A new theory of the functional organization of instinctive behavioral mechanisms in animals is proposed. The theory assumes a hierarchical drive structure consisting of Releasing Mechanisms and Selection of Releaser Mechanisms; this drive structure operates on, or calls forth, a repertoire of Behavior Units outside the hierarchy. A complex internal structure for each of these mechanisms has been defined.

Important differences exist between this scheme and the hierarchical scheme postulated by Tinbergen. Neurophysiological evidence has been found for the existence and localization of the postulated mechanisms in the CNS of mammals and birds. Evidence also exists for the specific kind of selective perception that the theory implies, i.e., an unstimulated superordinate RM blocks afferents to its subordinate RM's.

A computer simulation of an artificial animal in an environment is being synthesized to test these ideas. Behavior of the artificial animal is shown on film.

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WILLIAM G. HAND and D. DAVENPORT, *University of California, Santa Barbara.*

Dark adaptation in the phototactic behavior of the dinoflagellate *Gyrodinium* sp. (Motion picture)

Cultures of the dinoflagellate are raised under a 16-8 light-dark cycle, with the light phase being provided by a battery of 20-watt fluorescent tubes giving 340 ft candles of illumination at the culture. The cells are raised on a sea water enriched medium at 20°C. At the same time every day, samples are taken from a 5-day-old culture and placed on a lucite plastic well slide. This slide is made of black lucite and has a 3-mm square well. The bottom of the slide is clear lucite, as is one wall of the well. Through this clear wall the light stimulus is introduced. Through the clear slide bottom, the cells are scanned by a flying spot scanning device (Hand *et al.*, Biol. Bull., 128:90-101, 1965).

The samples taken from the cultures are immediately tested for the 'stop' response or shock reaction using a maximum intensity (50 ft candles), 470 millimicron, stimulus of a 2-second duration. The response is recorded by a 35-mm oscilloscope camera. The sample is then placed on a light adapting apparatus consisting of a high intensity light source (tungsten) filtered through a copper sulfate solution, this apparatus giving 1000 ft candles illumination on the sample. The cells remain here for five minutes, and then they are removed and 10 seconds later given the first stimulus at the prescribed intensity. This intensity is controlled by a neutral density filter. Stimuli are then given every 15 seconds until a response plateau is achieved.

This process is repeated, using a new sample each time, at continuously lower stimulus levels until no response can be observed over 10 minutes of stimulation.

The results indicate that a dark adaptation does appear to occur in the shock reaction phase of the phototactic response. Although the intensity range and time course of the dark adaptation is much smaller than that observed in other organisms, the characteristics of the response curve are identical. (Supported by grant 4222-03 from the Office of Naval Research.)

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RALPH G. JANES,* G. E. FOLK, JR., G. O. FRAZIER, L. ALLEN, and M. C. BREWER,

University of Iowa and Arctic Research Laboratory.

Funduscopic studies of eye pigment and retinal blood vessels of Arctic mammals in continuous light and continuous darkness.

Color photographs of the back of the eye were taken of fifteen species of Arctic mammals with a funduscopic camera. The animals were maintained outdoors and the eyes of the same specimens were compared in summer and winter at Point Barrow or at Fairbanks, Alaska. The shape of the optic disc (blind spot), the numbers and sizes of major vessels leaving the disc, and the color and density of the pigment in the back of the eye, were all characteristics of the individual species; there was no systematic relationship with size of eye, weight, or taxonomic family. For example, if the funduscopic camera functions with different-sized eyes of lower mammals in the same way as in human babies and adults, then the disc diameters were approximately: black bears, 2.55 mm; grizzly bear, 2.3 mm; wolves, 2.4 mm; husky dogs, 2.6 mm; dog-wolf hybrid, 2.36 mm; coyotes, 1.9 mm; lynx, 1.2 mm; wolverines, 2.8 mm; porcupines, 1.9 mm; red fox, 2.55 mm; and Arctic fox, 2.4 mm. The possibility that eye pigments may change when the animal is in continuous light or continuous darkness is being investigated. (Sponsored by the National Science Foundation and the Iowa College of Medicine Fund.) * Deceased.

179

RAYMOND G. STROSS, University of Maryland.

Photoperiodism and seasonal polymorphisms in *Daphnia*.

The readily recognized polymorphisms in *Daphnia* are the reproductive and the cyclomorphic. Both are associated with season; the former occurs in both short- and long-day, and the latter, in long-day photoperiods only. Both may be two-stimulus controlled, viz., photoperiods of permissive duration and a second stimulus which is associated with conditions of culture.

The control of reproductive polymorphism is known for a population of *D. pulex* which exhibits the phenomenon in autumn only. Short-day photoperiods in conjunction with a second stimulus are necessary (Stross and Hill, Science, 150:1462, 1965). The second stimulus determines the degree of shift from parthenogenetic to sexual reproduction, and it is associated with the density of the culture. The stimulus may be removed, at least in part, when the culture medium is aerated.

That cyclomorphosis may be conversely controlled by two stimuli is demonstrated in the work of other investigators. Turbulent culture (Brooks, P.N.A.S., 33:141, 1947) and exposure to constant light (Jacobs, Int. Rev. ges. Hydrobiol., 47:146, 1962) are essential for evocation of cyclomorphosis in *D. galeata*. Turbulence is here suspected to aid the loss of a volatile repressor which is produced in the culture. In that event the stimuli which promote the reproductive and repress the cyclomorphic polymorphism share a feature in common in that both may be volatile, at least in part. (Supported by Grant GB-339 from the NSF.)

180

JAMES A. BROWN, JR., WILLIAM L. WEST, and WILLIAM M. BANKS, Howard University.

Studies on the mode of action of the toxin of *Pasteurella pseudotuberculosis*.

Experiments were designed to study acute and chronic effects of the toxin on the cardiovascular system and to determine whether the observed effects were modified by pharmacological agents of known mechanism.

Toxin was produced by the method of Schar and Thal (Proc. Soc. Exp. Biol. and Med., 88:39, 1955) and each batch standardized. Blood pressure responses were recorded from the carotid artery of white albino rabbits (ca. 2 kg) which had been anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Blood pressure and EKG recordings were made on a Sanborn multichamber recorder. Toxin injections were made via cannulated jugular and washed in with saline. Direct effects on myocardium were studied using isolated heart and isolated atria preparations, perfused with oxygenated Tyrode's solution at 37°C. Changes in strength of isometric contractions and rate were recorded by use of a force displacement transducer attached to a Grass polygraph.

Following the administration of various doses of toxin (50-6000 μg/kg) a transient drop in blood pressure was followed by a return to normal. There was no dose size-response relationship to toxin administered. Repeated administration (15 minute intervals) of the same dose did not result in tachyphylaxis. Previously noted drop in blood pressure was not blocked by administration of atropine sulfate (1 mg/kg). Likewise, pyribenzamine (25 mg/kg) did not modify blood pressure response.

No major alterations were observed in EKG patterns acutely. Similarly, in *in vitro* preparations, no direct effects on the myocardium were noted. Chronically, the EKG appeared normal until just prior to death, at which time the animals passed into irreversible shock. In separate experiments in mice, the course of toxicity was not modified by either atropine or pyribenzamine.

181

F. BLAKE GRANT and GRACE E. PICKFORD, Yale University.

Ionic composition, urea, and serum osmolality in the blood of the coelacanth, *Latimeria chalumnae*.

Through the courtesy of Dr. Keith S. Thomson and the Peabody Museum of Natural History, we recently obtained samples of blood from the hepatic portal and renal veins, and the heart of *Latimeria*. The frozen specimen was just thawed and seemingly in good condition, but the blood was hemolyzed. Despite this circumstance, we believe that our data show, beyond reasonable doubt, that *Latimeria* employs urea to maintain serum osmolality at sea water levels. Total serum osmolality varied in the three samples, but was of the order of 1181 mOsm/liter. Inorganic ions were in the normal range for marine teleosts and elasmobranchs, or were affected by hemolysis (potassium). Mean values were as follows: Na, 178; K, 51; Ca, 3.5; Cl, 199; HCO_3, 4.6 mM/liter, respectively. The

sum, about 440 mOsm/liter, could only account for a part of the total serum osmolality. Serum urea contributed a major addition: 345 (246-384) mM/liter by the diacetyl monoxime method, confirmed by urease hydrolysis with direct nesslerization. Electrophoresis ($\mu = 0.075$, pH = 8.6) revealed some breakdown of serum proteins, and small molecular weight products presumably contributed to the total apparent serum osmolality. (Supported by Grant GB-132 from the National Science Foundation.)

182

ROBERT E. HENSHAW and ROBERT BIGGERS, Carnegie Institute of Technology.

Water balance in the wolverine.

In a captive colony of wolverines (*Gulo luscus*) maintained in large outdoor cages at Pt. Barrow, Alaska, water intake and output were quantified. Preliminary analysis indicates the following values for an animal of mean body weight of 13 kg. Intake: drinking, 50 g/kg; food, 20 g/kg. Output: fecal, 10 g/kg; urinary, 40 g/kg; respiratory, 20 to 100 g/kg. Cutaneous evaporative water loss was not measured directly. The biological half-life of injected T_2O was short indicating large water turnover. Urine concentrating capacity was not great: mean high concentration, 2360 mosm; concentration maximum, 2980 mosm (more than 3× plasma concentration). This carnivore might survive without sources of water other than in its food, however behavioral patterns cause excessive urination and defecation which would need to be supported by drinking. (Supported by Grant No. ONR 380 from the Arctic Institute of North America under contractual arrangements with ONR.)

183

KORIN GUSTAFSON and JOSEPH C. CURTIS, Clark University.

The effects of water deprivation on the Mongolian gerbil, *Meriones unguiculatus*.

Studies were undertaken to determine the tolerance of the Mongolian gerbil, a rodent which inhabits semi-arid regions in eastern Asia, to dried diets at 40% and 70% relative humidities. Total body water of young mature gerbils on dried Purina chow at 70% R.H. for 11-20 days was 73.3 ± 0.6% compared to 75.6 ± 0.3% for gerbils on the same diet receiving water *ad libitum*. Total body lipid of the animals on dried chow was 6.3 ± 0.5% whereas that of the controls was 17.4 ± 2.4%. Gerbils on the dried diet consumed 2.1 g/day compared to 4.9 g/day for the controls.

Gerbils fed dried oats initially lost weight at 40% and 70% R.H., but subsequently began to gain weight after 14 and 7 days, respectively.

In an attempt to determine the effects of initial weight on survival time and tolerance to body weight loss, three groups of gerbils with mean weights of 63.5 g, 86.7 g and 101.0 g, respectively, were maintained on dried chow at 40% R.H. and 25°C. Percent weight loss for the first group was 50.8 ± 1.9% while that of the third group was more than 60%. Survival time appears directly related to initial body weight, the mean survival time for the first group being 29 days while that of the third group was more than 48 days. In comparison to other rodents, the Mongolian gerbil appears to have a relatively high tolerance to weight loss. (Supported by Grant GY-877 from the NSF.)

184

PAUL W. WINSTON, Cambridge University and University of Colorado.

Water activity and a water pump in insect cuticle.

All arachnids and some insects are able to reduce their water loss below the loss that takes place when they are dead. It has been postulated that a pump mechanism reduces the water activity in the cuticle below that of the blood, thereby reducing the rate of evaporation from the surface. Last-instar nymphs of *Periplaneta americana* and *Locusta migratoria* of known age were anesthetized and a sample of blood taken for osmotic pressure determination. They were then decapitated and the prothoracic shield removed and cleaned of cellular debris and surface moisture. A 3.5 mm disc was punched out of the cuticle, weighed, and then exposed at 25 ± 0.05°C to the very high humidity over 0.175 Molal NaCl. Equilibrium with the water vapor over this solution, approximating to the osmotic pressure of the blood, was considered to be equivalent to equilibrium with the blood across the epidermal cell layer. Discs were weighed again after 24 hours. Discs of cuticle from cockroaches gained an average of 5% of the initial weight and those from locusts about 20%. There was no gain in specimens killed by electrocution. Gain in weight was proportionately less over 0.3 M NaCl and discs lost weight over all solutions below 0.5 M. The average point of equilibrium for both species lay between 0.45 and 0.5 M, much higher than any blood O.P. These data substantiate the hypothesis that water activity in the cuticle is reduced. It is clear that this reduction can be due only to a water pump of some sort operating in the cuticle of both these species. Further, the epidermal cell layer is the only possible site of the expenditure of energy for this phenomenon.

185

CHARLOTTE P. MANGUM and CHARLES D. COX, College of William and Mary and Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Mass.

A feeding response to chemical stimuli in the onuphid polychaete *Diopatra cuprea*. (Motion picture)

A feeding response to chemical stimuli has been detected by 1) visual observation, permitting qualitative characterization of components of the response, and 2) recordings of water currents created by one component, permitting quantitative comparisons of different stimuli.

The response is elicited by fluid expressed from many marine organisms, suggesting that the active substances are widely distributed. This absence of species specificity is correlated with the omnivorous habit of the worm in nature.

Investigation of the chemical nature of the stimulus has proceeded along two lines: 1) activity of synthetic substances, and 2) analysis of a representative extract derived from the lamellibranch *Mya arenaria*. 1% (w/v) solutions of several carbo-

hydrates, lipids and large proteins cause no response. 10^{-2} M solutions of each of 13 amino acids cause at least one component of the response. 10^{-4} M solutions of methionine, phenylalanine or valine cause a response which we believe to be identical with that elicited by clam juice—tentacle rotation, peristaltic movements creating water currents, parapodial clasping and mouth opening. Analysis of natural clam juice, however, indicates that other active substances are present.

Preliminary evidence suggests a seasonal change in sensitivity to chemical stimuli, possibly correlated with temperature. (Supported by grants from the Whitehall and National Science Foundations.)

186

GEORGE W. NACE, CHRISTINA M. RICHARDS and HAJIME SAMBUICHI, University of Michigan and University of Hiroshima.

Establishment of an amphibian facility.

The need for anurans and urodeles with biological and genetic definition for studies of development, genetics, and physiology is critical. Thus, the University of Michigan in cooperation with the University of Hiroshima has established a facility to maintain amphibian strains established in Hiroshima for some years, and to develop new strains for American species.

The colony now includes four American Ranidae and several urodele species. Twelve foreign species, including six Ranidae, plus hybrid and derived strains will be maintained. Axolotl (Siredon) strains developed by Dr. R. R. Humphrey of Indiana University plus other anurans and urodeles will be added. The development of strains derived from tumor bearing *Rana pipiens* and from Wisconsin and Vermont populations (Nace *et al.*, Ann. N. Y. Acad. Sci., 126:204, 1965) is receiving greatest attention.

Facilities for large scale maintenance under laboratory conditions include inverted truncated jugs provided with drain and overflow pipes for high density culture of tadpoles and aquatic urodeles in flowing water. Fish troughs divided into pens containing shelves provide aquatic and terrestrial areas for adults. Feeding regimes utilize lettuce, liver, mosquitoes, flies, crickets, and earthworms. Tattooing is used for identification. Health is monitored by the Animal Care Unit of the University of Michigan. Data on growth rates and ovulation cycles will be presented. The inadequacy of liver and the value of chilling will be indicated, and optimal conditions will be suggested. An offer of boarding services for valuable animals and a request for mutant forms will be made. (Supported by NSF GB 4677 and NSF GF 242.)

187

CHRISTINA M. RICHARDS and GEORGE W. NACE, The University of Michigan.

Homograft reactions among unrelated, sibling and parthenogenetic sibling frogs.

Homografts between recently metamorphosed *Rana pipiens* which were genetically unrelated were rejected in all cases. The median survival time (MST) for these grafts was 16 days.

Other frogs received simultaneous grafts from as many as eight of their normal siblings. Among these the grafts on an individual were not lost simultaneously, but 75-80% of the grafts were rejected as rapidly as homografts between unrelated animals (MST 17 days). However, the remaining 20-25% survived almost twice as long (MST 33 days).

Twelve parthenogenetic frogs received grafts from six of their parthenogenetic siblings. Of these, three remained alive and retained their grafts at 123 days, a fourth died with healthy grafts at 111 days. Among the remaining eight, two lost their grafts about the 24th day, three lost them about the 60th day and three lost them between the 85th and 113th day. The process of graft rejection took approximately 10 days and all grafts were rejected simultaneously.

The utility of this test system in developing genetically defined strains of frogs in our amphibian facility will be indicated. (Supported by NSF GB 4677.)

188

BRYAN M. GEBHARDT, Tulane University.

Immunity and tolerance of embryonic tail homografts in the leopard frog. (Introduced by E. Peter Volpe)

Portions of embryonic tail transplanted before the functional maturation of the immune system do not necessarily survive indefinitely. The rejection or persistence of the tail homograft is governed principally by the size of the transplant. Small homografts of tail tissue eventually evoke an immune response, whereas large tail transplants induce a state of tolerance.

Tail grafting was performed on Shumway stage 24 embryos. Some host embryos received transplants of whole tails (from cloacal pore to tip); others received a smaller amount, comprising only the posterior one-fourth of the tail. Small tail homografts were reacted upon by the hosts as early as Taylor and Kollros larval stage V and typically were completely destroyed by Taylor and Kollros stage XV. Large grafts (whole tails) persisted throughout larval development. Occasionally a partial host response to the large tail graft, characterized by mild vasodilation and hemostasis, was observed. Only rarely was a large tail homograft rejected.

Host larvae were challenged with second or test (dorsal skin) homografts from the original donors. In host larvae that had earlier rejected the small tail transplants, the test skin grafts were vigorously destroyed in typical second-set fashion. On the other hand, hosts bearing large viable tail homografts tolerated the subsequent skin grafts from the original donors. This study clearly indicates that the tolerance is a dosage-dependent phenomenon. (Supported by Grant GM-11782 from the USPHS.)

189

NORMA DOMBROCK, Lake Forest College, and D. H. BUCKLIN, University of Wisconsin.

In vitro culture of early embryos and embryonic cells of the grasshopper, *Melanoplus differentialis*.

An attempt has been made to develop reliable

in vitro culture techniques which will support normal growth and development and also permit experimental study of early embryos and embryonic cells of the grasshopper, *M. differentialis*.

Early embryos from stage 5½ through 6½ developed to stage 10 after 7 days of culture in hanging drops of Belar-Ringer with yolk. In hanging drops of H_5 medium (Horikawa and Fox, Science, 145:1437, 1964) the early embryos developed to stage 8 without yolk after 5 days of culture. These embryos were morphologically abnormal due to curling. Therefore, stage 5½ to 6½ embryos were then explanted in H_5 medium to millipore filters in organ culture dishes as developed by Grobstein (Exp. Cell Res., 10:424, 1956); Ball and Auerbach (Exp. Cell Res., 20:245, 1960). Embryos in the filter wells exhibited protocorm elongation and advanced one and one-half developmental stages after between 2 and 7 days of culture.

Culture of dissociated embryos was attempted to permit study of embryo formation from cleavage nuclei. Dissociation was achieved with a trypsin mixture followed by mechanical dispersion. Cells and tissue fragments from embryos at stage 10, 18, 20 and 23 survived for up to 58 days in culture in hanging and standing drops of Belar-Ringer solution with glucose, horse serum and peptone added. No significant cellular aggregation was observed in the cultures. However, cell groups organized themselves into thick and thin walled vesicles; carotenoid pigmentation developed in cultures from embryos dissociated at stage 10.

190

ROBERT L. SEARLS, University of Virginia.

Development of the embryonic chick limb bud *in vitro*.

One of the first procedures for the organ culture of embryonic rudiments was developed for the culture of the embryonic chick limb bud (Strangeways and Fell, Proc. Roy. Soc. B 99:340, 1926). It was reported that this procedure gave comparatively little cell degeneration and normal differentiation of cartilage, but that it gave very poor development of limb structures. This result suggested that some influence from a host embryo is required for limb patterning.

Terminal cartilagenous structures can be formed from precartilagenous limb buds when they are cultured by a simplification of the system of Bell and Merrill (Trans. Bull. 29:108, 1962). A Gelman filter of 0.45 μ porosity is placed on the chorioallantoic membrane of an 8- to 10-day chick embryo and a limb bud is placed on the filter. Approximately 30% of stage 19 limb buds develop digits under these conditions. Older limb buds develop digits with about the same frequency when the most distal 0.3 mm of the limb is cultured. When the most distal 0.06 to 0.1 mm of a stage 19 limb bud is cultured, digits form in about 40% of the cases.

One may conclude that the limb bud is capable of differentiation of terminal structures without a pattern of vascularization or any other directing influence from the host and that the proximal portions of the limb bud are not required in order that the distal portion form digits. (Supported in part by NSF GB 4846.)

191

JOAN ABBOTT and HOWARD HOLTZER, Columbia University and University of Pennsylvania.

Differences in phenotypic expression of chondrocytes grown in monolayers or in clones.

Isolated chondrocytes *in vitro* may exhibit either a fibroblastic or epithelioid phenotype depending on culture conditions.

Cultured as monolayers (10^5 cells/90 mm plastic petri dish) they multiply and after 20 days form a multilayered mat with modest patches of metachromatic matrix. Many cells are morphologically indistinguishable from fibroblasts. Chondrocytes when cloned (10^3 cells/90 mm plastic petri dish) may organize into an epithelioid sheet which morphologically and functionally serves as a *perichondrium*. Cloned cells divide and because daughter cells do not migrate establish compact epithelioid colonies. The crowded polygonal cells are not amoeboid. By mitosis new cells are added to these tight colonies both in the plane of the substrate as well as displaced upward from the substrate. Though anchored to the epithelioid sheet, the displaced cells round up and synthesize much metachromatic matrix which, if tagged with C-14 glucose, can be isolated as chondroitin sulfate. After trypsinizing and re-cloning many of these round chondrocytes form new epithelioid-perichondrial systems, others give rise to fibroblastic cells.

Chondrocytes cloned on a clot do not form epithelioid-perichondrial colonies but spread, migrate and transform into fibroblastic cells. After several generations on clots, such transformed cells when challenged by cloning on plastic do not reconstitute epithelioid-perichondrial colonies nor secrete detectable metachromatic matrix. The progeny of such transformed or dedifferentiated chondrocytes reproduce fibroblastic colonies. (Supported by grants from the NSF and USPHS.)

192

THOMAS C. MAYER, Rider College.

Migratory behavior of melanoblasts from piebald mice.

White-spotting patterns in mice have been attributed alternatively to a selective migration of melanoblasts into certain regions of the skin, or to a failure of the melanoblasts to differentiate in the areas which remain pigment-free. These two alternatives were tested experimentally in a series of grafting experiments in which embryonic 11-day mouse skin and 9-day neural tubes were grafted in combination to White Leghorn chick embryos. In one grafting series pigment production following 15 days of incubation was examined, and in the second series the migration of neural tube cells was followed using tritiated thymidine and autoradiographic techniques.

Grafts of normal skin and normal neural tubes produced pigmented hair, and the tissues of the host were colonized by melanocytes in the operated region. The combination of piebald skin-piebald tube did not produce pigment in the host tissues, and a large majority of the grafted skin cases produced pigment-free hair. The fate of the melanoblasts in both grafting combinations was followed by labeling the neural tube cells prior to grafting.

The pattern of migration of the labeled cells in the normal and in the piebald combination grafts was identical following incubation of 4 days. Labeled cells from normal neural tubes were identified in the same tissue environments where pigment was observed following 15 days of incubation. Piebald labeled cells were present in these same tissue locations, but the correlation with pigment development as in the normal series did not exist.

These results favor the conclusion that pigment-free areas under the conditions of these experiments develop due to a failure of melanoblasts to differentiate in certain tissue environments, and not due to a defect in migration. The hypothesis that white-spotting patterns of piebald mice develop due to a similar mechanism of preventing melanoblast differentiation in the prospective white-spotted regions is therefore supported. (Supported by grant GB4002 of the NSF.)

193

JOHN P. WOURMS, Stanford University.

Reaggregation of completely dispersed amoeboid blastomeres during the normal development of annual fishes. (Introduced by N. K. Wessells)

The normal development of annual fish eggs is characterized by a phase of complete dispersion and subsequent reaggregation of amoeboid blastomeres which is interposed between cleavage and embryogenesis. This report extends previous observations (Wourms, Amer. Zool. 5:662, 1965) on the dispersal of amoeboid blastomeres, to include an analysis of reaggregation in eggs of *Austrofundulus myersi* Dahl.

Two populations of blastomeres form during cleavage and segregate at the flat, hollow blastula. One population, composed of deep blastomeres, eventually disperses as amoeboid cells, while the other, an hemispherical shell of outer blastomeres, flattens to form the enveloping layer. As epiboly commences, the amoeboid blastomeres initially consolidate into a mass, then migrate away from that mass as individual cells and move into the space formed when the enveloping layer and periblast advance over the yolk.

The distribution of amoeboid blastomeres was studied quantitatively using the coefficient of dispersion (Salt and Hollick, J. Exp. Biol. 23:1, 1946). When epiboly is completed (Day 2) the amoeboid blastomeres are uniformly distributed. The blastomeres of the three-day egg are randomly distributed. In the four-day egg, aggregation of cells is found in a single region. Five- and six-day eggs manifest a progressive increase in the degree of aggregation. By day ten, a solid neural keel has formed in the aggregate. An embryo with characteristic teleostean morphology is formed almost exclusively from the reaggregated amoeboid cells.

The reaggregation mass can be considered to be developmentally identical to the embryonic shield of non-annual teleosts. Comparative studies on five other genera of annual fishes have yielded similar results. (Supported by USPHS Predoctoral Fellowship F1-GM-15,631.)

194

GERALD N. SMITH, JR. and MICHAEL J. GREENBERG, Florida State University.

Regeneration in a holothuroid *Leptosynapta crassipatina.*

Regeneration of the holothuroids is under investigation with *Leptosynapta crassipatina* (Apoda) from the mouth of the St. Marks River, Florida, as the chosen test system. H. L. Clark (Bull. U.S. Fisheries Comm., 19:21-31, 1901) had observed that, if the mouth and part of the adjacent digestive tract were intact, then anterior portions of *L. inhaerens* survived and regenerated a new posterior end. Tentacles also regenerated. In these experiments the nerve ring of the regenerating animal was always present.

We have confirmed and extended these observations in *L. crassipatina.* In most experiments the posterior portion died within three days following transversely into three equal segments. Both the middle and posterior ends died at about the same time; no gradient of survival time along the length of the animal was found. Longitudinal sectioning resulted in death in all cases. Animals with broken intestines regenerated the torn organ posteriorly along the edges of the mesentery.

Although untreated posterior segments soon die, we have recently been able to maintain posterior portions by adding nutrients and antibiotics to frequent changes of sea water. Preliminary studies suggest that nutrition is the vital factor in survival of the posterior ends. In this case nutrients must be taken up by the tissues directly from the medium since the gut is inoperative. (Supported by a contract with the USAEC and Grant HE-09283 from the NIH.)

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H. BERNARD HARTMAN and LOUIS M. ROTH, U. S. Army Natick Labs., Natick, Mass.

Stridulation by a cockroach during courtship behavior.

During courtship, the male of *Nauphoeta cinerea* (Olivier) raises his wings and tegmina exposing the tergum. The female responds to a pheromone (seducin), produced in the male's abdomen, by mounting and "feeding" on the tergum. The male grasps her genitalia and mating occurs. If the female is unreceptive, the male lowers his tegmina and wings, and stridulates. This is the first report of sound production by a cockroach during courtship behavior.

The stridulatory apparatus consists of parallel striae on the latero-posterior margins of the pronotum and on the proximal region of the costal veins of the tegmina. These structures are brought together for stridulation. The sound is produced by posterior, anterior, and side to side displacement of the pronotum rubbing against the costal veins.

The phrases comprising the sentence emitted by courting males begin with two to six complex pulse trains each having a 500-750 msec duration, followed by a series of disyllabic chirps, each of 40-80 msec duration. The phrases last 5-10 sec and are linked to form sentences lasting up to 3 minutes. The sound level at 1 cm above the cockroach ranges from 60-65 db for the complex pulse trains peaks and from 55-60 db for the chirp peaks. Audiospectral analysis indicates that no carrier frequency is present, but only a broad band of

frequencies extends to about 15 kHz. The courtship sound is probably detected via the substrate.

208

JAMES MULLIGAN, Saint Louis University.

The program in a long sequence of bird song.

In order to study sequential behavior and the programming of a small number of equivalent behavioral items an extended and continuous tape recording was made of the singing of a male Song Sparrow, *Melospiza melodia*. This individual, located in the interior of British Columbia, had a repertoire of nine distinct song types. The recording was made without playback or other stimulation, but the bird nevertheless sang an almost uninterrupted series of about 1,300 songs during five hours. Each song type was repeated in groups called bouts, of which there were 104. The latter were evenly distributed among the nine song types, and each song type recurred after an average of eight to ten others in the series. There was sufficient variability in this recurrence number, however, and in the linkage between particular song types, to make prediction of the sequence rather difficult. It is clear that these behavioral items are neither randomly nor rigidly ordered; they are arranged in such a way that each item normally recurs after most of the other members of the series have been interspersed. Some song types regularly follow certain others, but in general there is no consistent dependence in the occurrence of a particular song type on what has immediately preceded. (Supported by Grant GB-3433 from the NSF.)

209

WILLIAM L. THOMPSON, Wayne State University.

Song variation in a population of Indigo Buntings.

During the summer of 1965 1210 songs of 47 male Indigo Buntings were recorded on a study area slightly over 1300 acres in size near Pinckney, Michigan.

Sonograms of the songs showed that the birds produced a total of 93 distinct song elements. The maximum number of elements in the normal advertising songs of individual birds ranged from 6 to 24. There was very extensive sharing of elements in the songs of different individuals throughout the population and there were several instances of different birds sharing identical songs. One pattern was shared by six birds, two of them holding adjacent territories. One pattern was shared by three birds, and two patterns by two birds each, one of them by neighbors. All songs of any individual were of one basic pattern, although the full pattern was not always given.

The relative infrequence of neighbors sharing the same song pattern or even several elements suggests that the birds learn their song pattern before they settle on a territory during their first year probably from their father or males singing near the territory in which they are reared. It is possible, however, that birds hatching late in the summer may not develop their own song patterns until the following spring. The few instances found in this study of neighbors sharing a song pattern may be a result of this, or of birds returning to the immediate vicinity of the territory in which they grew up. (Supported by NSF grant 6B-3882.)

210

NANCY MUCKENHIRN, University of Maryland.

Vocalizations of the marmosets, *Saguinus oedipus oedipus* and *S. o. geoffroyi*. (Introduced by J. F. Eisenberg)

Vocalizations of two subspecies of *Saguinus oedipus* were divided into five morphological groups on the basis of sonograms: note bursts, chevrons, whines, slides and noisy vocalizations. Individual differences among the vocalizations of nine animals were identified but parameters of subspecific differences are not clear. Behavioral contexts of the calls were similar. Long calls or whine series produced by visually isolated animals were compared.

Two stereotyped vocalizations were elicited consistently from eight adult *S. o. geoffroyi* when presented with a snake or overhead objects. The number of slicing chirps (chevrons) and the number of looks at the box when a snake was present and absent were highly significant ($p = .01$ Wilcoxon). Marmosets reacted to overhead movement by trilling (a rapid note burst series) and flipping under a branch or jumping downwards.

Location and alarm functions of these calls were supported by observations on wild groups of *S. o. geoffroyi*.

211

STUART A. ALTMANN, Yerkes Regional Primate Research Center, Atlanta, Ga.

The basic communication network in a rhesus monkey society.

A society of animals is a communication network. Social communication in rhesus monkeys, studied for two years on Cayo Santiago, deviated strongly from what would be expected in a society of randomly intercommunicating individuals, in which the network of paired interactions among members of the age-sex classes could be predicted from the population structure. Deviations resulted from age and sex differences in the social reactivity of the monkeys, from differences in likelihood of being the recipients or targets of social signals, and from attractions and repulsions between monkeys of certain age-sex categories. Taking these deviations into account lead to a new model of interaction frequencies among members of the age-sex groups, but it, too, was inadequate to account for the distribution of particular behavior patterns: the likelihood of an interaction between two monkeys depended not only upon the age and sex of the individuals, but also upon the nature of the social behavior involved. (Supported by Grant MH 07336-01 from the NIH.)

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DONALD J. MULCARE, University of Notre Dame.

Induction of renal tumors in hybrids between *Rana pipiens* and *Rana palustris*. (Introduced by K. S. Tweedell)

The large mitochondrial fraction, derived by high speed centrifugation, from *R. pipiens* in-

clusion body tumors, was oncogenically active when inoculated into *R. pipiens* embryos (Tweedell, Amer. Zool. 5:171-172, 1965). Such studies have been extended to challenge *R. palustris* and reciprocal, interspecific hybrids between *R. pipiens* and *R. palustris* with a similar inoculum derived from a *R. pipiens* (Wisconsin) inclusion body, renal tumor.

The methodology employed involves suspension of the large mitochondrial pellet in five drops of Gomori 0.02 M phosphate buffer. Either this stock solution or its buffered ten-fold dilution was injected. A microliter syringe was used to place 1-4 μl of either inoculum into the region of the left pronephric swelling. The embryos were stage S18 to S21. *R. pipiens* (Wisconsin), *R. palustris* (Tennessee) and reciprocal hybrids between the two species.

Of 53 mid- to postmetamorphic experimentals, 11 possessed renal tumors. Two of 16 hybrids (*R. palustris* ♀ × *R. pipiens* ♂) and three of 10 of its reciprocal (*R. pipiens* ♀ × *R. palustris* ♂) were afflicted. Six of 20 *R. pipiens*, inoculated with the more dilute preparation, also developed renal tumors. The presence of tumors in seven surviving *R. palustris* was not demonstrated. Tumors were not observed in any of the controls.

Larvae of both species and both hybrid groups, receiving either dilution as embryos, developed a lethal syndrome characterized by subcutaneous, hemorrhagic lesions. Exactly 49% of each hybrid group receiving the concentrated inoculum, developed hemorrhagia, in comparison with approximately 23% of the dilute inoculated hybrids. The syndrome related death rates among the two species were: *R. pipiens*, 18% (concentrated) and 3% (dilute), *R. palustris*, 34% and 31%. The response to the inoculum was dosage dependent and became lethal in higher percents as a function of the host's taxonomic variance from *R. pipiens* (Wisconsin). Either species could provide the egg for the hybrid zygote without significant effect on the rate of hemorrhagia. By comparison with the controls, there were nine times as many syndrome deaths among the animals given the dilute dose and 15 times as many among the concentrated dosage group. (Supported by Grant IN 84A from the Am. Can. Soc. and Grant CA-07849-02 from the USPHS made to K. S. Tweedell.)

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G. LAWRENCE VANKIN and HENRY J. GRASS, Williams College.

Colcemid-induced teratogenesis in hybrid mouse embryos.

Primiparous C57BR/cdJ mice each were given a single intraperitoneal injection of colcemid (N-desacetyl-N-methylcolchicin; Ciba) 8¼ days after mating to BALB/Ci males. Dosage was 1.8 milligrams per kilogram of maternal body weight. Controls were injected with the vehicle. Females were sacrificed and fetuses dissected at 16½ days of pregnancy. Forty-one experimental litters produced 361 implantation sites, consisting of 195 resorptions (54.0% of implantations) and 166 survivors (46.0% of implantations). Among survivors, 128 (77.1%) were normal and 38 (22.9%) were grossly malformed. Distribution of major malformations was: exencephaly, 22 cases (57.9%); anophthalmia or microphthalmia, 10 cases (26.3%); gastroschisis with eventration, 4 cases (10.5%); tailless, 1 case (2.6%); kinky tail, 1 case (2.6%). Multiple malformations per fetus were fairly common, e.g., 8 exencephalic fetuses also exhibited anophthalmia or microphthalmia, and 2 of these had posterior-trunk spina bifida. Twenty-eight control litters yielded 233 implantations, which included 20 resorptions (8.6% of implanations) and 213 survivors (91.4% of implanations). Among surviving controls, 208 (97.7%) were normal and 5 (2.3%) were malformed, 1 with scoliosis and 4 with kinky tail, but none with multiple malformations. Although Thiersch (Proc. Soc. Exp. Biol. Med., 98:479, 1958) reported abortifacient action of colcemid without teratogenesis in rats, the data above and work of DeMyer (Neurology, 14:806, 1964) and Ferm (Anat. Rec., 148:129, 1964) demonstrate teratogenesis by metaphase inhibitors in laboratory rodents. Development of these malformations and mitotic blockage as a possible mechanism of teratogenesis will be discussed. (Supported by NSF Grant GY-114.)

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JOHN B. WINFIELD, Cornell University Medical College.

Actinomycin D teratogenesis in the young mouse embryo. (Introduced by Dorothea Bennett)

The intra-peritoneal injection of 100 μg/kg female weight of Actinomycin D, a specific inhibitor of DNA-dependent RNA synthesis, into pregnant Black Spot mice 6¼ days after mating produced mesodermal and ectodermal abnormalities in 100% of the embryos examined one, two, or three days after the injection. In young embryos (7¼ days), these included absent, small, or overgrown primitive streak; disorganized embryonic ectoderm; extra-embryonic membrane deficiencies, and growth retardation. In older embryos (8¼ and 9¼ days), absent or irregular somites, neural tube disorganization or degeneration, non-closure of the neural plate, hydrocephalus, enlarged pericardial sac, and decreased cephalic mesenchyme were observed.

Because the abnormalities were similar to those found in embryos homozygous for certain early acting lethal alleles at the T-locus on the ninth chromosome (Bennett, D., Science 144:263, 1964), an investigation was undertaken to study possible synergism between the teratogen and Brachyury (T), a homozygous lethal.

Female +/+ mice, mated to +/+ males and treated at 6¼ days after mating with 70 μg/kg Actinomycin D yielded no abnormal embryos at 7¼, 8¼, or 9¼ days. 37% of the embryos similarly exposed to Actinomycin D, but obtained from T/+ × +/+ matings were abnormal. The abnormal embryos did not differ from normal litter-mates in cytoplasmic RNA content as determined qualitatively by histochemical staining for RNA with Azure B.

The results have been interpreted as showing that Actinomycin D summates with T in converting T/+ heterozygotes into phenocopies of early acting t lethals. (Supported by a Grant from the Ford Foundation).

215

M. KERRIGAN, R. MEYER, and H. MAISEL, Wayne State University.

The effect of actinomycin-D on kidney development in the chick.

Mesonephric degeneration in the chick is characterized by a decrease in soluble protein concentration and enzyme activity after day 15 of incubation. In order to determine if this degeneration of the mesonephros is under nuclear control, 0.5 ml of actinomycin-D (30 mg/ml) was injected into fertile eggs at days 13, 15 and 17 of incubation.

After injection of actinomycin-D at day 15 of incubation, mesonephric degeneration was delayed for 108 hours as revealed by biochemical and histological studies. The soluble protein and enzyme concentrations of the mesonephroi from the treated animals remained at higher levels than in control animals. The wet weights of the mesonephroi from the treated embryos were higher than the wet weights of the mesonephroi from control animals.

Actinomycin-D had no comparable effect on the metanephros except for a retardation of increase in weight, a phenomenon also observed for the wet weights of the liver and whole embryo.

When actinomycin-D was injected on day 13 of incubation, a greater than normal increase in soluble protein and enzyme concentration was observed 12 hours later. When actinomycin-D was injected at day 17 of incubation the normal drop in soluble protein and enzyme concentrations failed to occur 12 hours after injection. The higher the dose of actinomycin-D injected at day 13, the greater the increase in soluble protein and enzyme concentration. At day 17 all doses of actinomycin-D prevented the normal drop in protein and enzyme concentration.

It would appear that the growth of the mesonephros occurs in the presence of a gradually increasing concentration of a mRNA dependent inhibitor substance which normally reaches a critical concentration at about day 15 of incubation. Thereafter, degeneration of the mesonephros ensues. (Supported by a Grant from the Michigan Kidney Foundation.)

216

LOUIS T. STABLEFORD, Lafayette College.

The effect of 5-fluorouracil on the early development of *Rana sylvatica*.

Continuing a study of the effect of 5-fluorouracil on early amphibian development (Stableford, Amer. Zool., 4:397, 1964), embryos of *R. sylvatica* were raised for varying periods of development in 1/10 Holtfreter's soln. containing 100 μg 5-fluorouracil and 200 μg thymidine per ml. Embryo exposed starting at blastula, gastrula, or neurula stage developed normally until tail-bud stage, Pollister & Moore's (1937) stage 19. From this point until hatching (stage 20-22) the forebrain and optic cups showed progressive dedifferentiation and retardation; neural and optic cup cells migrated into the neurocoel and surrounding mesenchyme, somewhat resembling neural crest cells. Formation of the lens, olfactory organs, and ear vesicles, however, was unaffected.

Embryos were also exposed to the 5-fluorouracil medium for limited periods of development then cultured in 1/10 Holtfreter's soln. until stage 20-22. Regardless of how early the stage when exposure started, the effect of 5-fluorouracil depended on exposure during stages 15-17, middle to late neurulation.

These results tend to support the view of Saxen & Toivonen (1962) that after primary induction there must be subsequent inductors that stabilize structures and control further differentiation, if certain assumptions are accepted: that 5-fluorouracil interferes with RNA synthesis, probably that of messenger RNA; that RNA is intimately involved in induction. They further suggest that inducing agents are laid down well in advance of the time that they become effective. (Supported by a Grant from the Lafayette College Research Fund.)

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EILEEN D. HICKEY and NORMAN W. KLEIN, Marquette University.

Sequence of changes in DNA and protein content of regions of the explanted chicken embryo following insulin treatment.

Attempts were made to determine if biochemical tissue specificity could be demonstrated corresponding to the known morphological specificity of insulin's teratogenic action.

Chicken embryos of 11-13 somites were explanted onto whole-egg agar media containing insulin. After 48 hours of culture, insulin treated embryos showed morphological defects, which, at low concentrations, were localized in the posterior trunk, and at 4 u/ml, involved the whole posterior somitic region and the brain. In embryos on 4 u/ml, protein nitrogen was 29% of the controls, and DNA, 23%. At lower insulin concentrations this reduction was less marked. In embryos exposed to 4 u/ml of insulin for 2-3 hours, and transferred to control medium for 46 hours, the morphological response was comparable to the above, although the reduction in protein nitrogen and DNA was only about 40%.

Insulin treated and control embryos cultured in the latter manner were collected every 6 hours for the first 24 hours of explantation, and dissected into 5 regions: the head up to the otic vesicles, the anterior trunk through the eleventh somite, the posterior trunk, the heart, and the membrane. DNA and protein were measured, and in all tissues responding to insulin treatment, the DNA was reduced first. Significant differences from the control appeared in the head and posterior trunk at 12 hours, and in the anterior trunk at 18 hours, followed after several hours by reduction in protein. The response of heart and membrane were not consistent. (Supported by NSF Grant GB-2388 to N. W. Klein.)

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JOSEPH G. KUNKEL, Western Reserve University.

Developmental sequences in synchronous cultures of cockroaches. (Introduced by M. Locke)

Large cultures of the German cockroach, *Blattella germanica* are maintained in synchronous development by controlling food availability (Kunkel, J. Insect Physiol., 12:227, 1966). Samples of animals are taken from the synchronous culture

and assayed for various developmental events. Plotting the proportion of each sample past a given event against the time of assay determines a probit regression line, which defines the distribution of the event in time. A t-test is used to infer the sequency of two events in the developing culture.

A number of developmentally critical periods were assayed for. The brain critical period is defined as the time after which the brain is no longer needed to support a molt. The prothoracic critical period is defined similarly as the time after which the prothoracic gland is no longer needed for a molt. The critical period for regeneration as described by O'Farrell and Stock (J. Biol. Sci., 6:485, 1953) is defined as the time after which leg regeneration is postponed to the next molt.

It was inferred that the critical period for regeneration is not significantly different from the brain critical period. Also the critical period for the prothoracic gland was determined to be closely associated with the end of epidermal mitosis and the detachment of the epidermis from the cuticle.

This type of approach will lead to a developmental map of the synchronous culture. Subsequent experiments may be designed to determine the causal relationships of the events whose sequence has been determined. (Supported by Grants GM 09960 and 1-FI-GM-33, 259-01 from the USPHS.)

219

JERRY J. KOLLROS, University of Iowa.

Influence of thyroxine treatment upon limb regeneration and onset of the corneal reflex in *Rana pipiens*.

Three groups of tadpoles, initially in stages V-X, were used to study the influence of thyroxine on limb regeneration, with transection at the thigh. One group was untreated, serving as a control; another was placed in thyroxine solution (30 μg/l) immediately after amputation, while the third group was placed in hormone first, and amputated at the end of thyroxine treatment. In both treated groups exposure to hormone was for 6 or 7 days, during which time metamorphic progress was advanced about 3 stages (e.g., VI to IX, IX to XII, etc.). Those animals treated with hormone immediately after amputation showed regeneration capacity almost identical to that of the controls, although hormone treatment resulted in limbs of more advanced stage. Those animals in thyroxine prior to amputation showed distinctly reduced regenerative capacity, essentially that characteristic of control animals of the same stages as those attained by the experimental group at the time of amputation, and perhaps less. Animals were observed over a 15-22 day period, this being sufficient for the attainment of forelimb emergence in the most highly stimulated. This interval was also sufficient in most hormone treated animals to permit maturation of the corneal reflex center. Since a substantial number of animals displaying the reflex were not yet close to experiencing forelimb emergence, these results contrast with those reported earlier (Kollros, '42), in which reflex onset *followed* forelimb emergence in animals stimulated by continuous immersion in thyroxine solutions. (Supported by Grant AM02202 from the USPHS.)

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WILLIE M. REAMS, JR., University of Richmond and the Medical College of Virginia, and BARRY E. SCHAEFFER, University of Richmond.

X-ray effects on intramuscular pigment cells in the mouse.

It is well established that X-rays provoke hyperpigmentation in the skin of mammals and much has been speculated on the relative roles of radiation, pigment cells and the epidermis in bringing about this hyperpigmentation. The present study was initiated to investigate the response of pigment cells to X-rays in an extraepidermal tissue environment. To this end, the legs of newborn mice of the PET (Pigmented Extraepidermal Tissues) strain were irradiated with from 200 to 7000 r from a GE 1000 KVP Maxitron machine. Ten days after irradiation the mice were killed and the legs amputated. Non-irradiated legs served as controls. The gastrocnemius of each leg was dissected free and studied either as a cleared whole mount or in histological section. DOPA was employed to augment the pigment cell examination.

For the sake of comparison, the leg skin was examined also. As expected, the skin showed a progressive hypertrophy and hyperplasia of the melanocytes with increasing doses of radiation. In contrast, there was a progressive decrease in the intramuscular pigment cell population with increasing irradiation. There was no pigment cell stimulation in muscle at any dosage level. At higher levels of radiation, melanocytes damage and loose melanin granules were evident.

Hence, additional evidence is tendered in support of the epidermis as the mediating agent for positive pigment cell responses to X-radiation. The interrelationship between X-ray and -SH inhibition of pigment cell melanogenesis in muscle is currently under study. (Assisted by NIH Grant ITI AM 5508-01 at MCV.)

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GEORGE SZABÓ, Harvard Medical School and Marine Biological Laboratory, Woods Hole.

Electron microscopical studies of the ink gland of the squid (*Loligo pealii* Les).

The ink gland is located in the "caudal" end of the ink sac. It is a cavernous, heart shaped organ with an opening on its apical end. The inside of the organ consists of folds and septa of connective tissue lined by secretory epithelium. This epithelium is simple, columnar secretory epithelium, the ink-epithelium. The ink cells are typical secretory cells, with a large nucleus, tubular mitochondria, a very extensive granular endoplasmic reticulum and several Golgi areas. The ink cells show a well developed basal interdigitaion. At the apical end of these cells there are numerous vacuoles of varying diameter which may be full of small, spherical particles, the ink (melanin) granules. There may be a dense matrix present in between these melanin granules. The melanin granules are excreted into the lumen of the gland, where they are suspended freely. There are cilia and microvilli at the free margin of the ink cells. Septate desmosomes are also present in between the epithelial cells. The connective tissue of the gland consists of several cell types and extracellular material. There

are myoepithelial cells attached to the base of the secretory epithelial cells keeping the whole organ in a gentle pulsatory movement. There are also general connective tissue cells and perineural cells present. The blood vessels are lined by endothelial cells with relatively large gaps in between these cells. There are collagen fibers in the extracellular spaces.

Histochemical and biochemical studies indicate that tyrosine and DOPA are incorporated into the ink vacuoles and there is also free tyrosinase in the lumen of the ink gland and also in the large reservoir of the ink sac itself.

The ink gland can be maintained in organ cultures (Szabó and J. Arnold, Biol. Bull. 125:393-394, 1963) for at least 10 months. (Supported by Grant CA-05401 Natl. Cancer Inst., USPHS and Cancer Development Award USPHS Ke-GM-14,987.)

222

ELIZABETH L. BROWN, W. THRELFALL and F. A. ALDRICH, Marine Sciences Research Laboratory, Memorial University of Newfoundland.

Cestode fauna of the squid *Illex illecebrosus illecebrosus* (LeSueur) in Newfoundland waters.

Squid were obtained during the months July through October, 1966, from Holyrood Arm, Conception Bay, and Freshwater Bay, Newfoundland. The specimens were obtained by jigging and immediately upon capture they were placed in a container of crushed ice and so transported to the laboratory for examination. The squid were later examined for their cestode burden according to a definite plan. An incision was made in the median ventral mantle wall, and the internal organs were then examined for parasites. Any helminths found were preserved in 10% formalin prior to staining with borax carmine. As well as being investigated for parasites, measurements of squid total length, standard length, and mantle length were made. The sex and maturity of each squid was also noted.

The cestodes found were of two genera, Phyllobothrium and Dinobothrium. The incidence of these was tested for correlation with standard length, total length, mantle length, sex and size of the host squid as well as relative numbers of squid in the bays at the time of their capture. Also found in the caecum and mantle walls were several nematodes. (Supported by NRC Grant A-1368.)

223

JOHN C. BOYKIN, University of Washington.

Somatic musculature of *Trachydemus ilyocryptus* (Kinorhyncha). (Introduced by R. P. Higgins)

The somatic muscles of *T. ilyocryptus* Higgins, 1961, are cross-striated as reported for several other kinorhynch species. The number of cells per muscle could not be determined.

Longitudinal muscles: Each trunk segment except the last contains a dorsal and a ventral pair of longitudinals. Six pairs of anterior retractors (of the head) originate on the pachycyclus of the exoskeleton of segment iv, inserting posteriorly in the head. Segments iii and iv each contain a pair of oblique muscles laterally. A third pair of obliques runs from sternite v to tergite iii. Pairs of outer retractors originate on the exoskeleton dorsally in segments iii and v-viii, and ventrally in iii and vi-viii; all insert in the forebrain.

Inner longitudinals: Pairs of inner retractors originate, dorsally and ventrally, in segments vi-viii. They insert in a symmetrical circle in the hindbrain. Ten protractors of the pharynx originate in the mouth cone, while 4-6 others originate in the hindbrain; all the protractors insert on the posterior end of the pharynx. Ten head longitudinal muscles run just beneath the external cuticle of the head from the level of scalid circle six to the first scalid circle.

Other muscles: In each trunk segment is a pair of dorsoventral muscles. Two cutaneovisceral muscles originate on tergite xii, inserting on the endgut. A circular muscle of the neck forms a transverse circle, incomplete ventrally, just beneath the cuticle of the neck or posterior portion of the head.

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JULIAN F. HAYNES, University of Notre Dame.

The ultrastructure of the muscles of *Hydra viridis*.

Relaxed *Hydra viridis* were flooded with a cold two per cent glutaraldehyde solution in a phosphate buffer. After glutaraldehyde fixation they were post-fixed in two per cent osmic acid and imbedded in araldite. The sections were stained with lead citrate and examined with an RCA-EMU 3G electron microscope.

The muscular system of *Hydra* is formed from myonemes in the bases of both epidermal and gastrodermal cells. The myonemes of the epidermis are formed from masses of long fibers running parallel to the axis of the polyp. There are two distinct fiber sizes within the myoneme. The thick filaments are approximately 200 Å in diameter and the thin filaments are 60 Å in diameter. The two types of filaments are randomly interspersed within the myonemes. The only attachments of the myonemes to the membranes of the epithelial cells are found at the ends of the filaments. They are never attached to the bases of the epithelial cells, and contraction of the myonemes results in extensive foldings of the basal cell membranes. In regions where the myonemes of adjacent cells contact each other the cell membranes are modified to form a junctional complex similar to the intercalated discs of cardiac muscle. The gastrodermal myonemes are of essentially the same structure but are less well developed. (Supported by USPHS Training Grant 5T1-HD21-04 and NSF Grant GB 3262.)

225

JANE A. WESTFALL, University of California.

Electron miscroscopy of the basitrich and its associated structures in *Obelia*.

In the hydranth of *Obelia longissima* only one type of nematocyst is found: the basitrich. The undischarged thread of this nematocyst is differentiated into a relatively straight, wide, shallowly folded, heavily spined proximal tube and a narrow, slightly coiled, lightly spined distal tube with deep folds in its wall. The discharged thread of the nematocyst appears isodiametric, but bears

numerous spines proximally and, contrary to its name, a few spines distally.

The basitrich is closed by an operculum; nearby lies a cnidocil ringed basally by nine long finger-like processes. Additionally, nine short processes surround the opercular region of the nematocyst. A delicate lattice-like fringe of material lies on the outer surface of the processes. The dense cores of all 18 processes continue as rods beneath the surface of the cell where they are interconnected by a ladder-like arrangement of cross-connecting fibrils. The rods fray into longitudinal fibrils, probably microtubules, which surround the nematocyst and join basally in a fibrous band. The latter structure connects with half-desmosomes that attach the cell to the mesoglea.

The cnidocil and interconnected rods are believed to function in the discharge of the nematocyst. If stimulation of the cnidocil by a prey caused a mechanical stimulus to pass via the fibrous arms of the kinetosome to the fibrills interconnecting the dense rods causing them to contract, this might constrict the nematocyst sufficiently to spring open the operculum. Simultaneously chemical stimulation might be effected by way of the fringe and membrane covering the processes. (Supported by a USPHS Grant to Richard M. Eakin.)

226

JERRY M. NEFF, Duke University.

Ultrastructure of the calcium-secreting glands of *Pomatoceros caeruleus* (Schmarda), (Annelida: Polychaeta: Serpulidae). (Introduced by K. M. Wilbur)

Pomatoceros caeruleus produces a tube of calcite by means of two epithelial calcium-secreting glands located in the ventro-lateral peristomium under the collar. These glands are tubulo-racemose. The cells of the secretory acini are elongate with basal nuclei. They have a well developed rough endoplasmic reticulum concentrated in the perinuclear region and extending into the middle and apical regions. A well developed Golgi zone with many secretory vacuoles fills much of the region between the nucleus and apex of the cells. Mitochondria are most abundant in the perinuclear region. Most of the secretory cells have a well defined intracellular channel free of cell organelles and filled with a fine granular material extending from the nuclear region to the apex. These channels are bordered on one side by the cell membrane and on the other by the rough endoplasmic reticulum. The apices of the secretory cells have long microvilli extending into the gland lumen.

The lumina of the acini are filled with small dense spheres, 0.3 micron or less in diameter, composed of minute calcite crystals embedded in a fine granular matrix, probably a mucopolysaccharide. Also present in the lumina are large membrane bound vacuoles filled with a granular material of variable electron density.

No calcite crystals were detected intracellularly in the glands, indicating that crystallization takes place in the gland lumen. (Supported by Grant 5TI DE 92-05 from NIDR-NIH.)

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GRETA E. TYSON, University of California, Berkeley.

Gross morphology and fine structure of the maxillary gland of the brine shrimp, *Artemia salina*. (Introduced by Richard M. Eakin)

Using the procedure described by Boyer (Anat. Rec., 100:191, 1948), a plastic reconstruction was prepared from serial sections of the adult maxillary gland of *Artemia salina* (L.). Each gland consists of two parts, an end-sac and an efferent tubule. The end-sac is centrally located and around it the efferent tubule coils, making three loops before leading to the outside via a slender terminal duct.

Whole glands were fixed in cacodylate-buffered glutaraldehyde, postfixed in osmium, embedded in epon, and sectioned for electron microscopical examination. The end-sac of *Artemia* is very similar in ultrastructure to the coelomic sac of the crayfish antennal gland (Kümmel, Zool. Beiträge, 10:227, 1964). The most striking feature of the end-sac epithelium is the presence of numerous cytoplasmic projections at the bases of the cells. The arrangement of these basal "feet" is highly reminiscent of the podocyte processes in Bowman's capsule of a vertebrate nephron. The wall of the efferent tubule is made up of large, flattened, epithelial cells, which posses an apical microvillous border and deep, basal foldings of the plasma membrane. Numerous mitochondria are associated with the basal configurations which are, in part, due to complex interdigitations of neighboring cells. An abrupt change in cell type occurs at the beginning of the terminal duct. The duct is lined by a thin layer of cuticle, and the epithelial cells here are smaller and more regular in shape, lacking both microvilli and basal foldings. (Supported by USPHS Grant RG-6025 and a USPHS predoctoral fellowship.)

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VICTOR SPRAGUE and SANFORD H. VERNICK, Chesapeake Biological Laboratory and Georgetown University.

Light and electron microscope study of developing stages of *Glugea* sp. (Microsporida) in the stickleback *Apeltes quadracus*.

Glugea sp. is common in sticklebacks in the Patuxent River near Solomons, Maryland. It produces white cysts approximately 3 mm in diameter, usually retroperitoneal or subcutaneous. Each cyst is a greatly hypertrophied and multinucleate host cell as shown by Weissenberg (9th Congress Intern. de Zool. Monaco, 380-389, 1914), which contains the parasites and is encapsulated by connective tissue fibers. Schizogony and sporogony occur in the periphery, and vacuoles containing mature spores coalesce in the central portion of the cyst. In this study which may be the first to deal with the fine structure of microsporidean development, the sporogonic stages are identified and studied with the lectron microscope. Vacuoles containing these stages are limited by two double membranes, and are devoid of host cell ground substance. The parasites themselves are limited by a double membrane. The cytoplasm of these stages is characterized by smooth surfaced endoplasmic reticulum in the form of vesicles, tubules, and flattened sacs. Mitochondria, Golgi material, and ribonucleoprotein particles appear to be lacking. The nuclear envelope is interrupted by pores, and centrosomes

and spindle fibers related to karyokinesis are present. The cytoplasm of the hypertrophied host cell contains the normal complement of cellular fine structure. In addition structures possibly representing abortive reduplication of the host cell nuclear envelope are visible. These are sometimes seen in malignant or virus infected cells, and may be a result of the stimulation of the cell by the parasites.

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STEPHEN A. ROTH and JAMES A. WESTON, Western Reserve University.

Relative measurement of intercellular adhesion between labeled cells and unlabeled aggregates in shaker cultures.

Current theories concerning sorting out of cells in mixed aggregates all involve some assumptions about differential cellular adhesions. There is, however, no unambiguous measure of adhesions which could be used to test these assumptions.

A method has now been devised to measure relative intercellular adhesive stabilities by circulating unlabeled tissue aggregates in H^3TDR-labeled cell suspensions. The number of labeled cells collected by aggregates of equal size while in shaker culture is taken as a comparative measurement of their adhesive stability with the cells of the suspension. Specifically, liver and neural retina aggregates from 7-day chick embryos are introduced into aliquots of labeled liver or neural retina suspensions in iso- and heterotypic combinations. Counts of labeled nuclei in radioautographs of sectioned aggregates reveal that liver aggregates collect up to 100 times more labeled liver cells than neural retina aggregates in the same suspension within six hours after the labeled tissue was dissociated. Conversely, neural retina aggregates collect up to 100 times more neural retina cells than liver aggregates in the same suspension within the same time period.

These results are interpreted to mean that, at least for liver and neural retina cells, adhesive specificity does exist, that it exists among cells in suspension before they become assimilated into aggregates, and that isotypic associations are more stable than heterotypic associations. (Supported by Grant GM-13072 from the USPHS and Developmental Biology Training Grant 2T1HD20 from the USPHS.)

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JOSEPH T. BAGNARA, University of Arizona.

Changes in pigments of amphibian xanthophores during larval development.

Recent work has revealed that pteridines are a dominant pigment of amphibian xanthophores and erythrophores. Pteridines appear with the onset of differentiation of these bright-colored chromatophores and their pattern is more or less species specific. Generally, sepiapterin is prevalent in xanthophores and considerable amounts of the drosopterins are found in erythrophores. For a time, it was thought that pteridines were the exclusive pigments of these chromatophores during larval development and that carotenoids, long considered their major pigment type, were utilized in post-metamorphic stages. By the use of differential solubilities and with the aid of the sulfuric acid test it was revealed that carotenoids are already present in larval stages. In the newts, *Pleurodeles waltlii, Taricha torosa,* and *Diemictylus viridescens,* carotenoids first appear in xanthophores when the larvae are about two-thirds grown *(Pleurodeles* stages 52-54). In *Rana pipiens* they appear much earlier and are already present at Taylor-Kollros stage II. In *Xenopus laevis,* xanthophores first become numerous at mid-larval life and are marked by the presence of both pteridines and carotenoids. In the toads *Bufo alvarius* and *Scaphiopus couchi,* carotenoids are present by mid-larval life. Riboflavin is another xanthophore constituent. In adult newts it is a major yellow pigment making its first appearance just before the onset of metamorphosis. Within the xanthophore, carotenoids are present in small droplets which seem to be evenly distributed between the smaller pteridine containing granules. Occasionally they coalesce into large droplets. In some xanthophores, notably those of *Bufo* and *Xenopus,* carotenoids are also present in small spindle shaped bodies. The cytological distribution of riboflavin has not been observed. (Supported by Grant GB-4923 from NSF.)

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FRED A. DIEHL, Western Reserve University.

Morphogenesis in irradiated Cordylophora.

Coenosarc from *Cordylophora caspia* stolons was mechanically separated from the perisarc and isolated as epidermal and gastrodermal masses. These masses were left in isolation or recombined with tissues of the complementary layer. Both normal and irradiated (4,500 R) animals were employed in the study. Normal epidermis alone produced complete animals, as reported originally by Zwilling (Biol. Bull., 124:368-378, 1963). Irradiated epidermis rounded-up and secreted perisarc around the periphery of the masses but never exhibited hydranth production or other morphogenetic activity. Normal gastrodermis also formed rounded balls but the cells began to slough off after a short interval. The cells apparently lost cohesiveness and the masses had completely disintegrated 24-36 hours after preparation. Irradiated gastrodermis behaved in a similar manner.

Normal epidermis combined with irradiated gastrodermis yielded complete, viable animals in all cases. Moreover, normal gastrodermis combined with irradiated epidermis produced normal animals in some cases. Presumably the irradiated epidermis acted as a protective layer which allowed certain gastrodermal cells to differentiate into epidermal cell types. In the absence of such a protective layer the cells did not remain in contact for a sufficient period to allow for expression of their differentiative capacity. Irradiated epidermal masses placed with irradiated gastrodermis formed hydranths and stolons, in some cases 10-12 days after irradiation. Although capable of feeding for an additional period of 15-20 days these animals eventually died. Thus, in order for hydranths and stolons to form, normal or irradiated gastrodermis must be present. Isolated normal epidermis did not develop these structures until after the gastrodermal layer was produced. Also, irradiated epidermis, which never developed a gastrodermal layer, did not form

hydranths or stolons. (Research conducted while author was NIH Postdoctoral Fellow at the University of Virginia.)

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ARTHUR LAVELLE and PIERSON J. VAN ALTEN, University of Illinois College of Medicine.

Antigenic differences in isolated regions of brain.

We have been studying the reactions of antigens obtained from different regions of young and adult hamster brains. The antiserum used was prepared by subcutaneously injecting saline homogenates (combined with Freund's adjuvant) of perfused, whole, five-day postnatal hamster brain into Polish rabbits. The following regions of five-day brain were used as sources of test antigens: the olfactory bulb, forebrain, cerebellum and medulla. The test antigens from the adult were obtained from brain sections which had been previously cut in a cryostat and dried *in vacuo*. The areas isolated from these sections were the pyramidal motor tract, ascending sensory tracts and the motor nuclear areas of the 7th and 5th cranial nerves. Each isolated region or area was separately pooled, weighed, homogenized in physiological saline and then run against the antiserum on micro-gel-diffusion plates.

In general, for either the young or adult material, different areas of the brain differ in the number and pattern of precipitin lines resulting when reacted against the same antiserum. Thus far our evidence seems to indicate that the differences in precipitin reactions among the various areas tested are due to differences in cell types constituting the areas. Therefore, the total number of precipitin lines that result when whole brain is reacted with the antiserum reflects the accumulative reactions of separate cell populations constituting brain tissue. (Aided by a grant from the United Cerebral Palsy Research and Educational Foundation.)

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WESLEY J. BIRGE and PAUL F. DOOLIN, University of Minnesota, Morris; Veterans Administration Hospital, Hines, Illinois, and Stritch School of Medicine, Loyola University, Hines, Illinois.

Ultrastructural differentiation of the endoplasmic reticulum in the epithelial cells of the avian choroid plexus.

Toward the end of the first week of embryonic development, the epithelial cells of the avian choroid plexus begin to show incipient changes in their chemical composition. Basic dye-binding methods indicate an increase in cytoplasmic ribonucleic acid (RNA). This build-up is progressive, and from the tenth day RNA basophilia is intense, especially in the basal (subnuclear) cytoplasm where much of the RNA is sharply localized. During early stages (6-8 days) of differentiation, this build-up of RNA closely correlates with a heavy concentration of free ribosomes. Subsequent to the eighth day of development, however, this build-up of RNA closely parallels the differentiation of an elaborate endoplasmic reticulum which by later embryonic stages takes the form of a tight "whorl arrangement" of ribosome-laden membranes and cisternae localized largely in the basal cytoplasm.

In the differentiation of the endoplasmic reticulum (ER), smooth vesicles appear to form in close association with the nuclear membrane. These vesicles apparently fuse progressively to form a typical pattern of ER cisternae. Ribosomes become associated with the developing cisternae, giving the endoplasmic reticulum the classical "rough" appearance. Though the principal concentration of ER elements is localized in the basal cytoplasm, a looser network of ER tubules extends into the apical cytoplasm. The tubules of this network appear to become continuous with elements of a profuse Golgi complex.

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JOHN R. COLEMAN, ANNETTE W. COLEMAN, and HARRY ROY, Brown University.

Myosin-containing mononuclear cells in myogenic cell cultures from chicken embryo leg muscle.

Stockdale and Holtzer (Exp. Cell Res. 24:508, 1961) have described myosin-containing mononuclear cells in chicken embryo somites, but did not report them in myogenic cell cultures. Using histochemical procedures, Roy (Am. Zool. 5.213, 1965) found rare mononuclear cells exhibiting myosin-like ATPase activity in monolayer cultures (Konigsberg, Circulation 24:447, 1961) grown on coverslips. We have employed immunofluorescence to determine (a) if mononuclear myosin-containing cells are indeed present in our cultures as suggested by the histochemical analysis, (b) whether they can synthesize DNA and divide, and (c) whether they are contributed by the inoculum or are produced from cells proliferating *in vitro*. The indirect staining technique was used as follows: cultures grown on collagen-coated coverslips or slides were fixed in ice-cold acetone, rinsed with buffered saline, exposed to rabbit antiserum against chick skeletal muscle myosin (Baril *et al.*, J. Biol. Chem. 241:822, 1966) or to control serum, rinsed again with saline and exposed to fluorescent goat anti-rabbit gamma globulin. Specificity of the antisera was shown by intense staining of A-bands in contractile muscle fibers from 7-day cultures, very light staining of most mononuclear cells, and very light staining when control rabbit serum or saline was used instead of antiserum.

Rare mononuclear cells are present which are brightly fluorescent after antimyosin treatment. After a 30-minute incubation with ^{3}H-thymidine, followed by fluorescent staining and autoradiography, 30% of the non-fluorescent mononuclear cells in a 3-day culture exhibit nuclear grains, but no grains are found over brightly fluorescent cells or over nuclei in syncytia. Exposure to labeled thymidine for 24 hours or more results in grains over the nuclei both of fluorescent mononuclear cells and of syncytia. Thus, mononuclear cells which react with antimyosin sera do not make DNA, but are produced from cells which have made DNA *in vitro*. Their role in myogenesis is currently being studied both in monolayer and clonal culture. (Supported by Grant No. HD-00047 from NIH.)

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WILLIAM H. TELFER, University of Pennsylvania.

Trypan blue uptake and post-pinocytotic growth in the Cecropia moth oocyte.

The validity of trypan blue uptake as an indicator of pinocytotic yolk formation by insect oocytes (Ramamurty, Exp. Cell Res., 33, 1964) was confirmed in the Cecropia moth. After injection into the blood, the dye was concentrated in two cell types, oocytes and pericardial cells, both of which are known to be pinocytotic. In the oocyte, the dye was concentrated only in the yolk spheres being produced in the cortex.

An unexpected finding was that the largest oocytes incorporating trypan blue were only two-thirds the volume of mature eggs. This suggested an extensive terminal phase of oocyte growth not entailing pinocytosis. The suggestion was confirmed by the finding that the amount of blood-derived yolk proteins in extracts of the largest trypan blue stained oocytes nearly equaled that extractable from mature eggs. While the timing of the cessation of other components of yolk deposition is not yet certain, present indications are that the post-pinocytotic volume increase is due primarily to fluid uptake, while synthesis, along with pinocytosis, is greatly suppressed.

The cessation of pinocytosis is the first of a series of steps in converting the oocyte cortex from vitellogenic to embryogenic functions. At a later time basophilic cytoplasm emerges from the yolk mass to form a ten to twenty micron thick stratum underlying the remnants of the pinocytotic cortex. This forms the bulk of the "periplasm" that will ultimately be invaded by cleavage nuclei. (Supported by Phi Beta Psi Sorority and NSF Grant GB 4463.)

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ERWIN GOLDBERT, JOAN M. WHITTEN, and LAWRENCE I. GILBERT, Northwestern University.

Changes in soluble protein during development of *Sarcophaga bullata* foot pads.

Giant cells, two in each foot pad, were discovered by Whitten (1964) in the developing feet of Diptera Cyclorrhapha. These epidermal cells contain polytene chromosomes which clearly show sequential puffing activity associated with development and appear to be ideally suited for studies on the biochemistry of differentiation. The present report is concerned with the qualitative analysis of soluble proteins appearing in these foot pad cells during the 12 days of development from the cryptocephalic pupal stage to the day of emergence.

Soluble proteins, extracted by homogenization of pupal foot pad cells in 0.05 M phosphate buffer (pH 7.0), were resolved by disc electrophoresis on polyacrylamide gels. There was a qualitative increase in protein components up to six days of development followed by a decrease. On day 6, the most complex protein pattern was observed with at least 15 protein species detectable. At this stage the chromosomes show a changing and striking pattern of puffing activity, and dramatic changes occur in the ultrastructure of the cells.

The most dramatic molecular change in this system concerned a single protein component. This protein, representing 30-40% of the total soluble protein in the cell, with a relative mobility on disc gels of 0.4-0.5 (cf. origin to front = 1.0) was first detectable at 6 hrs of pupal development. It then increased in amount through day 5 and abruptly disappeared at about 6½ days of development. These changes are associated with the onset of functional activity in this cell type; i.e., initiation and continued sequential secretion of the cuticular layers. The possible physiological significance of these data will be discussed. (Supported by grants from NSF and NIH.)

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ROBERT GILMORE McKINNELL, Newcomb College of Tulane University.

Renal tumors obtained from pre-breeding Minnesota lake frogs.

Spontaneous renal tumors of *Rana pipiens* are of considerable interest because of their possible viral etiology, high incidence, and wide geographic distribution. Little is known about the natural history of tumorous frogs because most laboratories purchase their animals from commercial dealers. The investigator is thus deprived of precise knowledge concerning the geographic origin, habitat, and biological condition of the animal at capture.

Studies made of collected and purchased frogs over a period of three years suggest a seasonal fluctuation in the incidence of renal tumors. Few tumors are found in summer populations of frogs in contrast to the abundance of afflicted animals during the cold months. Minnesota was selected as a collecting area because of a renal tumor incidence of about 8.5% reported by McKinnell (Ann. N. Y. Acad. Sci., 126:85, 1965).

It was the purpose of the present study to examine renal tumors fixed immediately after frogs were captured from their natural habitat. Tumor microscopic anatomy might thus be related to conditions of season, temperature, and other ecological factors.

Frogs were collected in Minnesota prior to their departure from the lakes for Spring breeding during the first half of April 1966. The collected animals were autopsied within a few hours of their capture. Tumors varied in size and histology. They were composed of multi-layered epithelium which formed tubules containing much cellular detritus. Many nuclei were characterized by clumped chromatin. The tumors resemble those obtained from cold-maintained laboratory *R. pipiens*. (Supported by Grant E-369 from the American Cancer Society, Inc.)

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DAVID W. DUNHAM, K. KORTMULDER, and J. J. A. VAN IERSEL, University of Leiden.

Agonistic displays in the two spot barb.

Study of agonistic encounters between male *Barbus ticto* in aquaria shows that both threat and appeasement displays lower the probability of the displaying fish being attacked. Reaction to the displays indicates that this common function is achieved by different means: threat appears to inhibit attack by the reactor, whereas appeasement evokes a "loss of interest" in the displaying animal. (Supported by USPHS fellowship 5FZMH-17,487-02.)

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BENJAMIN D. SACHS, University of California, Berkeley.

Sexual-aggressive interactions among pairs of quail (*Coturnix coturnix japonica*).

Eight male quail, known to copulate vigorously and with short latency when presented with receptive females, were paired in 49 two-minute encounters in an incomplete round-robin. Both males generally approached and attempted to grab each other with their beaks at the back of the head or neck. Usually within 15 seconds, one of the pair (B) broke off contact and ran from the other male (A) for the duration of the test. In only two of twelve retests was there a reversal of the original outcome. The results of the 49 encounters yielded a straight-line "peck order."

The behavior in these "aggressive" encounters is very close to that observed at the beginning of male-female pairings. Indeed, in some of the male-male pairings the B male actually was mounted by the A male, who then treaded and depressed his tail as if mounted on a female; this behavior occurred only if the B male stopped and crouched. Apparently the approach-peck-grab behavior pattern, which is known to be androgen-dependent (Beach, F. A., & Inman, N., Proc. Nat. Acad. Sci., 54:1426, 1965; Selinger, H., & Bermant, G., Behaviour, In press), can serve both aggressive and sexual functions, depending on the behavioral response of the partner. The behavior may be related to quail's territoriality, mating system and minimal sexual dimorphism. (Supported by NIMH Predoctoral Fellowship MH-21,586 and by NIMH Grant MH-04000 to Frank A. Beach.)

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JAMES A. LLOYD, Albert Einstein Medical Center, Philadelphia.

Relationship of social structure to physiological response in a freely-growing population of house mice (*Mus musculus*).

A population of house mice (*Mus musculus*) was established by placing two sibling females with a sibling male into a 19 cubic-foot cage. Food, water and nesting materials were provided *ad libitum*. The population was censused every two weeks and daily records of births and deaths were made. The population was maintained on alternating white and red light with the red light on between 6 a.m. and 6 p.m. daily. Observations of the behavior of individually marked mice were made during 20-minute intervals between 9 a.m. and 6 p.m. Data recorded included distances moved, areas where individuals were observed, aggressive encounters and sexual activity. From these data, individuals were assigned positions in the social structure. Individuals maintained definite positions in the social structure, with some of the individuals' positions changing markedly as population density increased. Position in the social structure was reflected in some instances in physiological parameters such as weights of adrenals and testes and in the reproductive performances of individual females. (Supported by grants HD-00096 and GM-10530 from the NIH.)

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CHARLES H. SOUTHWICK and LINDA H. CLARK, Johns Hopkins University.

Aggressive behavior and exploratory activity in fourteen mouse strains.

Male mice of 14 strains were weaned at 21-23 days of age, isolated individually for 3 weeks, and placed together in groups of 4 at 42-44 days of age. All groupings were of the same strain. During and after one week of grouping, data were obtained on agonistic, grooming, defecation, and exploratory behavior, operant activity, social organization, and physical condition. Data were tested by Duncan's Multiple Range Analysis. Significant interstrain differences were found in all parameters studied. Group aggression scores (chases, attacks, and fights/hour) were: C57Br (82.2), AKR (69.6), CFW (66.4), Balb/c (62.7), C57Bl/6 (49.7), DBA/1 (47.7), C57L (41.5), DBA/2 (40.8), RF (40.2), C3H/He (27.1), SWR (25.9), A/J (14.5), CBA (12.1), and A/He (11.3). Individual openfield exploratory scores (6 in. squares/10 min.) were: C57Br (245.0), RD (237.8), C57L (198.0), C57Bl/6 (182.6), CFW (141.2), AKR (136.8), SWR (122.4), DBA/2 (105.9), DBA/1 (100.5), C3H/He (94.2), CBA (90.0), Balb/c (82.5), A/J (47.1), and A/He (17.2). Individual spontaneous operant scores (bar presses/hour after 24 hours of water deprivation) were: RF (154.2), C57Br (122.4), C57L (116.0), CeH/He (66.5), CFW (62.2), A/He (56.6), DBA/2 (56.1), DBA/1 (42.9), AKR (40.6), Balb/c (38.5), C57Bl/6 (38.4), A/J (32.6), CBA (26.7), and SWR (17.6). Aggressivity and activity were not consistently correlated throughout all strains, but some strains stood out in the tests as aggressive and active (e.g., C57Br, CFW), whereas others were passive and inactive (e.g., A/J, CBA). Balb/c's were aggressive but relatively inactive in exploratory and operant behavior. RF's were nonaggressive, but very active in exploratory and operant responses. (Supported by USPHS Grant HD 00365-03.)

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SUSAN L. DONALDSON, W. C. BLACK, and J. L. ALBRIGHT, Purdue University.

The effects of early feeding and rearing experiences on dominance, aggressive, and submissive behavior in young heifer calves.

Seventeen Holstein and 17 Holstein-Red Danish Crossbred calves were assigned upon birth to one of four feeding-rearing conditions: (1) fed separately-reared together, (2) fed separately-reared separately, (3) fed together-reared together or (4) fed together-reared separately. Three testing situations were conducted with each calf. In test #1, three calves of a similar feeding-rearing condition that had never been together were placed together; in the #2 phase one calf from each feeding-rearing group were placed together, and the #3 trial all 34 subjects were placed together. Dominance, aggressiveness, and submissiveness scores were calculated for each test. An analysis of variance was computed for each score.

The results revealed that the dominance order was significantly ($P<.05$) changed as a function of feeding, rearing, and test conditions. On test #1 calves fed together and reared separately were

most dominant; on tests #2 and #3 those calves fed separately and reared together were most dominant; on test #3 the calves fed together and reared together were most dominant.

There was only one significant ($P<.05$) difference in aggressiveness—Holsteins fed separately were more aggressive than Crossbreds fed separately.

Submissiveness changed significantly ($P<.05$) as a function of feeding, rearing, and test conditions. On test #1 calves both fed and reared separately were least submissive; on tests #2 and #3, those calves fed separately and reared together were least submissive.

Dominance and submissive hierarchies were not complementary. With two exceptions of 12 cases, position in the dominance hierarchy did not predict position in the submission hierarchy. (This research was financed in part from a trust agreement between Purdue University and Normandy Farm, New Augusta, Indiana.)

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JEANNE ALTMANN, STUART A. ALTMANN, and PENELOPE J. BANNISTER, Yerkes Regional Primate Research Center, Atlanta, Ga.

Models of aggression: the bison hierarchy.

Agonistic interactions within groups can be tallied in matrix form, with each individual represented in the rows as "winner," and in the columns as "loser." Cell entries give the frequency of the agonistic encounters among every possible pair of individuals. Such matrices differ from usual contingency tables in that the main diagonal is undefined, thus entailing the use of special mathematical techniques for analysis. Underlying assumptions in such analysis will be made explicit and the analysis will then be applied to data obtained during a study of bison in Waterton National Park, Canada. It will be shown that paired agonistic encounters in these animals cannot be predicted solely on the basis of their relative aggressiveness and submissiveness. The bison interacted most frequently with those closest to them in the hierarchy; the relation between frequency of agonistic interaction and proximity in the hierarchy was linear. (Supported by grants GB 2879, 3903, and 4415 from the NSF.)

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BRUCE K. ALEXANDER, ROBERT O. DODSWORTH, and HARRY F. HARLOW, Wisconsin Regional Primate Center.

The effects of peer deprivation on mother-reared rhesus monkeys.

The hypothesis that social contact with peers is necessary for normal behavioral development in the rhesus monkey (see Harlow & Harlow, Sci. Amer., 1962) was tested. Three groups of mother-reared rhesus monkeys, differing in the amount of peer contact which occurred during the first eight months of life did not differ in most aspects of social behavior. However some affective differences were detected. The peer-deprived monkeys engaged in more aggressive behavior and less affiliative behavior than the controls. The hypothesis must be modified. The more tenable position is that most aspects of social behavior develop normally in young monkeys provided either maternal contact or peer contact. Affective traits are, however, modified by peer privation.

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WILLIAM A. MASON and K. MUROFUSHI, Tulane University.

Interspecific contrasts in temperament: a comparison of three species of macaque monkeys.

Previous reports have described differences in docility or emotional responsiveness among *Macaca mulatta, M. speciosa* and *M. irus,* but conclusions have been based on nonsystematic observations or a few measures obtained in a limited number of situations. The present research employed a variety of measures and a wide range of situations in order to achieve a broader base for the characterization of interspecies contrasts. Thirty adolescent animals balanced as to sex and species served as subjects. Measures were obtained on social behavior in the living cage, on responses to novel stimuli, both social and inanimate, and on adjustment to new situations. High, but not perfect, consistency of interspecies differences was obtained across tests, with *M. irus* and *M. speciosa* occupying extreme positions on most measures. Results suggest that species differences can best be accounted for in terms of three major descriptive dimensions—response output, response variability and temporal change.

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ARISTIDE H. ESSER and THOMAS L. ETTER, Rockland State Hospital.

Automated location recording on a psychiatric ward.

We are developing a relatively simple and inexpensive device for continuously determining and recording the spatial location and body orientation of all persons on our psychiatric research ward (about 30 patients, staff and visitors). Data provided by this device will be used in studies of territoriality and social interaction.

Each person carries a 40 knz oscillator which periodically "broadcasts" a brief signal through an induction coil. His location is automatically computed by an on-line computer from the respective strengths of his signal as received by several fixed induction coils. As such a "fix" depends only on the ratio of several received amplitudes, it is neither affected by variations in transmitting power from one patient to another, nor by the ageing of transmitter components. We find that the low-frequency induction coils provide a signal path which is almost completely unaffected by the presence of large metal objects, other persons, etc.

The various persons transmit in a fixed periodic sequence; a person is identified by his place in the cycle. Each person's transmitter is turned on at its characteristic time by a delay mechanism triggered by a pulse broadcast at the beginning of the cycle from a fixed transmitter; the timing of this pulse is governed by the computer. Thus the fixed receiving and data handling equipment handles the various signals in exactly the same way, except for the assignment of code names on the basis of cycle position. This makes it easy to accommodate changes in the ward population.

(Supported by General Research Support Grant FR 05561-03.)

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J. R. CORTELYOU, D. J. McWHINNIE, and L. LEHRER, De Paul University.

The effect of temperature on parathormone-influenced phosphorus metabolism in amphibians.

Previous studies (Cortelyou, J. R., and Quipse, P., Amer. Zool., 6 (3):344) showed that parathormone (PTH) elicits hypophosphatemia in *R. pipiens*. However, urine phosphorus (Pi) did not increase, but showed a tendency to hypophosphaturia. In this study, it was found that PTH-mobilized P_i accumulated in liver, eye, skin, and muscle. On a protein basis, liver mitochondria incorporated twice as much P^{32} as controls. It is evident that the hypophosphatemia and tendency to hypophosphaturia are accompanied by accelerated rates of P_i accumulation in these organelles. Parallel experiments with cold-acclimated frogs (6-8°C, 3 days) showed that PTH-treatment caused a significant hyperphosphaturia; simultaneously, liver mitochondria incorporated half the P^{32} of those from normal cold-acclimated animals. Thus, the increased urine P_i of cold-stressed, hormone-influenced frogs is reflected by loss of P_i from soft-tissue mitochondria.

Normal cold-acclimated frogs differed from those maintained at 18-22°C by P_i conservation by way of a decrease in renal release and doubled mitochondrial incorporation. These changes are associated with variations in carbohydrate metabolism during cold stress; muscle and liver glycogen increased significantly. Increased P^{32} incorporation may represent an elevation of base triphosphates (Rossiter, R. J., and Nicholls, D., Rev. Can. Biol., 16:249), which could contribute via UDP-glucose to the increased glycogen levels. Since PTH-treatment of cold-acclimated frogs induced hyperphosphaturia and decreased intracellular P_i, it is clear that hormone reverses the phosphate-sparing response to cold stress. These results may provide a rationale for the degeneration of amphibian parathyroid glands in winter, and the apparent discrepancy between heterotherms and homoiotherms with respect to PTH-induced changes in phosphorus distribution. (Supported by Grants G-13403 and GB-1485 from the NSF.)

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IRWIN PESETSKY, Albert Einstein College of Medicine.

Developmental arrest in the central nervous system of thyroidectomized *Rana pipiens* larvae.

Brain development is generally retarded in vertebrates rendered hypothyroid early in life. In addition to histological and chemical deficits demonstrable in adults, gross deficiencies in brain size are evident, and relative proportions of the organ tend to resemble those of the newborn. Such sequelae of the hypothyroid state are frequently designated as *indirect* effects on brain. It was recently suggested (Eayrs, Proc. 2nd Intern'l. Congress Endocrinol., 1965) that in the absence of thyroid hormone, cranial cavity size fails to keep pace with brain growth and that consequent increases in intracranial pressure inhibit neural development.

Current investigations into endocrine influences on neurogenesis in *Rana pipiens* include experiments with head-grafts and with tadpoles thyroidectomized in embryonic stages. In grafts which become hydrocephalic, neural tissues are obviously compressed. Thyroidless larvae, however, display no evidence of unusual intracranial pressure, and their cranial cavities are essentially similar in size to those of controls ($p > .2$). Nevertheless, representative cross-sections through brains of thyroidless animals are consistently smaller in size than those of controls ($p < .001$). The administration of thyroxin (333 μg/l water) permits the brain to attain normal size. Such recovery occurs in larvae as old as 7 months and is unlike the situation in mammals where replacement therapy must be instituted during temporally restricted "critical" developmental periods. Observations indicating that brain growth is directly influenced by thyroid hormone will be discussed. It is suggested that hypothyroid retardation of brain development is attributable to thyroid-hormone-dependence of developing neural and neuroglial elements. (Supported by Grant NB 04555 from the USPHS.)

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DAVID B. KING and JAMES G. SNEDECOR, Franklin and Marshall College and the University of Massachusetts.

Effect of TSH on the liver glycogen level of the hypophysectomized chick.

Hypertrophy and enhanced glycogen content are characteristic of the liver of the chick following radiothyroidectomy or goitrogen administration. Evidence is presented that suggests the liver changes result not from the lack of thyroid hormone alone, but from the concomitant extrathyroidal effect of TSH. Three- to four-week-old hypophysectomized cockerels injected with propylthiouracil to block thyroid hormone formation did not have enlarged livers although the glycogen values were intermediate between non-operated controls and similar controls injected with PTU. The administration of 2 USP units of thyrotropin per day for 5 to 7 days to PTU-treated hypophysectomized chicks resulted in liver parameters similar to the PTU-injected non-operated controls. If approximately ¼ the normal daily secretion rate of thyroxin were administered to PTU-treated hypophysectomized chicks in addition to TSH, liver weight and glycogen content were normal; thus the TSH effect on the liver occurred only when thyroid hormone was very low or absent. Significant comb growth occurred in TSH-treated hypophysectomized chicks indicating some contamination of the TSH with gonadotropin. To investigate the possibility that endogenous androgen might be involved in the liver response to TSH, hypophysectomized, castrated cockerels were treated with PTU and TSH. The liver responses were similar to non-operated PTU-treated control chicks, which produce very little androgen. The data support the conclusion that TSH in the absence of thyroid hormone is responsible, at least in part, for the liver hypertrophy and liver glycogen increase in the hypothyroid chick. (Supported by Grant AM 01266 from the USPHS.)

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RICHARD L. SWALLOW and WARREN R. FLEMING, University of Missouri.

Effect of starvation, ACTH, and glucose injections on liver glycogen levels of *Tilapia mossambica*.

The effect of ACTH on liver glycogen, muscle glycogen, glucose-6-phosphate phosphatase activity, and liver weight/body weight ratios has been studied. Well-fed animals, animals starved for a minimum of 12 days at 33°C, and animals starved for intermittent periods were used. ACTH did not affect the liver wgt/body wgt ratios of well-fed animals or of animals starved for 12 or more days. Between these two periods, ACTH (0.4 IU/day) injected fish had significantly higher liver wgt/body wgt ratios than did their controls. ACTH did not, however, cause a net synthesis of glycogen for no changes in glycogen, expressed as per cent of liver weight, were observed.

Neither ACTH nor starvation affected glucose-6-phosphate phosphatase activity.

A marked net synthesis of liver glycogen may be observed following a single injection of glucose (15 mg) into a starved control animal. Starved animals given chronic injections of ACTH showed little or no net glycogen synthesis after treatment with glucose. Preliminary experiments using C^{14} labelled glucose show that ACTH does not block the metabolism of glycogen, for C^{14} glucose is incorporated into liver glycogen rapidly. The relative rates of glycogen formation and utilization are being studied.

To date, we have not observed any effect of ACTH on muscle glycogen. (Supported by NSF Grant GB 2264 and by a NASA fellowship to the senior author.)

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JACK E. YOUNG and WALTER CHAVIN, Wayne State University.

Histopathology of the islets of Langerhans, adrenals, liver and kidney of control and alloxan diabetic goldfish, *Carassius auratus* L.

The sequential histopathology of organs related to carbohydrate regulation were studied in alloxan diabetic goldfish and compared with control and normal fish. 240 goldfish (1.5-4 g) maintained under constant conditions (25°C, 12-hour photoperiod, diet) were untreated, or injected IP with 0.58% saline or alloxan (600 mg/k) and sacrificed 1 minute to 12 days post-injection. Histological examination of liver glycogen (PAS) and serially sectioned fish (AF-trichrome-hematoxylin) was made.

Pancreatic beta cells degranulated maximally 15 minutes post-saline, returning to normal at 2 hours. Alloxan produced beta cell degranulation (1 minute) followed by hydropic degeneration and necrosis (2-4 hours). Islet recuperation began at 8-12 hours.

Adrenal cortical hypertrophy occurred 1 minute to 2 hours post-saline. Chromaffin cells were initially shrunken, then hypertrophied (15 minutes) but normal thereafter (30 minutes). Following alloxan, cortical cell hypertrophy and vacuolation appeared (1-5 minutes). Shrunken chromaffin cells (1 minute) preceded hypertrophy (5 minutes). Both cell types gradually returned toward normal.

Maximal liver glycogen depletion occurred 15 minutes post-saline or alloxan. Alloxan produced degenerative foci (4 hours) and massive liver necrosis (12 hours). Necrotic areas were restricted by 12 days.

Cellular edema of kidney tubules (90 minutes) led to progressive tubular necrosis (2-12 hours) only after alloxan. Recuperation beginning at 8 hours continued to 12 days.

Thus, alloxan produces transient beta cell necrosis and both accelerates and intensifies the responses found in control fish. This extra-islet cytotoxic action of alloxan may affect serum glucose levels. (Supported by Grant AM-04876-05 from the USPHS.)

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MELVIN WEISBART and JAMES C. FENWICK, University of British Columbia, Vancouver.

Effect of pinealectomy on osmotic and ionic regulation in the goldfish, *Carassius auratus*. (Introduced by W. S. Hoar)

In view of the current controversy concerning the role of the pineal 'organ' in osmotic and ionic regulation, a study was made to determine the effect of pinealectomy on the osmoregulatory ability of the goldfish. From the literature, it was hypothesized that extirpation of the pineal would result in the loss of an aldosteronotropic factor or the removal of a pituitary antagonist, or both, with the result of a net increase in plasma concentration. From Pflugfelder's work (Wilkelm Roux' Arch. Entwicklungsmech. Organ. 146:115-136, 1953) a change in the concentration of plasma calcium was also expected.

Pinealectomized, sham operated and unoperated goldfish were held in brackish water (14‰) for a period of 23 days at room temperature (20-23°C) and under normal summer photoperiod (16 hr light-8 hr darkness). Measurements of Na, Cl, Ca and total osmotic concentration revealed no significant differences among the 3 groups of animals. However females showed a consistently higher value of plasma Ca than males ($P<.10$). No differences were observed in the hematocrits nor in the amount of melanization at the end of the experiment, although there was a suggestion of greater melanization earlier in the normal and sham operated animals. (Supported by NRC grants-in-aid of research to Dr. W. S. Hoar.)

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DONALD M. MEISMER and WENDELL W. LEAVITT, University of Cincinnati.

Further studies on the action of steroids and triphenylethylene derivatives on the neonatal rat.

Earlier work had shown that neonatal administration of a triphenylethylene derivative, clomiphene citrate (MRL-41), altered the pituitary-gonadal relationship in the female rat (Leavitt and Kanerva, Am. Zool. 6:345, 1966). A continuation of these studies has revealed a similarity in the mechanism of action of estrogen and clomiphene on the 5-day-old rat.

Female, 5-day-old, CD rats were injected in groups of 10 or more with clomiphene (100 or

1000 μg), MRL-37 (1000 μg) or estradiol (1000 μg). Controls were given either the vehicle or no injection. Vaginal smears were taken daily after introitus, and the animals were sacrificed at 100 days of age.

A significant reduction in ovarian weight occurred in animals treated with estradiol and both levels of clomiphene. Ovaries were entirely follicular, estrous cycles were absent and vaginal smears were predominantly of the proestrous type. Ovaries were normal in size and appearance in the MRL-37 group, but vaginal smears revealed abnormal estrous cycles in these animals. Uterine weight was significantly lower after clomiphene, MRL-37 or estradiol.

It is well-known that a persistent anovulatory "estrous" condition develops when appropriate doses of various steroids are given during the neonatal period. It is now evident that the non-steroidal compound, clomiphene, has a similar activity. Of interest is a close structural analog, MRL-37, which did not produce the anovulatory condition but did alter the pituitary ovarian axis when injected at day 5. Work is in progress on clomiphene isomers and other non-steroidal estrogens. (Supported by grant HD01568 from the USPHS.)

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LARY V. DAVIS, University of Hawaii.

Inhibition of growth in *Hydra* by methylglyoxal.

Studies by Freedlander and French (Cancer Res., 18:1286, 1958) have demonstrated that several low-molecular-weight α-ketoaldehydes possess a marked carcinostatic activity. More recently, it has been shown that these compounds inhibit mitotic activity, and it has been suggested that one (or more) of these ketoaldehydes may be an important growth regulator (Szent-Györgyi, Science, 149:34, 1965).

The present investigation shows that the α-ketoaldehyde, methylglyoxal, when added to culture media at concentrations of 10^{-3} to 10^{-4} *M*, will inhibit both distal regeneration and asexual budding in *Hydra littoralis, H. pirardi,* and *Chlorohydra viridissima.*

In addition, the presence of methylglyoxal in the tissues of these organisms was established indirectly, by manometrically determining the total glyoxalase activity of tissue extracts in the presence of added methylglyoxal, according to the methods of Platt and Schroeder (J. Biol. Chem., 104:281, 1934).

It is suggested that this compound, or perhaps some derivative of it, also may regulate mitotic activity and hence contribute to the over-all control of morphogenesis in hydroid polyps. (Supported by an institutional grant from the American Cancer Society.)

260

R. BLANQUET, Duke University and the University of Miami.

Properties of the acontia nematocysts and toxin of the sea anemone *Aiptasia pallida.* (Introduced by H. Lenhoff)

Large quantities of undischarged nematocysts were rapidly obtained by placing acontia threads in 1.0 molar sodium citrate. This solution caused massive extrusion of nematocysts from the acontia and the suspended nematocysts were collected by centrifugation. Using isolated, pure nematocyst suspensions, it was possible to observe discharge in response to various stimuli and compare these results to discharge *in situ.*

Isloated nematocysts were comparatively insensitive to stimuli which normally caused their discharge when situated in the acontia. This was true for alcohols such as butanol or isopentatol solutions of low pH or high potassium ion concentration. Discharge *in situ* could be largely or completely abolished by pretreatment of the acontia with magnesium chloride. This inhibition was reversible. Isolated nematocysts placed in sea water or approximately isosmotic salt solutions showed little discharge. However, increasing dilution of these solutions with distilled water caused a progressive increase in discharge. Divalent cations, particularly calcium, caused a marked inhibition of this effect. Calcium was also effective in reducing discharge due to increasing temperature.

The toxicity of the nematocyst contents was determined by bioassay using the fiddler crab *Uca pugilator.* A protein, possessing a paralytic activity was isolated using electrophoresis and Sephadex thin layer chromatography. This protein had a molecular weight of about 130,000 and was stable to drying. Activity was destroyed by boiling, organic solvents such as ether, chloroform or acetone, and enzymatic digestion by trypsin. Compounds such as quaternary amines or hydroxy-indoles were not detected in the toxin. (Supported through a Physiology Traineeship at Duke University.)

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ROSEVELT L. PARDY, University of Arizona.

The use of disc acrylamide electrophoresis in the study of enzymes of sea anemones. (Introduced by P. E. Pickens)

By the technique of disc electrophoresis esterases and acid phosphatases have been demonstrated in sea anemone muscles.

Retractor and columnar muscles were removed from the sea anemones and homogenized in cold sucrose. Samples of the homogenate were incorporated into a polyacrylamide sample gel. The samples were run for 70 minutes in a linear electrophoresis apparatus at pH 8.3.

Esterases were detected in the sample gels by the azo dye technique using alpha-napthol acetate as a substrate and fast blue B salt as an indicator. Acid phosphatases were likewise detected using alpha-napthyl phosphate for the substrate.

The esterase and acid phosphatase patterns of two populations of *Phyllactis concinnata,* a burrowing sea anemone, were compared as were the patterns of three other species. Preliminary results indicate that the enzyme patterns elucidated by disc electrophoresis may be used to distinguish between different populations of the same species and may also show relationships between different species. (Supported by Grant GB-3745.)

262

WILLARD D. HARTMAN and THOMAS F. GOREAU, Yale University, New York Zoological

Society and University of the West Indies, Kingston, Jamaica.

Ceratoporella, a living sponge with stromatoporoid affinities.

The rediscovery in Jamaica of *Ceratoporella nicholsonii* (Hickson), first described as a coenothecalian octocoral but now shown to be a sponge, has reopened the question of the relationships of an enigmatic group of fossils, the stromatoporoids. *Ceratoporella* has a skeleton composed of siliceous spicules, a network of fibrous protein and a solid basal mass or aragonite intrinsic to the sponge and enclosing the first two skeletal elements. The living tissue, supported by siliceous spicules joined by a network of fibers, forms a veneer, 1 mm thick, over the surface of the aragonitic mass. Among the cells present may be recognized choanocytes arranged in chambers. Water enters the animal by way of surface pores and leaves by oscules fed by stellate patterns of raised exhalant channels as in many encrusting sponges of the class Demospongiae.

The skeleton of *Ceratoporella* bears a striking resemblance to that of stromatoporoids. The exhalant channels of *Ceratoporella* sometimes leave imprints of their form in the calcareous basal skeleton; these patterns are reminiscent of the astrorhizae of stromatoporoids. Spicules have been described in at least two species of Paleozoic stromatoporoids. The siliceous spicules of *Ceratoporella*, after becoming enclosed in aragonite, slowly dissolve away in the deeper parts of the matrix. A comparable process may explain the absence of spicules from most fossil stromatoporoids. The microstructure of the calcareous skeleton of *Ceratoporella* shows a fasciculated fibrous pattern similar to that of certain Mesozoic stromatoporoids.

These facts suggest to us that the fossil stromatoporoids (or at least some species of what may be a polyphyletic assemblage) constitute a new class of the phylum Porifera with an ancestry that at one time in the history of the phylum had common ties with the class Demospongiae. *Ceratoporella* is a surviving representative of this ancient class. (Aided by ONR contract NR 104-854 and by NSF grant GB-516 and GB-3542.)

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DAVID A. EGLOFF, Hopkins Marine Station, Stanford University.

Commensalism and parasitism in the *Thyca-Linckia* association. (Introduced by D. P. Abbott)

One to five *Thyca crystallina* Gould (Mollusca: Gastropoda) were found attached to 14% of the *Linckia laevigata* Linnaeus (Echinodermata: Asteroidea) collected in Fiji. The snails are located proximately on the ventrolateral side of a sea star's arm and to the right of the ambulacral groove when viewed from the sea star's oral side. The female *Thyca* is endoparasitic on its host; its proboscis penetrates into the region of the ambulacral ridge and into the perivisceral coelom in some specimens. These findings will be discussed in relation to the possible sources of nutrients in the ambulacral ridge of the sea star.

The commensal *Thyca* males are located on the right dorsal side of the female's head and concealed beneath her shell. The male's proboscis leads ventrally to the anterior dorsal side of the female's attachment disc. Although the proboscis is attached to the anterior margin of the disc, it is open at the distal end. As a consequence, the male may remove food from the female's respiratory currents, which are strong in this region.

The sexual dimorphism and the absence of males on the smaller females suggests protandric hermaphroditism. However, the absence of intermediate forms in transition from male to female and the dissimilarity of the larval shells is evidence that this species is dioecious. (Supported by Grant G-17465 from the NSF.)

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C. W. HART, JR. and DARNEY G. HART, Academy of Natural Sciences of Philadelphia.

New Australian entocytherid ostracods and their relation to North American species.

A new subfamily of the commensal ostracod family Entocytheridae has been proposed to include twenty-four new species, representing six new genera, found living on some seven crayfish species and an isopod in Australia. Details of their known distribution in Australia, and their relation to North American species will be discussed. This study is based on approximately 400 specimens collected by and made available to us by Dr. E. F. Riek (C.S.I.R.O.). (Supported by Grant GB-4197 from the NSF to the senior author.)

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EDWARD TILLINGHAST, Duke University.

Studies on the transition from ammonotelism to ureotelism in the earthworm *Lumbricus*. (Introduced by K. M. Wilbur)

Feeding *Lumbricus* is ammonotelic but becomes ureotelic with fasting. The quantity of ammonia and urea excreted, as well as the time of transition to ureotelism while fasting was shown to be strongly dependent upon the temperature and water volume in which worms were immersed. A rapid elevation in urea output and decline in ammonia production while fasting at 23°C led to a transition to ureotelism in 2 to 3 days. This transition did not occur until a week or more at 8°C, nor was the maximal urea output as high as that at 23°C. Significantly larger quantities of ammonia were excreted initially by worms in 5.0 ml of water compared to those in 1.0 ml of water, but the urea output was not affected. Worms completely immersed in water excreted significantly less urea than those in 1.0 to 5.0 ml at 23°C.

The posterior halves of bisected worms, feeding prior to amputation, increased their urea output, contrary to the existing view that the anterior half controls the urea output. Nevertheless, a significant difference in the ratio of urea to ammonia excreted was observed, with anterior halves excreting more urea and less ammonia than posterior halves.

It was demonstrated that while urea is voided by the nephridia, ammonia is voided via the gut. The distribution of ammonia in the gut in turn was shown to account in part for the foregoing observation that the posterior halves of bisected worms void larger quantities of this waste than isolated anterior halves. (Supported by Grant 81-1184 from NIH.)

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AARON M. TAUB and WILLIAM A. DUNSON, Pennsylvania State University.

A new gland in sea snakes, (Squamata, Reptilia).

A salt secreting gland has not previously been found in the marine sea snakes (Hydrophiidae). In the course of physiological studies on the sodium metabolism of *Laticauda semifasciata,* significant extrarenal sodium excretion was demonstrated.

After salt-loading, an attempt was made to collect fluid from the nasal vestibule. This was unsuccessful. Concentrated salt solutions were collected from the anterior portion of the mouth, between the mental and rostral scales. Upon dissection, a median tubuolo-acinous gland was found in the inter-palatine groove. The gland lies in the soft tissues underlying the premaxilla and is anterior and medial to Jacobsons organ. It lies anterior to the internal choanae. The gland appears to empty into the inter-palatine space via paired ducts. The secretions are then pushed out of the mouth by the movement of the tongue.

No gland in this region has been previously described for any ophidian (Taub, J. Morph. 118: 529-542. 1966). The gland may be a homologue of the *glandula vomeris* which Farhenholz (1937, In Bolk, *et al.,* eds. Handbuch der Vergleichenden Anatomie der Werbeltiere, Vol. III, pp. 147-152) reports as being absent in snakes. In view of the uncertainty of homology and the apparent demonstration of extrarenal salt secretion (Dunson and Taub, Amer. Zool., 1966, and in preparation). this gland is named the natrial gland (*natrium* Latin, sodium). Dissections of *Pelamis platurus* also revealed a well developed gland in this position.

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CHARLES A. REED, University of Illinois, Chicago, and WILLIAM SCHAFFER, Yale University.

Evolutionary implications of cranial morphology in the sheep and goats (Caprini, Simpson 1945).

Within the Tribe Caprini evolution appears to have taken two major directions: first, there has been selection for greater climbing ability; and second, the horns and related parts of the skulls of the males have become particularly specialized. The Caprini are thus distinguished from their more primitive allies by the following characters: increased massiveness of the horns; hypertrophy of the frontal bones posterior to the supraorbital foramina; reduction of the parietals; heightening of the skull as measured from the plane of the vertex to the foramen magnum; a sharp downbending of the frontals between the bases of the horn cores; and the bending of the basicranial axis in several places with resultant ventrad rotation of the posterior parts of the braincase. Additionally, the air sinuses within the frontal bones and horn cores are enlarged and complicated by numerous bony struts.

The structural details in different caprine groups appear to be correlated with the intensity of the contests between males, which form an important part of the rutting behavior. Aside from the obvious enlargement of the weapons employed (the horns), downbending of the braincase prevents violent rotation of the head and hence of brain injuries of the whip-lash variety at the time of impact. Furthermore, the expanded frontal sinuses, with their crenulated bony cross-struts, probably attenuate compression of the brain case and concomitant damage to the medulla. Experiments are planned to clarify not only the nature of these adaptations basic to the group, but also that of the differences among the various genera, which are quite consistent and no doubt also of functional significance. (Supported by NSF grant GE6141 for Undergraduates, to the Department of Biology, Yale University.)

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HERBERT I. ROSENBERG, State University of New York at Buffalo.

Hemipenial anatomy of some amphisbaenids (Amphisbaenia, Reptilia). (Introduced by Carl Gans)

The amphisbaenids, a group of specialized squamates provisionally placed with the Sauria, generally have cylindrical, bifurcate hemipenes.

In section, the hemipenis is composed of several, more or less, concentric structures. A peripheral sinus forms part of the outer wall. Enclosed by this composite system are the fibers of the retractor muscle which are arranged around a central sinus. A third sinus system fills the lips of the *sulcus spermaticus.*

It is interesting to note that South American and Antillean amphisbaenids have smooth unornamented hemipenes, while the African genus *Chirindia* has hemipenes covered with minute epidermal papillae. All sinuses comprising the hydrostatic system of the hemipenes are filled with erythrocytes and no separate lymph spaces are seen. The complex hemipenis of the African species *Chirindia rondoensis* is unique in that only half of it contains fibers of the retractor muscle.

Analysis of thirteen species indicates four distinct patterns: 1) Antillean forms exhibit relatively simple, bilobed hemipenes; 2) South American forms, classically assigned to different subfamilies, have bilobed hemipenes with tips that are subdivided into distinct lamellae; 3) The specialized South American genus *Anops* shows two elongate processes projecting from the bases of the terminal lobes of the hemipenes; 4) A fourth, drastically distinct, pattern is seen in two species of *Chirindia.* Here the hemipenes are morphologically complex being either tri- or quadrifurcate.

Some of the patterns seen in this inadequate paradigm remind of lizards, some of snakes, supporting the concept of an independent status of the amphisbaenids, but until more material becomes available it is impossible to characterize an "amphisbaenid" pattern. (Supported by Grant GB-2460 from the NSF to C. Gans.)

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KEITH STEWART THOMSON, Yale University.

Mobility of the skull and fins in the coelacanth (*Latimeria* chalumnae Smith). (Motion picture)

A specimen of the coelacanth fish that had been preserved by freezing immediately after capture (rather than formalin fixation) has provided an opportunity to study the range of mobility of

the intracranial mechanism of the skull and the range of possible movements of the fins. Movements produced by manual operation of the various structures were analyzed through the use of still and motion picture records. It was ascertained that the anterior division of the skull could be moved through an angle of approximately 15 degrees relative to the posterior division (see also Thomson, K. S., Science, 153:999, 1966). The pectoral fins could be rotated through a total of almost 300 degrees (approx. 200 degrees forwards and 100 degrees backwards). The pelvic fins could be brought up vertically so that the dorsal surface lay against the flank of the fish and could also be rotated through 90 degrees in either direction. The first dorsal fin could not be moved laterally to any great extent, but the tip of the second dorsal could be brought forward on either side through an arc of more than 90 degrees. The anal fin could be rotated through about 90 degrees. The mobility of the intracranial joint and the fins provides a useful insight into the mode of action of homologous structures in the rhipidistian crossopterygian ancestors of the tetrapods. (Supported in part by Grant B-4814 from the NSF.)

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HERBERT R. BARGHUSEN and THOMAS S. PARSONS, Loyola University and University of Toronto.

Variation in the skull of *Trionyx*.

This report presents the initial results of a study of intra- and interspecific variation in linear skull measurements within the turtles of the genus *Trionyx* from both a taxonomic and a functional point of view. The most striking variation is in the width of the maxillary alveolar surfaces. Although historically this has been used to distinguish taxa even at the generic level, it is now considered unreliable because within many species, as defined by other criteria, this character varies greatly. Since such variation indicates possible differences in diet and feeding methods within the species concerned, we are attempting to rigorously define the variation and to see if correlations exist with other skull variables involved in the feeding apparatus. Further, there are differences between certain species in the relative width of the maxillary alveolar surface which may also be of functional significance. For example, although both *Trionyx cartilagineus* and *T. ferox* sometimes have greatly expanded alveolar surfaces in large specimens, other *T. ferox*, including all small specimens, have narrower surfaces relative to basicranial length than those of any *T. cartilagineus*.

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WALTER J. BOCK, Columbia University.

Mechanics of one- and two-joint muscles.

The mechanics of one-joint and two-joint vertebrate muscle-bone systems under static and dynamic conditions were analyzed with free-body diagrams, using the assumptions that the bones are rigid elements, that the articulations are ideal, frictionless, non-energy storing joints, and that no force is lost through friction at the points of application.

For both one-joint and two-joint muscles under all conditions, the muscle force and the articulation force *do not* constitute a force couple. Earlier statements in the literature to the contrary appear to have resulted from an incomplete analysis of all forces, real and fictitious, acting on the bone.

No simple relationship exists between the direction of rotation of the central bone and the intersect angle of the force vector of a two-joint muscle and of the longitudinal axis of the central bone. The movement of the central bone in a two-joint muscle-bone system cannot be ascertained by inspection of the morphology, but can be determined only after analyzing all forces acting upon the distal bone. A two-joint muscle-bone system is not stable under static conditions; an additional one-joint muscle between the proximal and central bones is required to maintain static conditions.

The misleading notion of "shunt" and "spurt" muscles is incorrect and should be discarded.

In general, the consequences of a muscle within a bone-lever system is not inherent to the muscle, but depends upon all of the forces acting upon the bones. Free-body diagrams provide a simple, direct approach to the analysis of vertebrate muscle-bone systems. (Supported by Grant GB-3802 from NSF.)

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JAMES M. MOULTON, Bowdoin College.

Further studies on the teleost ear: Mid-water and demersal fishes.

The inner ear of a variety of teleost fishes has been studied, the fishes being mid-water and pelagic ones from the deep Carribean and Gulf of Mexico and demersal ones from the west coast of South America. Inner ear morphology lends itself in several ways to an estimate of behavioral possibilities, and in addition can be of taxonomic value in teleost systematics. There is considerably greater variation than commonly recognized.

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GEORGE M. HUGHES, University of Bristol, England and CARL GANS, State University of New York at Buffalo.

Electromyographic analysis of respiratory movements in *Testudo graeca*.

Intrapulmonary pressure and the mechanical movements of the limbs were recorded simultaneously with the electrical activity in a variety of muscles of unrestrained Greek tortoises. The records show that all phases of ventilation are active in this genus, even though it lacks the *M. diaphragmaticus*. It is powered both by the "respiratory" abdominal muscles that attach to the connective tissues closing off the posterior apertures of the shell and by muscles that rotate the pectoral girdle in and out of the anterior aperture.

The shell of *Testudo* is domed and rigid. It lacks hinges. The lungs lie within the carapace above the other viscera. The *M. transversus abdominus* and *M. pectoralis* act to reduce the volume available for the viscera; their contraction raises the pulmonary pressure and induces expiration when the glottis is open. The *M. obliquus abdominis* and *M. serratus major* (= *M. testocoracoideus*) respectively act as direct antagonists to these (as indicated by reciprocal inhibition).

Tortoise respiration consists of periods of apnea of varying lengths (observed range 4 sec to 23 min) interrupted by biphasic (expiration-inspiration) ventilating cycles. The pressure records of the cycles are triphasic (pressure rise, pressure fall to below atmospheric, recompression), but records taken from the muscles that open and close the glottis indicate that the terminal recompression is not a flow event since the glottis closes before the pressure rise.

Differential pressure recordings (front to rear of one lung) gave no evidence for recirculation. The pulmonary pressure also provides an excellent reflection of the cardiac pulse. (Supported by NSF GB2460 and N.E.R.C. grant for equipment.)

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DOUGLAS B. WEBSTER, New York University.

Morphology of the vibrissae of the kangaroo rat.

The most prominent tactile sense organs of *Dipodomys merriami* are the facial vibrissae. These large hairs on the upper lip attain a length of six cm and number between 14 and 18 on each side. There is a continuous gradation in size between large vibrissae and normal pelage hairs on the upper lip. As with pelage hairs there are sometimes two vibrissae sharing a single follicle.

The vibrissae roots are characterized not only by their large size but also by large superior and inferior venous sinuses within the hypertrophied dermal sheath. These sinuses are separated by a prominent ring of connective tissue, the ringwulst. Arterial supply to these sinuses is from the infraorbital and external maxillary arteries with drainage from the corresponding veins.

The external and internal root sheaths contain the same concentric layers of cells found in most mammalian hair. Each follicle is innervated by a substantial branch of the infraorbital nerve. Upon piercing the outer part of the dermal sheath the nerve bifurcates and courses up opposite sides of the hair root. During its course it travels through the connective tissue trabeculae of the inferior venous sinus just outside the glassy layer of the outer root sheath. Individual fibers leave the nerve, pierce the glassy layer and terminate among external root sheath cells. Some fibers continue past the ringwulst and form a group of fibers that encircle the hair between the ringwulst and the sebaceous glands. (Supported by grant #NB 05800 from NINDB.)

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DENZEL E. FERGUSON, HOBART F. LANDRETH, and JAMES P. MCKEOWN, Mississippi State University.

Sun compass orientation of the northern cricket frog, *Acris crepitans*.

Acris crepitans uses celestial cues to maintain a compass course (Y-axis) that is perpendicular to the home shore. To successfully use this mechanism the frog must possess information concerning a familiar shore position, a view of a useful celestial cue, and a clocking mechanism phased to local time.

Cricket frogs kept in extended darkness are dephased to local time and do not score on a Y-axis measured from the home shore. If a light cue is presented in phase with the local sunrise and sunset, after such a period in darkness, the animals again orient to the original Y-axis. The association of time can be rephased by daily fluctuations of temperature and/or humidity, by a rhythmic daily exposure to 10 minutes of light, or by a 12-hr photoperiod from a reduced, indirect light source. A light-dark regime 6 hr out of phase with local time produces a predictable 90° shift in directional responses.

The time required to evolve the degree of adaptive refinement exhibited by *Acris crepitans* and the increasing probability that the Y-axis phenomenon is common to most anurans, suggests a basic mechanism rather than a specialization derived by recent taxa.

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LESTER TALKINGTON, University of Wisconsin, and LOUIS GRAUE, Bowling Green State University.

Helicopter observation of pigeon homing.

Experiments indicate that the small helicopter is both suitable and efficient for the observation of pigeon navigating behavior. Maps of homing flight paths will be shown and the techniques for obtaining them described. Some general observations on the navigating behavior of the birds will be given. (Supported by grant GB5239 from the NSF.)

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LOUIS GRAUE, Bowling Green State University, and LESTER TALKINGTON, University of Wisconsin.

Initial orientation and homing paths.

What is the relationship between the vanishing direction of homing pigeons, observed from ground at release point, and the actual flight paths home, observed from aircraft? Examples will be given. (Supported by Grant 4331 from the ONR and Grant GB5239 from the NSF.)

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KENNETH S. RAWSON, Swarthmore College.

Goal directed orientation in the homing behavior of mice (Genus *Peromyscus*).

Deermice (*Peromyscus maniculatus rufinus*) were released at distances up to 3000 feet from borders of their known home ranges. Individual mice were released several times so that new pathways were traversed in returning to their home ranges. 650 homing releases were made. Selection for homing performance occurred with 66% returning from the first release and 82% and 84% from the second and third releases. Contrary to expectations for random search homing, the percentage of successful returns did not decrease with distance of the release point. Mice released in the center of a uniform array of traps and caught en route showed a statistically significant homeward orientation (1% level by Rayleigh test for uniform distribution) with 78% of the captures in the homeward semicircle. Records of rapid homing and subsequent exploration support the conclusion that these mice exhibit goal directed orientation. (Supported by grants RG-18106 and GB-2635 from the NSF.)

288

JACK P. HAILMAN, Department of Zoology, University of Maryland.

Four color preferences of the Laughing Gull (*Larus atricilla*)

The way in which the visual system encodes chromatic information may be studied not only by physiological means and by behavioral comparisons among species, but also by comparisons among several different color preferences in the same species. The newly-hatched gull chick pecks most frequently at red and blue stimulus objects. When the stimulus object is achromatic, it receives highest pecking rates against green and yellow backgrounds, such that chromatic preferences for stimulus and background are spectral mirror-images of one another.

The adult gull during the nesting season removes from its nest objects such as egg shells. Utilizing metal models painted the same colors as the pecking stimuli, it was found that the red response mode occurred at higher frequencies (shorter wavelengths) than the pecking preference. Using colored wooden egg models set at the edge of the nest, it was found that gulls roll models into the nest according to a color preference shifted still further to higher frequencies.

The spectral preference curves resulting from these studies resemble somewhat the family of spectral response curves recorded by others from single units in the monkey's visual system. (Supported by a USPHS postdoctoral fellowship.)

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A. H. SCHULMAN and E. B. HALE, Pennsylvania State University.

Characteristics of visual stimuli for initial approach response in neonatal chicks.

This study evaluated two theories proposed to account for the stimulus conditions resulting in initial approach in precocial neonates: (1) the stimulus intensity theory, proposed by Schneirla (Nebr. Symp., 1959) and interpreted by Moltz (Psychol. Rev., 70, 1963) to mean that retreating, rather than approaching, objects yield approach and following behavior and (2) the stimulus conspicuousness theory (Bateson, Biol. Rev., 41, 1966; Matthews & Hemmings, Nature, 198, 1963), which maintains that the effectiveness of the initial approach stimulus is correlated with its ability to channel visual attention.

Newly-hatched chicks, kept in dark isolation prior to testing, responded to a red sitmulus disc in an open field. The following results were obtained: (1) initially, newly-hatched chicks moved toward approaching objects rather than followed retreating ones; (2) the effectiveness of an approaching stimulus was positively correlated with its size; (3) newly-hatched chicks approached an oscillating stimulus significantly more often than the same stimulus when stationary; and (4) size and distance modified the effectiveness of an oscillating stimulus. Large oscillating objects were more effective than small ones, and small oscillating objects became more effective at close distances.

The results tend to disprove the assumptions derived from the stimulus intensity theory and lend support to the stimulus conspicuousness theory as it applies to moving objects. Attempts to increase the conspicuousness of stationary objects failed to increase stimulus effectiveness. It was concluded that stimulus motion is the most consistent determinant of stimulus effectiveness and may mask the effects of other qualitative stimulus dimensions.

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PHILIP H. GRAY, ALLEN T. YATES, GEORGE T. DAVIS, and CHARLES J. MODE, Montana State University, Bozeman.

Some aspects of the genetics of imprinting.

A hierarchical experimental design was used to test for genetic aspects of imprinting in the domestic chick. Eight sires were mated to four dams each. Four full-sibs were used from each mating, of which two were given a 20-minute exposure session (E) on the first day of life (1) to a moving green box (GB) and (2) to a moving red cylinder (RC). Discrimination tests (T) between strange and familiar models occurred within 24 hours after the imprinting, or exposure session. This design was repeated on another set of chicks on the fifth day of life (5). Behavior was measured in terms of number of minutes spent within a certain distance of a model minus minutes spent within a certain distance of an empty but corresponding area (E), or minutes within a certain distance of the familiar model minus minutes within an equal distance of the strange model (T).

Using the above symbols, a hierarchical ANOVA yielded significances for Sires ERC1, TGB1, and ERC5, and for Dams within Sires ERC1, TRC5, and TGB5. A complete mixed model ANOVA for exposure to a single model (E) showed that sires, dams within sires, models, and days were all significant. The same analysis of variance for discrimination of familiar from strange model (T) indicated that sires, dams within sires, models and both interactions dealing with days were significant. Estimates of genetic variance components, and factor analysis of the 32 matings, further the conclusion that imprinting is differentially inherited. (Supported by NIMH Grant HD00877.)

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H. B. GRAVES and P. B. SIEGEL, Virginia Polytechnic Institute.

Quantitative inheritance of the approach response of chicks.

Response and approach tendencies toward a distant audio-visual imprinting apparatus were compared in several closely related lines of chickens from the American class. At 24 $\pm$ 1 hours post-hatching there were significant differences among lines for times to respond and for time to approach. The mean of reciprocal crosses of lines differing in their responsiveness toward the stimulus was intermediate to the means of contemporary progeny from the parental lines. Bidirectional selection for time to respond toward the stimulus has been practiced for three generations. A realized heritability of .18 $\pm$ .03 was obtained for the divergence of the selected lines demonstrating a significant amount of additive genetic variation for this trait.

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P. B. SIEGEL and F. C. WILEY, Virginia Polytechnic Institute.

Behavior of game chickens to 24 weeks of age.

The development of a social hierarchy and subsequent development of social inertia within sexes is typical of flocks of immature game chickens and adult females. In flocks of adult males, however, the peck-order suddenly disintegrates because of an apparent loss of submissiveness. Such agonistic behavior appears to have been developed through artificial selection and makes game cocks desirable for studies of aggression. This report involves the ontogeny of behavior in male and female flocks prior to the age when submissiveness disappears in the former. Data were obtained for stretching, running, frolicking, sparring, threat-avoidances, peck-avoidances, and fighting. Although these patterns appeared in the same sequence observed in other breeds of domestic chickens, their appearance and frequency varied in time. For example peck-rights in flocks of game chickens were established about two weeks earlier than in flocks composed of other breeds of domestic fowl.

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HENRY C. BROWNING and M. PERLEY, The University of Texas, Dental Branch.

Effect of single prenatal dosage of androgen on female mice.

CBA mice received 10 mg of testosterone propionate on the 17th, 18th, or 19th days of gestation. Offspring were transferred to untreated mothers at birth (*17, 18,* or *19 day treated*). Offspring of the latter were fostered by treated mothers (*17, 18,* or *19 day, untreated*). Some other untreated untransferred offspring received 2.5 mg of androgen when 5 days old (*injected*): others did not (*controls*). All female offspring were ovariectomized at puberty and received subcutaneous ovarian isografts. Vaginal smears were followed through the 5th month of life.

Controls and approximately half of the *18* and *19 day treated* and of *17* and *19 day untreated* showed vaginal cyclicity and grafts with vesicular and luteinized follicles. (No *18 day untreated* mice survived.) The *injected* mice and the other *18* and *19 day treated* and *17* and *19 day untreated* showed continuous metestrus and grafts with vesicular follicles only. On the other hand, *17 day treated* mice had distended upper, and absent lower, vaginas; half of their grafts had vesicular, and half vesicular and luteinized follicles.

Apparently, hypothalamic-pituitary cyclicity is being established on the 17th day of fetal life and, as in the newborn, is labile to androgen. (Supported by USPHS Research Grant CA-02880.)

294

R. H. NAQVI and M. X. ZARROW, Purdue University.

Induction of sexual precocity in the female rat with testosterone propionate.

The current investigation is concerned with the action of testosterone propionate (T.P.) on the development of precocious puberty in the female rat. Vaginal opening and 1st estrus occurred on days 42 and 45 respectively in the control rats whereas treatment with 1.25 mg T.P. on day 21 of age caused premature vaginal opening (day 27) and no effect on time of onset of 1st estrus (day 45). Treatment with 0.1 mg T.P. on day 21 or day 28 as a single injection was without effect. Treatment with 0.1 mg T.P. on days 21 and 23 caused both precocious vaginal opening (day 34) and precocious 1st estrus (day 37). However, if daily treatment with 0.1 mg T.P. was carried out day 21 through day 30, precocious vaginal opening was obtained (day 28) but the 1st estrus appeared somewhat delayed (day 51).

The possibility of the action of T.P. being mediated by the ovary was explored in two separate experiments. In the 1st experiment premature vaginal opening (day 33) was obtained following treatment of ovariectomized rats with 0.1 mg T.P. on days 21 and 23. In a 2nd experiment, 0.01 mg T.P. was injected into the perivaginal region on days 21 and 23. Premature vaginal opening (day 30) was obtained but the time for the 1st estrus (day 42) was not greatly affected. Perivaginal treatment with 0.01 mg T.P. on day 21 only was without effect. It would appear that the peripheral action of testosterone is independent of the ovary. (Aided in part by a grant, HD-02068, from the NIH.)

295

NANCY B. CLARK, University of Connecticut.

Influence of endogenous or administered estrogens upon serum calcium, phosphate and protein concentrations of fresh-water turtles.

Single doses of estradiol benzoate as low as 20 μg induced significantly higher concentrations of total calcium, inorganic phosphate and total protein in serum of both sexes of fresh-water turtles *Pseudemys scripta* and *Chrysemys picta*. At this low dose, statistically significant hypercalcemia occurred by 2 weeks post-injection, hyperproteinemia 3 weeks post-injection and hyperphosphatemia by 6 weeks post-injection. When higher doses of estrogen were administered (100-750 μg) the latent periods were shorter: 1 week for significant hypercalcemia and 3 weeks for hyperphosphatemia. These higher levels generally persisted at least 8 weeks after the time of injection. Protein levels were tested only in response to a 20 μg hormone dose and its effect lasted only 3-4 weeks.

A study of these fractions of calcium, phosphate and protein in serum of *C. picta picta* caught in local Connecticut ponds from early April until mid-July (sampled within a day of capture) revealed that hypercalcemia occurred in normal females from about mid-May until mid-June, hyperphosphatemia occurred from approximately mid-May until early June and hyperproteinemia from about mid-May until early July. The levels to which these substances rose in normal females during this period of yolk production and egg-laying (A. Carr, Handbook of Turtles, Cornell University Press, 1952), presumably in response to endogenous estrogens, were greater than those induced by a 20 μg dose of estradiol benzoate. The concentrations of these substances in serum of normal males generally remained low at this time of the year.

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R. A. MEYER, JR. and H. J. BRINKLEY, University of Maryland.

Reduced serum calcium during proestrus in the mouse.

In all classes of vertebrates, excluding mammals, an elevated serum calcium is associated with reproductive activity and/or estrogen administration. In cattle the serum calcium is slightly elevated at estrus; whereas, in rats there is no fluctuation during the estrous cycle. In male mice injections of large quantities of estrogen cause endosteal bone proliferation and a reduced serum calcium. It was of interest to determine whether the fluctuation of estrogen during the estrous cycle would affect the serum calcium of mice.

Albino mice (MBR/ICR: 16 and 22 weeks old at sacrifice) were given Rockland Mouse/Rat Diet and water *ad lib*. Stages of the estrous cycle were determined each evening for at least three weeks by the vaginal smear technique (lavage). The two groups were decapitated (unanesthetized) on different evenings after a ten-hour fast. The entire blood drainage from the trunk was collected and the serum samples were stored in flame sealed ampules at –25°C. The calcium analyses were performed by the Kingsley and Robnett technique. For statistical comparisons, the Mann-Whitney (rank sum or U) test was used throughout. Since significant differences were not observed between the age groups, the results from the two groups were combined.

Although not significantly different from those showing estrus smears ($\bar{x} = 5.62$ mN; $n = 7$), the serum calcium of those showing proestrus smears on the evening of sacrifice ($\bar{x} = 5.57$ mN; $n = 11$) was significantly lower than those showing metestrus smears ($\bar{x} = 5.89$ mN; $n = 17$; $p<.01$) or diestrus smears ($\bar{x} = 5.91$ mN; $n = 12$; $p<.05$).

297

F. HUNTER, L. C. KREY, and G. ROBBIN, Brown University.

Glucose-6-phosphate dehydrogenase (G6PD) in constant estrus rats.

G6PD provides reduced NADP, which is essential in several steroid reactions, including A-ring reduction. Changes in the concentration of this enzyme may, therefore, play a role in gonadal feedback mechanisms. G6PD concentrations were measured in liver, kidney and neural tissues from CFE rats. Liver G6PD concentrations vary significantly with the stage of the estrous cycle. The highest concentrations of liver enzyme are found in proestrus and metestrus.

To determine the effect of altered hormonal status on this enzyme, G6PD concentrations were measured in constant estrus animals. Constant estrus was induced by electric coagulation in the preoptic area of the hypothalamus. In constant estrus animals, liver G6PD concentrations stabilize at relatively low levels. These enzyme levels approximate those found in the livers of nonlesioned animals in estrus. However, the characteristic cyclic changes of liver G6PD concentrations are not evident in constant estrus animals. Kidney and hypothalamic G6PD also respond to the induction of constant estrus. Increased concentrations of G6PD are observed in these tissues, and the concentrations exceed those seen in any stage of the estrous cycle. Comparable changes in G6PD concentrations are not found in sham operated animals.

It appears that the hormonal status of the female rat does affect tissue G6PD concentrations. Furthermore, it appears that the response depends upon the tissue examined. (Supported by ACS Institutional Research Grant IN-45F.)

298

NATHAN LAVENDA and GINO DI VIRGILIO, North Adams State College, Massachusetts.

Microbial interactions in the estrous cycle of the guinea pig.

It has recently been reported by Di Virgilio and Lavenda (Am. J. Obst. & Gynec., 93:491, 1965) that in the normal human vagina the relative concentrations of the microbial elements (bacteria and viruses) are altered in the chronic inflammatory states of unknown etiology, pre-cancerous states and incipient carcinoma. Microbial interrelationships have also been studied in ten cases of primary human syphilis (Di Virgilio and Lavenda, submitted for publication). In the present study 35 guinea pigs were used (a total of 80 estrous cycles) to determine the feasibility of experimenting with this species to investigate gynecological problems of current interest, e.g., the effects of antibiotics and oral contraceptives in altering the microbial concentrations of the vagina. Using aseptic procedures, daily smears were prepared consisting of the following stains: Papanicolaou, Gram and an original cytovirological technique for the identification of viral elementary bodies. The observations with respect to the microbial interrelationships occuring in the vagina in the normal estrous cycle will be presented. The implications derived from these data with regard to evaluating the long-range effects of antibiotics and oral contraceptives on the female reproductive tract will be discussed.

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G. C. KENT, JR., D. R. DICKERSON, and G. W. TATE, JR., Louisiana State University.

Luteolysis and the pseudopregnant uterus in the golden hamster.

Complete hysterectomy at any time prior to about noon of Day 8 of pseudopregnancy results in prolonged hyperemia of the corpora lutea and delay in ovulation until aproximately the term of normal pregnancy. Total uterine resection followed by immediate rejunction *in situ* of the pseudopregnant uterus on Day 1 of pseudopregnancy had the same effect. However, complete resection and immediate rejunction at the end of Day 4, or on Days 5, 6, 7, or on the morning of Day 8 resulted in luteolysis earlier than in hysterectomized controls. Circulation had been reestablished by the 18th hour after resection and rejunction. It is assumed that uterine innervation had not been reestablished by the time luteolysis occurred. It is tentatively concluded that, in order for the pseudopregnant hamster uterus to participate in evoking the luteolysis essential for terminating pseudopregnancy at the normal time (Day 9), uterine innerva-

tion must be intact during some specific interval prior to the end of Day 4, and uterine vascular connections must be present, except for short interruptions, throughout the entire period encompassing Days 5, 6, 7, and the morning of Day 8 of pseudopregnnacy. (Supported in part by Grant GY-767 from the NSF.)

300

BARRY R. KOMISARUK, PETER G. McDONALD, and CHARLES H. SAWYER, University of California, Los Angeles.

Non-specific effects of progesterone and sensory stimuli on brain activity.

The effects of intravenous injections of progesterone (100-200 μg) and sensory stimuli such as vaginal probing, pain, and cold on neural activity in the hypothalamus, cortex, and thalamus were investigated in urethane-anesthetized rats. Changes in neural activity were assessed by simultaneously recording cortical EEG, single-unit firing and integrated multiple-unit activity in these brain regions. Changes in single- and multi-unit activity from regions studied in cortex and thalamus were closely synchronized with alterations in cortical EEG, while hypothalamic activity was relatively independent of the cortical EEG. Intravenous progesterone quickly induced a sleep-like EEG which lasted about 20 minutes, and single- and multi-unit activity changed to the patterns which they characteristically showed in deep spontaneous sleep. During this period all types of sensory stimuli were partially or completely blocked in their arousing effect throughout the brain. Progesterone is thus considered a non-specific anesthetic in its effect on altering neural activity in this preparation. (Supported by grants from NIH and the Ford Foundation.)

301

WILLIAM B. LANGAN and THOMAS V. GETCHELL, Villanova University.

Effects of steroids on oxygen consumption of *Rana pipiens* ovarian follicles during ovulation.

Although *in vitro* ovulation is readily induced with homoplastic pituitary preparations and several steroids, observations indicate differences in response. Pituitary induced ovulation has been shown to be oxygen dependent (P. Rondell and P. Wright, Science 125:644-45). A significant difference in oxygen consumption between pituitary and steroid induced ovulation would support the hypothesis of different underlying mechanisms.

Rana pipiens (40-60 grams) were obtained during February from Vermont. Ovarian fragments consisting of 20 follicles were excised, placed in the outer well of the Bronwill Warburg apparatus with the appropriate test solution, shaken at 80 cycles per minute while maintaining a temperature of 22°C. Test solutions were: homoplastic anterior pituitary homogenate, progesterone, deoxycorticosterone acetate, estradiol benzoate and controls-Ringers solution. All test systems were closed for the duration of the experiments, up to 24 hours. Average microliters of oxygen per hour were determined for each substance, along with standard deviation, standard error of the mean, coefficient of variability and the 95% confidence interval. Grafts prepared showed linear correlations with oxygen consumption and straight lines were fitted using the least square method, checking goodness of fit by chi square.

The final plots showed no significant difference between controls and estradiol and no ovulation was observed. The anterior pituitary preparation induced 25% ovulation and the greatest increase in oxygen consumption. Both deoxycorticosterone and progesterone induced 50-56% ovulation and showed oxygen consumptions intermediate to the pituitary and control experiments. A t-test indicates a closer affiliation with the controls.

302

W. ST. AMAND, University of Mississippi.

Radiation-induced changes in Ehrlich ascites tumor cell populations.

Following *in vivo* X-irradiation of Ehrlich ascites tumor cell populations there is an increase in average cell size. For doses of 250 to 7500 r the increase is linearly related to time for 24-36 hours after treatment. Larger doses result in greater inceases, but the dose-effect curve is not linear. Ten cell size classes, comprising three series, have been followed, and each shows a characteristic pattern of change after treatment. Maximum effects occur at different times for the various size classes. The radiosensitivity differences are not ascribable to differences in original cell surface areas or cell volumes. (Aided by Grant No. IN-60E from the American Cancer Society.)

303

GLENN GENTRY, Donelson, Tennessee.

Twenty-five years of collecting amphibian eggs and adults for research.

In 1939, 500 *Ambystoma* eggs were supplied to Washington University, St. Louis, beginning a service of supplying amphibians and embryos for research which has continued to date. The advent of commercial airlines made it possible for eggs to be delivered within 20-36 hours, and the use of plastic bags sealed in heavy cardboard boxes maintained temperatures around 40 degrees Fahrenheit, thus greatly increasing the availability of early-stage eggs.

The increase in demand for amphibian material necessitated the location of many ponds and the study of the factors that stimulated egg laying. It was found that fluctuations in temperature, rainfall, and altitude in Tennessee extended the egg laying period, which usually occurs when the temperature rises above 50 degrees for about two days with about one-half inch of rainfall. Dry and cold periods interrupt migrations to the ponds.

The *Ambystoma opacum* egg laying period is short, beginning the last week in September and ending in mid-October. *Ambystoma tigrinum* begins laying about December 1 and continues to mid-February. *Ambystoma texanum* eggs also occur during this period. *Ambystoma maculatum* usually lays during February and March.

Newts are usually available year round but may become scarce in an extended drought. They usually occur in large numbers evidently because they have few predators. Red efts and adults begin to migrate to the ponds in early fall. One observed migration was estimated at 5000.

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DAVID T. RAY and CHOPPALA D. JOHNSON, Howard University.

Oogenesis of *Mormoniella vitripennis.*

Architectural and cytochemical descriptions of the gonial follicles of the ovaries in *Mormoniella* compare very favorably with those in *Drosophila* as described by R. C. King and his colleagues; the egg tubes, however, are longer and taper more gradually revealing movements of nurse cells, follicle cells and developing oocytes. The ovaries of *Mormoniella* consist of 2 clusters of four parallel tapering egg tubes, each of which is differentiated into an anterior germarium followed by a series of successively more developmentally advanced egg chambers. Each egg chamber contains 16 cytoblasts produced by four divisions of a single oogonium. The most posterior cell of the 16 differentiates into the oocyte; the next four or five cells migrate around the oocyte and divide several times to form the enveloping follicle cells. The remaining cells serve as nurse cells which nourish the developing egg. Intercommunication among the nurse cells is conducted by a system of canals. During the first stages all 16 cells grow at identical rates after which vitellogenesis occurs. The oocyte now grows much faster than previously at the expense of the nurse cells which shrink and degenerate. Mutations resulting in sterility and semisterility assume various structural and physiological variations. In some egg chambers tumorous cells fail to nourish the oocyte. Individual tumorous egg chambers have been observed in eye-color mutants not considered sterile indicating a type of deleterious effect probably associated with the mutant eye pigment. In other sterile types the chorion of the egg fails to develop. Normal contour is maintained until the rupture of the follicle envelopment. A third type of mutant reveals the abortion of development in the germarium. (Supported by grant GM 12193 from the USPHS.)

305

SARA H. FRYE, Irvine, Kentucky.

Cytogenetic analysis of an X-ray induced, double-marker mutant in *Drosophila melanogaster.*

Young P_1-males (Y/y^+ ac^+ In49 B^{M1}) were exposed to 2000 r (delivered at 400 r/min, 210 kvp, 15 ma) then mated to virgin-females containing attached-X-chromosomes. One mutant F_1 male, phenotypically yellow (*y*) and achaete (*ac*) was recovered from an irradiated series. Genetic analysis of the exceptional male consisted of tests (among others) for fertility and transmissibility, allelism, $X\text{-}Y^S$ exchanges, response to $sc^S.Y$ and/ or scute-19i insertion, and X-ray induced reverse phenotypes. The deduced genotype was Y/y ac In49 B^{M1}.

A cytological examination was made by E. S. Gersh. The tip of the y ac In49 B^{M1} chromosome is extended beyond that of the Oregon-R normal X-chromosome and the banding distal to 1A5.6 is very unclear. Band 1A4 may be missing. Part of band 1A5.6 may also be missing, as this band often appears thinner in the Oregon-R normal X-chromosome. A photograph of the double-marker mutant's salivary gland chromosomes will circulate during the presentation of the paper, and predictions concerning the implications of this double-marker mutant for radiation genetics will be risked. Experiments in X-ray and spontaneous mutagenesis, functional and recombinational analysis of the X-chromosomal genetic regions responsible for the two mutant phenotypes, yellow (body color, bristles, wings) and achaete (absent posterior dorsocentral thoracic bristles) indicate that they can mutate independently, are of two different functional cistrons, and are very closely linked yet structurally unrelated.

A continuing multiple perspective analysis of this double-marker mutant would require a determination of DNA content as compared to DNA content in normal X-chromosomes perhaps by the method employed by D. Wolstenholme (Genetics 53: 357-360, 1966), and a cytological analysis of the y ac In49 B^{M1} chromosome via electron microscopy. (Supported by USPHS and the estate of the author's deceased father.)

306

F. G. BIDDLE and M. L. PETRAS, University of Windsor, Ontario.

The inheritance of an erythrocytic esterase in *Mus musculus.*

A variant erythrocytic esterase has been detected in feral populations of the house mouse, *Mus musculus,* using a modification of the starch gel electrophoretic technique described by Pelzer (Genetics, 52:819, 1965) and α-naphthyl acetate as substrate. Sixty-three animals from several natural populations in the Windsor, Ontario, area all exhibit this band of esterase activity, whereas three inbred strains, C57Bl/10, C3H and CBA/J, do not. An examination of 298 offspring from all possible matings involving wild and C57Bl/10 mice has revealed that the inheritance of this zone of esterase activity is consistent wtih a two-allele, single autosomal locus hypothesis. The presence of the band is controlled by what appears to be a completely dominant allele and the absence by the recessive allele. This esterase locus, which has been tentatively designated *Ee-3,* is not closely linked with the agouti locus nor with two serum esterase loci: *Es-1* described by Popp and Popp (J. Heredity, 53:111, 1962; J. Heredity, 56:107, 1965) and *Es-5* described by Petras and Biddle (Am. Zool. this issue). Also, the effect of the *Ee-3* locus on esterases of other tissues will be discussed. (Supported in part by grants from the National Research Council of Canada and the Ontario Research Foundation.)

307

HORTON A. JOHNSON and EDWARD M. EISENSTEIN, Brookhaven National Laboratory, and State University of New York, Stony Brook.

Dense particles in axons and presynaptic boutons of the prothoracic ganglion of the cockroach.

Dense particles about 150 Å in diameter have been found within certain axons and pre-synaptic boutons within the neuropile of the prothoracic ganglion of the cockroach, *P. americana.* These occur in addition to the profiles of neurotubules, neurosecretory droplets, and synaptic vesicles usu-

ally found in these locations. The most striking feature of these particles is their discrete distribution. In a given section the axons and synapses in which they are seen make up only a few per cent of the total. The few axons and synapses in which they are found, however, contain them in large numbers. Typically they occur in clusters of about 5-10 scattered along the length of an axon or among secretory vesicles.

These particles are electron dense in unstained sections and their density is enhanced by uranyl ions, tending to rule out the possibility of their being glycogen granules and indicating that they are either ribosomes or some uncharacterized granule.

The presence of aggregates of these particles in just a few axons and synapses suggests that they may be related to specific neural functions rather than to the metabolic activity of the tissues as a whole. (Supported by USAEC and NIH 1-R01-NB05827-02 and M. H. 11012-01.)

308

RAMON S. GRILLO and ROSEMARY POLSKY, Adelphi University.

Interference with normal cell division following kinetin treatment in *Triturus viridescens*.

Adult newts, *Triturus viridescens*, were placed singly in glass bowls containing 200 cc of aged tap water and 20 mg kinetin. The kinetin solutions were changed every three days and newts were treated in this manner for approximately 14 days. Animals were killed by decapitation at various intervals, several pieces of liver and small intestine were processed according to the standard paraffin technique, and iron alum hematoxylin sections and Feulgen stained autoradiograms were prepared. Two hours prior to decapitation each newt received a 1 μc/g intraperitoneal injection of tritiated thymidine (1.9 C/mM).

The mitotic indices of the perihepatic cells and intestinal nest cells of the kinetin treated animals were significantly higher than control values according to t-tests. However, determination of per cent labeled cells (number of cells within a population undergoing DNA synthesis prior to mitosis) revealed no significant differences according to t-tests between the kinetin treated and control animals. Moreover, the chromosome abnormalities of the kinetin treated newts were strikingly similar to the effect of colchicine, and were as follows: (1) no observable spindle, (2) supercontraction of chromosomes, (3) precocious separation of chromatids, (4) polyploidy.

These data indicate that kinetin does not stimulate mitosis in the newt but interferes with the normal cell division process.

Attempts to increase the amount of kinetin in solution by using a .01 N NaOH solution revealed no significant differences in mitotic indices or per cent labeled cells between newts so treated and their controls. These results may be due to an interaction between NaOH and kinetin. Experiments are in progress to determine the manner by which kinetin affects the cell. (Supported by Grant GE 6267 from NSF and GM 12880-01 from USPHS.)

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RODERICK A. SUTHERS and JULIA CHASE, Indiana University.

Visual pattern discrimination by an echolocating bat.

A tropical nectivorous, echolocating bat, *Anoura geoffroyi* Gray (Phyllostomidae) was trained by operant conditioning to obtain food by visually distinguishing between two stationary patterns consisting of a 15 $\times$ 280 mm vertical rectangle and a disc (73 mm in diameter) of approximately equal area. These patterns were presented simultaneously at one end of the test apparatus as silhouettes spaced 30 cm apart against a diffusely illuminated background. Positive and negative reinforcement was provided by sucrose and quinine solutions associated with the disc and rectangle, respectively. Each solution was presented in a glass feeder projecting about 2 cm through a small hole at the center of the pattern. Pattern positions were interchanged semi-randomly, A sheet of clear acrylic plastic containing an identical small hole for each feeder, was placed between the bat and the patterns to prevent possible discrimination by echolocation. Control trials ruled out possible olfactory discrimination. On each trial the bat was required to fly toward the pair of patterns from a distance of about 1.5 m and to taste one of the solutions while hovering at the feeder. The experiments were performed with one bat which, after a two-day learning period totaling 52 trials, selected the sugar solution in 72.5, 92.6, and 93.3% of 40, 27, and 30 trials, respectively, during the next three successive days ($p<0.01$ for each of these days). (Supported by grant GB 4885 from NSF.)

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LLEWELLYN T. EVANS, Troy, New Hampshire.

Morphology and kinetics of courtship in certain Testudinidae.

In *T. elephantopus, T. denticulata* and *Gopherus* the male plastron is deeply grooved antero-posteriorly, to fit closely upon the female carapace. The gular "horns" protrude anteriorly and are used to prod mates and rivals. In their courtship, the male: a) slides forward and back upon the shell of the female with each thrust of rear feet:)b nods his head; c) vocalizes during courtship. These genera diverged from a common stem testudine, *Hadrianus corsoni,* in Upper Cretaceous (Williams '50).

In the aquatic *Clemmys insculpta,* the male plastron is similarly grooved. The male clasps the marginals of the female in his claws and slides forward, back, and sidewise on her carapace. He also pulls the latter in loud percussion against his plastron, while striking her nuchals noisily with his jaws. He also pumps water into the face of the female (Evans, A. Zool. 1, '61; 3, '63).

In *Terrapene,* the male plastron is concave in the center of the abdominal and femoral scutes. His rear claws are larger, more abruptly curved than the female's and are held by the female's plastron in early courtship as his plastron rests upon her posterior vertebral scutes. The males of the above mentioned species are larger than the females.

In *Chrysemys, Pseudemys* and *Graptemys,* the males are smaller, have flat plastrons, and have greatly elongated foreclaws which are used by most males to titillate the neck of females in very subtle courtship. The Terrapene male's concave plastron, powerful claws (and red eyes of *T. c. carolina*) and the foreclaws of male *Pseudemys* are hormonically controlled, secondary sex characters (Vertebrate Speciation, Texas U. 148-178, '61).

311

WILMA H. LEHMANN, Michigan State University.

The nasal airway in platyrrhine primates.

The purpose of this study is to (1) qualitatively ascertain the extent of inter-generic variation of the structural components of the nasal fossa and (2) examine the extent of angulation of the epiglottis with respect to the floor of the nasal cavity in the cebid genera *Alouatta, Aotus, Ateles, Cacajab, Cebus, Lagothrix,* and *Saimiri* and in the marmoset genera *Callithrix, Cebuella,* and *Saguinus.*

Within the nasal fossa the most conspicuous structural variation involves the turbinate bones, particularly the modification of the characteristic triangular form of the ethmoturbinate. The nasoturbinate, usually reduced in size and form in primates, is extremely well developed in *Alouatta* and *Aotus.* The maxilloturbinate is the most constant in form.

The medioventral elevation of the epiglottis usually lies in or nearly in a direct line with the dorsal end of the hard palate (i.e., the floor of the nasal cavity and the roof of the buccal cavity) and the dorsocaudal end of the laryngeal cricoid cartilage. In such instances the ligamenta hyoepiglatticus and glossoepiglotticus are well developed. Exceptions to the epiglottal angulation and ligamental development occur in *Alouatta, Ateles,* and *Lagothrix.* Negus (Mechanism of the Larynx, 1930) reported that in *Alouatta* the epiglottis is independently immovable. In the absence of effective ligamentary function, the present study suggests that a mechanically advantageous straight-line epiglottal relationship with the buccal cavity is achieved via elevation and protrusion of the tongue in vocalization.

312

W. P. LUCKETT, University of Wisconsin Medical School.

The ovarian cycle of the tree shrews (family Tupaiidae), with reference to the phylogenetic relationships of the tupaiids. (Introduced by H. W. Mossman)

Recent neuroanatomical, serological and osteological examinations suggest that tree shrews may not be as closely related to Primates as previously believed and that their similarities may be due to convergence and parallelism. Due to the absence of unquestioned fossil tupaiids and the lack of agreement concerning the relative merits of various neontological characteristics in assessing the phylogenetic affinities of higher taxa, it is imperative that the greatest number of characters possible to be examined to determine the relationships of the Tupaiidae to Insectivora and Primates.

Ovaries were examined histologically from several species of four genera of tree shrews: *Tupaia, Urogale, Lyonogale* and *Dendrogale.* Stages ranged from fetal to immature to pregnant specimens. The general nature of the ovarian cycle will be described, and quantitative data will be presented on the occurrence of accessory corpora lutea, cortical epithelial cords and "testis tubules."

This study indicates that the tree shrew ovary shows certain similarities to that of the soricid Insectivora, Primates and Carnivora. Other than these general affinities, the tupaiid ovary does not appear to be a good characteristic for determining the more explicit phylogenetic relationships of this group. This is in agreement with Mossman (J. Mamm., 34:289, 1953) who concluded that ovarian morphology is a useful characteristic in studying interrelationships of genera and families, but that they are less conservative than the fetal membranes in showing affinities between higher taxa, such as orders and suborders. (Supported by NIH Fellowship 5-F1-GM-19, 136.)

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VIRGINIA A. WEBER, GEORGE L. WEBER, and HULDA MAGALHAES, Bucknell University.

Use of computer for development of programmed instruction in vertebrate morphology.

An IBM 1620 Digital Computer has been used for automated measurement of learning. Approximately two thousand questions have been stored in a computer memory for retrieval according to their level of difficulty, or 99 subject categories, including ten traditional anatomical systems. 50 different organs, five body regions, and 20 basic morphological concepts, such as evolution, regeneration, differentiation. Questions pertaining to specific animals, classes, or larger groups may be retrieved in the category desired.

In addition to storage and retrieval of questions, the computer prints out copies of individual questions and tests. It prepares the original from which duplicates can be made for large classes. The computer grades student answer sheets at a rate 50 times faster than a human instructor. Simultaneously, for each question it provides a statistical analysis of the responses of each student.

Routine computer testing of learning of basic information provides released faculty time for instruction at more sophisticated levels. (Supported by Carnegie Foundation Grant coordinated by Dr. J. William Moore.)

327

PAUL C. SCHROEDER, Hopkins Marine Station of Stanford University.

Morphogenesis of epitokous setae during normal and induced metamorphosis in *Nereis grubei* (Polychaeta) (Introduced by A. C. Giese)

The "pelagic" setae of the mature, swarming heteronereid arise from a new proliferation of the parapodial epidermis at a time when the major oocyte component has reached a diameter of about 95 μ. Serial sections of segments 44-46 of normal and decapitated animals stained with Mayer's haemalum and celestine blue were examined. Setal morphogenesis was found to be divisible into four distinct stages based upon the successive appearance within the parapodium of the paddle ends and shafts of the setae. Each

setal region bears a characteristic sculpturing easily detected with phase contrast optics. The period between the first appearance of the epidermal proliferation and the appearance of the paddle-shaped tips of the heteronereid setae is designated Stage I (average oocyte diameter about 95 μ-130 μ). The period during which only this portion of the new setae is present is designated Stage II (about 130 μ-165 μ). The interval between the appearance of the setal shafts and the eruption of the setae to the exterior is designated Stage III (165 μ-180 μ), and the period after eruption to the exterior Stage IV (over 180 μ).

Examination of a series of young, gameteless animals 15 days or more after decapitation (which removes the source of the metamorphosis-inhibiting hormone) revealed that even the youngest responded by production of a few small heteronereid setae. Six young females with oocytes under 95 μ responded similarly. The parapodial epidermis is thus competent to differentiate long before the stimulus to do so is given in normal metamorphosis. Decapitation of animals with oocytes up to 140 μ in diameter has produced accelerated setogenesis, but no acceleration was noted in older animals. The natural hormone withdrawal which regulates metamorphosis has by this time probably progressed to a point where decapitation has little effect on the hormone level. (Supported by PHS Fellowship 5F1-GM-17,007.)

328

VICTOR J. BROOKES, DERRELL S. CHAMBERS, and ROGER DEJMAL, Oregon State University.

Oocyte development in isolated abdomens of *Leucophaea maderae.*

Implantation of corpora allata (CA) or treatment with synthetic hormone (J. Law, C. Yuan, and C. M. Williams, Proc. Nat'l. Acad. Sci. 55:575, 1966) stimulated development of the oocytes in isolated abdomens of *L. maderae.* Development began 10 days after implantation. In a small percentage of abdomens treated, oocyte development was virtually complete but for the most part development ceased when the oocyte was approximately one-half the mature size. The yolk proteins of normal oocytes and oocytes developing in isolated abdomens have been characterized by polyacrylamide electrophoresis. No obvious differences in the qualitative characteristics have been found. Developing oocytes showed a band which appeared very soon after development began and intensified during growth. This band may correspond to a protein which appears in the hemolymph only during deposition of yolk (F. Engelmann, Amer. Zool. 5:673, 1965).

The fat body has been implicated in the synthesis of yolk proteins and suggested as a target organ for the hormones of the CA. When uridine-H^3 was injected into isolated abdomens the incorporation into RNA was 2-fold greater in those implanted with CA than in controls. This difference was evident 2 hours after treatment but after 48 hours the difference was much less. No significant difference in protein synthesis was observed until 5 days after the operation.

The evidence so far accumulated suggests that the failure of isolated abdomens to produce fully developed eggs is due to deficiencies in the amounts of stored material available for complete synthesis of yolk. (Supported by grant GB 3818 from the NSF.)

329

NED A. SMITH and C. L. RALPH, University of Pittsburgh.

Fractionation by centrifugation of heart accelerating substance from the corpora cardiaca of a cockroach.

Dissected corpora cardiaca from adult male *Periplaneta americana* L. were washed and placed in cold Pringle's insect saline solution (Pringle, J. Exp. Biol. 15:101, 1938) and homogenized in glass tissue grinders. In various experiments such extracts were centrifuged at 2°C at speeds of 1000 *g*, 11,000 *g*, and 25,000 *g*. The fractions were subsequently tested for their heart accelerating activity on the isolated heart preparations as described by Ralph (J. Ins. Physiol. 8:431, 1962). In all cases, most activity was associated with the soluble fraction, which initiated marked heart acceleration in the first 30 seconds of the assay. Activity of sediment fractions was much lower and slower acting. Changing the extraction medium to .4 M sucrose had no significant qualitative or quantitative effects on activity localization. Ultracentrifugation of the 1000 *g* supernatant on a 4 to 40% sucrose gradient revealed two active peaks. Most acceleration is associated with a peak which indicates that the active material is a relatively small molecule. This peak is characterized by rapid acceleration after application to an assay heart. A second less active peak is associated with a much heavier material, which is slower acting in biological assays. The results suggest that water-soluble accelerating factors from these glands may occur in two fundamentally different forms, having qualitatively different actions.

330

AVERETT S. TOMBES, Clemson University and DAVID S. SMITH, Univ. of Miami.

Ultrastructural studies on the corpora cardiaca-allata complex of the adult alfalfa weevil, *Hypera postica.*

The organization of the corpora cardiaca and corpora allata of the female alfalfa weevil has been examined in the electron microscope. The structure of both glands generally parallels that reported for other insects with extrinsic neurosecretory axons, intrinsic secretory cells and interstitial or glial cells appearing in both glands.

Within the main nerve trunk connecting the brain with the cardiacum are the extrinsic neurosecretory axons with the diameter of the contained granules ranging between 600 and 2200 Å. Similar granules are found in the intrinsic cardiaca cells with their maximum diameters reaching 4000 Å.

From the distal region of the lobed corpus cardiacum pass axons and glia along the short nerve trunk into the ovoid corpus allatum. A striking characteristic of this gland which has not appeared in previous ultrastructural studies in insects is the presence of a layer of axons, ensheathing most of the allatum beneath an external basement membrane. These axons are believed to have their origin in the protocerebral neurosecre-

tory cells, since they contain granules similar in size and appearance to those observed in the main nerve trunk proximal to the cardiacum. Although the allatum is known to be engaged in the release of hormonally active material, the cytoplasmic organization does not strongly reflect such activity. (Supported by NIH Postdoctoral Fellowship 7-F3-GM-28, 152-01A1 and NSF grant GB-5445.)

331

RONALD R. NOVALES and BARBARA JEAN NOVALES, Northwestern University.

Electron microscopic studies of pigment movements in melanophores.

The precise mechanism by which pigment movements occur in melanophores is still largely unknown. It was hoped that electron microscopic studies might shed light on the ultrastructural basis for melanin movements. Skin from *Rana pipiens* frogs was either darkened *in vitro* by MSH or lightened with adrenalin. After glutaraldehyde fixation, osmium post-fixation and embedding in Araldite, stained sections were examined with an Hitachi HS 7 electron microscope. The area immediately about the nucleus is relatively devoid of melanin granules in the MSH-treated cell, but granules are thickly present in peripheral regions. The cytoplasm is largely free of organelles and no structures obviously associated with melanin movements were found. In adrenalin-treated melanophores, processes with melanin-free cytoplasm could be found. Dense aggregations of mitochondria were often present peripheral to the mass of melanin.

Melanophores of the killifish, *Fundulus heteroclitus,* were also studied. Scales were treated with NaCl to produce dispersion or KCl to produce aggregation of pigment. Other methods were as above. Microtubules were observed in the processes of the *Fundulus* melanophores. Melanin-free cytoplasm was also seen in melanophores with aggregated melanin. The microtubules probably serve largely as "cytoskeletal" elements in the processes. However, they could be associated in some manner with the movements of melanin granules. In any case, it is likely that melanin movements result from biochemical or biophysical changes below the limit of resolution of the methods used in the present study. (Supported by Grant G-24017 from the NSF.)

332

JEFFREY NICHOLS, CHARLES SCHNEEBECK, and MAC E. HADLEY, Brown University.

Comparative *in vivo* response of embryonic, larval, and adult melanophores of *Fundulus heteroclitus* to melatonin.

In vitro (Fain and Hadley, this volume), melanophores of adult *Fundulus heteroclitus* are unresponsive to melatonin, but their melanin granules rapidly become aggregated in the presence of noradrenaline. Experiments reported here were directed toward determining whether, *in vivo,* these melanophores are similarly unresponsive to melatonin.

Fish (8-10 cm) were adapted to a black background for several hours and then injected with a saline solution or saline containing either 1 mg of melatonin or 1 mg of noradrenaline. Other fish were placed on a white background at the time of injection. Melanophores of fish adapted to a white background or injected with noradrenaline became punctate (melanin granules aggregated) within a matter of minutes. The melanophores of control-injected and melatonin-injected fish remained completely dendritic as assessed microscopically at sacrifice. This experiment was repeated, but this time fish were first adapted to a white background for several hours and then transferred to a black background after being injected with the test solutions. All fish became black within a few minutes except those injected with noradrenaline. Thus, the failure of melatonin to bring about melanin granule aggregation cannot be ascribed to a possible inability to override a high circulating level of "MSH." Small fish (3-4 cm) gave similar results.

In contrast, the body and yolk sac melanophores of *Fundulus* embryos and larvae rapidly became punctate when placed in solutions of melatonin (100 μg/ml and higher). These results are similar to those obtained by Wyman (1923, 1924) using pineal extracts. (Supported by Grants No. CA-06097-06 and GM-00582-06 from the USPHS.)

332a

JOHN ZAVODNI and R. T. HOULIHAN, Pennsylvania State University.

Adrenocortical response to increased oxygen tensions.

This investigation was initiated to determine the extent of adrenocortical adaptation in rats subjected to increased oxygen tensions. Adult male rats were maintained in a 100% oxygen environment, and in a 66% oxygen, 34% nitrogen environment at 750 mm Hg absolute pressure. The corticosterone content of the serum and 3-hour adrenal gland incubates was determined at 1, 2, and 3 days for the animals in the 100% oxygen environment and at 2, 7, and 14 days in the 66% oxygen environment.

The steroid was identified by chromatography and fluorescent spectral analysis; and quantitated with sulfuric acid-ethanol induced fluorescence. The results of this study show that in a 100% oxygen environment, serum corticosterone levels had increased significantly at days 2 and 3. The corticosterone produced by excised adrenal glands on a μg per mg wet weight basis also increased significantly, with very high levels just prior to death. In the 66% oxygen environment, there were no detectable changes in either the serum corticosterone levels, or in the μg per mg corticosterone produced by the excised glands until day 14, at which time a decreased serum corticosterone was evident, suggesting that these rats could successfully adapt to this particular oxygen tension. (Supported by NONR contract NR102-654.)

332b

GOTTFRIED FRAENKEL and CATHERINE HSIAO, University of Illinois.

Pupal diapause in *Sarcophaga falculata* (Diptera).

Robaud (Bull. Biol. 56:455, 1922) recognized a pupal diapause in a few specimens of a blowfly, *Sarcophaga falculata.* We have experimentally pro-

duced this diapause under two different circumstances. 1. When flies are raised at 14°C, some emerge as adults after about seven weeks, but the majority enters diapause. Diapausing pupae resume development at temperatures of 20° and over. Some pupae have remained in diapause for over a year at 14°, and successfully resumed development at a raised temperature. However, even at 14° development usually resumes spontaneously after 6 to 12 months. 2. Larvae raised at 20° and 8 hours light-16 hours dark often enter diapause which persists for several months at 20 and 25°. In diapause, development stops shortly after evagination of the pupal head, 36 to 40 hours after puparium formation, as judged by the development of the ganglia and the antennae. This is apparently also the stage in which pupal development stops in the hind part of a fly which was ligatured after the prepupal "critical period." Diapause is probably initiated, as is pupal diapause in the Lepidoptera, by a lack of ecdyson production. It occurs at the age when (Shaya and Karlson, J. Ins. Physiol. 11: 65, 1965) ecdyson is virtually absent from the blood between two peaks of secretion. The same condition of diapause at 14° was also, apparently for the first time, observed in *Sarcophaga bullata*. (Supported by USPHS grant AI00533-15.)

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ALBERT C. SMITH, University of California, Irvine.

Electrophoretic studies of soluble protein from lens-nuclei of bluefin tuna, *Thunnus thynnus*, from California and Australia.

Extracts of lens-nuclei from sixty California-caught and forty southern Australia-caught bluefin tuna produced a total of seven patterns on cellulose acetate electrophoresis. California fish produced two patterns and Australian fish produced six (one pattern was produced in common). Fifty-eight of the sixty California bluefin produced one pattern. The uniform appearance of this pattern was impressive since the California fish were collected in samples one year apart. This homogeneity also supports the validity of the heterogeneity of patterns produced by the Australian fish. Morphologic differences between bluefin of these two regions are also reported in the literature, consistent with the electrophoretic findings.

Tagging and serology are the most widely used methods of identifying separate populations of the same fish species. The reliability of the electrophoretic method and the stability of nuclear lens protein suggest that electrophoretic analysis of this protein is a potentially valuable procedure for this purpose. (Supported by the California Department of Fish and Game.)

333a

GARY FREEMAN, University of Illinois and Bermuda Biological Station.

Studies on regeneration in the Ctenophore *Coeloplana*.

Coeloplana is a biradially symmetrical animal which has a statocyst and two tentacular pouches. A part of the animal containing a statocyst, one tentacular pouch, or a statocyst and one tentacular pouch will regenerate the whole animal. If the statocyst is removed from an animal it is regenerated.

By cutting animals through the statocyst it is possible to produce a half animal containing a statocyst and one set of tentacles in about 10% of the cases. A part of a half animal that contains a statocyst will regenerate a half animal. A part of a half animal that does not contain a statocyst will regenerate a whole animal. If one removes a statocyst from a half animal it will regenerate a statocyst. A part of this animal containing the regenerated statocyst will regenerate only a half animal. If one removes the statocyst from a half animal and replaces it with a statocyst from a whole animal the part containing the statocyst will regenerate a whole animal. If one does the reciprocal experiment in which one removes the statocyst from a whole animal and grafts in the statocyst from a half animal the statocyst piece will regenerate a whole animal. (Supported by Grant BG 3588 from NSF.)

334

G. W. BROWN, JR. and SUSAN G. BROWN, University of Texas Medical Branch, Galveston.

Intermediary nitrogen metabolism of the coelacanth, *Latimeria chalumnae*.

Studies on coelacanth liver (frozen 6 months) suggest a metabolism resembling that of Elasmobranchii. High concentrations (1-2%) of plasma and tissue urea in elasmobranchs aid in maintenance of plasma tonicity *vis-a-vis* sea water. Liver homogenates of the coelacanth contained a large quantity of water-soluble material giving a strongly positive ureido color reaction (to about the same extent as sharks) with 1-phenyl-1,2-propanedione-2-oxime (E_{max} 540 millimicrons). Chromogenicity was abolished almost completely by treatment of the homogenate with Sigma Type V urease. Calculated as urea, the material represented 1.8% of liver by weight. Rat liver stored frozen for 16 months had less than 0.1% urea.

Ornithine carbamoyltransferase (E.C. 2.1.3.3) was assayed in water homogenates of coelacanth liver: found, 25 micromoles citrulline per min per gram liver (25°). This corresponds to production of citrulline at a rate comparable to that of liver homogenates of the lungfish, *Protopterus aethiopicus*, but is lower than in sharks. The reaction was absolutely dependent upon added L-ornithine, carbamoylphosphate, and unboiled enzyme. This enzyme of the ornithine-urea cycle was chosen for initial studies because of its ability to retain activity for a number of months in frozen preparations. (Specimen No. 1482 of the Fish Collection, Peabody Museum of Natural History, Yale University.) (Supported by the University of Texas Medical Branch and the Tiburon Co., Biomarine Preparations, Galveston.)

334a

ABNER B. LALL and ROBERT M. CHAPMAN, Institute for Behavioral Research, Silver Spring, Maryland and Walter Reed Army Institute of Research, Washington, D. C.

Spectral sensitivity comparisons of the lateral eye and the median ocellus in *Limulus*.

The spectral sensitivities of the lateral eye and the median ocellus of Limulus were determined in the dark and chromatic adapted preparation by recording ERG's elicited by monochromatic stimuli ranging from 320 mμ to 675 mμ. The responses were analyzed for (a) the presence of steady-state as well as transient components, (b) the slopes of amplitude-energy functions at various wavelengths, and (c) the wavelength specific waveform characteristics, i.e., response area, rise time, and fall time. The spectral sensitivity functions were obtained by applying a criterion amplitude to the amplitude-energy curves obtained at the various wavelengths. The sensitivity peak for the lateral eye lies near 520 mμ. The median ocellus has two maxima, one in the UV (360 mμ) and the other in the green (530-535 mμ). The UV peak is about 200-300 times more sensitive than the green. In the median ocellus, violet light adaptation selectively suppressed the near ultra-violet peak, while orange light adaptation selectively suppressed the green peak. No such differential effects were observed in the lateral eye. The data are interpreted as a single spectral mechanism in the lateral eye and two spectral mechanisms in the median ocellus.

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GEORGE H. FRIED, MARTIN P. SCHREIBMAN, and KLAUS KALLMAN, Brooklyn College, New York Zoological Society.

Alpha-glycerophosphate dehydrogenase and glucose-6-phosphate dehydrogenase activities in teleosts.

Determinations of activities of alpha-glycerophosphate dehydrogenase (AGPD) and glucose-6-phosphate dehydrogenase (G-6D) were carried out in homogenates of liver and muscle of *Xiphophorus maculatus, X. milleri, X. helleri* and *Poecilia formosa* to demonstrate a possible significance of glycerophosphate shuttle and pentose shunt mechanisms in metabolic synthesis in teleosts. A modified tetrazolium method was utilized and enzyme activities expressed as micrograms formazan per microgram tissue nitrogen. In all species, G-6D activity in liver was almost five-fold greater than corresponding levels of AGPD. This is the reverse of the situation obtaining in mice. In muscle, AGPD activity was less than that of liver in all cases, while G-6D activity was barely measurable. In selected instances, lactic dehydrogenase activity was determined and found to be many times higher in muscle than AGPD activity, which is generally characteristic of other vertebrate muscle types. Incubation of homogenates at 27°C rather than the usual 37°C resulted in appreciable lowering of activity, suggesting that enzyme temperature optima for these poikilotherms are not markedly different from those for mammalian forms. Effects of removal of cofactors on enzyme activity were similar to previous findings with mice. High levels of G-6D activity in teleost liver, coexistent with the absence of an appreciable adipose tissue mass, suggest that the liver may be the major source of lipid synthesis in teleost fish. (Supported by Grants A-5905 and CA-06665 from the USPHS and GB-3843 from the NSF.)

335a

TRACY L. SIMPSON, Tufts University, and RADOVAN BOROJEVIC, University of Strasbourg.

The origin of basal epithelial cells during the attachment of explants of microcionid sponges.

Basal epithelial cells in the outgrowth region of explants of microcionid sponges are morphologically and cytochemically identical to nucleolate cells (Simpson, 1963 and in press). These data indicate that these new cells originate from mesenchymal nucleolate cells (= acheocytes of other authors). Considering all of the other cell types present in these sponges, the only other logical cell type which could give rise to new basal cells are the preexisting pinacocytes—exopinacocytes which cover the outer surface, and endopinacocytes which line the canals. The remaining cell types—choanocytes, gray cells, rhabdiferous cells, and globoferous cells—differ morphologically and cytochemically from basal cells to such an extent that it is most improbable that they give rise to a new basal epithelial cell.

Three types of explants of *Microciona atrasanguinea* were made in order to investigate the possible origin of new basal cells from preexisting pinacocytes: (1) normal explants with the original basal area in contact with the slide, (2) upside down explants with the outer surface in contact with the slide, and (3) explants which lacked both the basal region and the surface region. In all three cases, new basal cells attach to glass slides after 18 hours and are morphologically identical to nucleolate cells. This happens even in (3) above in the absence of exopinacocytes. If endopinacocytes in (3) gave rise to new basal epithelial cells these basal cells would differ morphologically from those in (1) and (2)—they do not differ. This evidence supports our contention that mesenchymal nucleolate cells (= archeocytes) give rise to new basal epithelial cells when explants of microcionid sponges attach and grow. (Supported by Grant GB-4094 from NSF and the French National Research Council.)

336

M. A. McWHINNIE and R. J. KIRCHENBERG, De Paul University and Concordia College.

Phosphatase activity in the hepatopancreas of the crayfish, *Orconectes virilis.*

Previous hepatopancreas phosphatase studies in decapod crustaceans (Kugler and Berkner, 1948; Travis, 1955 and 1957) employed histochemical techniques measuring the enzymatic activity at a single pH value. A change of metabolic pathways in the hepatopancreas of *O. virilis* has been reported by McWhinnie and Kirchenberg, 1962; McWhinnie and Corkill, 1964; and McWhinnie and Chua, 1964.

In vitro studies of homogenized hepatopancreas tissue using disodiumphenyl phosphate as substrate, measured at 20°C across the pH range of 5.50-10.00, suggest the presence of two enzymes (or two isozymes) with activity peaks at 6.80-7.30 and 8.00-8.50. Activity associated with the latter range appears to be cyclic, being low in winter and cold-adapted animals as well as in the D_4 and A stages of the intermolt cycle. Prior to the spring premolt stages activity in the 8.00-8.50 range increases progressively to a degree surpassing that of the lower range. Subsequent reduction of this activity is pronounced at the D_4 stage. There appears to

be little, if any change in the specific activity in the lower pH range throughout the growth cycle.

Changes in relative concentrations of these two groups of phosphatase enzymes correlate with the intermolt hexosemonophosphate shunt to a premolt glycolytic shift in metabolic pathways. Increased levels of total enzyme activity may also reflect the storage and synthesis roles played by this organ. (Supported under Grant GB-1474 from the NSF.)

336a

STUART J. COWARD and BYRON GALLIS, University of Georgia.

Dissociation and reaggregation of the turbellarians *Dugesia* and *Bdelloura.*

Entire planaria, *Dugesia dorotocephala,* were minced and then dissociated by trypsin in calcium-magnesium-free saline (0.1 dilution of Niu-Twitty saline). The suspension of washed single cells was adjusted to a concentration of 2-3 $\times$ 10^6 cells/ml and incubated on a rotary shaker at 25°C. Visible aggregates formed within the first hour of incubation. Little growth and no visible differentiation occurred within a week of observation. Supplementation of the basic glucose-saline medium with calf serum, chick embryo extract and amino acids, either singly or in combination, did not improve significantly the growth and had no grossly observable effects on differentiation. The maximum size attained by these aggregates was about 7 mm.

The marine triclad, *Bdelloura candida,* was dissociated more readily than *Dugesia,* using trypsin in calcium-magnesium-free artificial sea water. Within 24 hours, aggregates of 1 mm or larger had formed. These aggregates continued to increase in size through both further aggregation and by cell proliferation. The minimal requirement for this growth activity appears to be artificial sea water supplemented with glucose and a complete array of the amino acids. It has been possible to subculture *Bdelloura* aggregates and obtain continued good growth; however no gross signs of differentiation were noted.

The patterns of aggregation are distinctively different for the two species. The entire pattern of aggregation in *Bdelloura* can be altered by providing an artificial "condensation center," which in our experiments was simply a sterile cotton fiber.

Further studies are currently in progress, including the histological comparison of aggregates and intact animals.

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JOHN B. BUCK and ELISABETH M. BUCK, National Institutes of Health.

Mechanism of synchronous flashing of fireflies.

Photometric and cinematographic records of the synchronous flashing of huge aggregations of fireflies in riverbank trees in Thailand showed that the interflash period averaged 560 $\pm$ 2.5 (s.d.) msec and that the range of variation in population flash coincidence was $\pm$ 16 msec. Darkroom observations showed that the synchrony is maintained by visual feedback operating over less than 6 feet and requiring continual reinforcement. Possible mechanisms of entrainment or coupling of the individual flashing rhythms to achieve synchrony will be discussed and evidence will be presented suggesting that the control depends on performance in the preceding mass flash rather than in the current episode.

337a

MALCOLM S. STEINBERG and R. EUGENE GRANGER, The Johns Hopkins University.

The re-acquisition of adhesiveness by trypsinized chick embryonic cells *in vitro.*

We have devised a sensitive method for following the kinetics of cell aggregation in suspension cultures. It involves deposition of samples of aggregating cell populations onto membrane filters which are then fixed, stained and mounted beneath cover slips. The mean number of cells per aggregate can then be directly counted with considerable accuracy. In studying thus the aggregation kinetics of trypsin-dissociated neural retinal cells from 6-day chick embryos, we found no detectable aggregation during the first 30 minutes of agitation at 37°C in Eagle's MEM with 10% horse serum. (A 10% increase in the mean number of cells per aggregate would have been detectable.) Aggregation became demonstrable by 40 minutes and progressed thereafter. Because this lag occurred both in the absence of serum and in medium reclaimed from an aggregated preparation, the possibilities were excluded that it was due to either an inhibitor of aggregation in the serum or a requirement, in the external medium, for materials emanating from the cells themselves. Cells dissociated by divalent cation withdrawal aggregated with no lag, as did trypsinized cells mechanically redispersed after aggregation had begun. Initial aggregation rate progressed from barely detectable after half an hour to maximal when redispersal was carried out after 1½ hours at 37°C. Warmth was shown to be essential for abolition of the lag; it was also essential at every moment for the continued aggregation of even a fully adhesive cell population. Possible interpretations of these and related observations will be discussed. (Supported by Grant GB-2315 from the NSF.)

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H. C. PITOT and SISTER M. ROSELLEN THEINE, University of Wisconsin.

An automated assay of catalase employing computer techniques.

An assay was developed for the enzyme, rat liver catalase, using automation and computer techniques. The assay was based on the catalytic decomposition of hydrogen peroxide by catalase. The automated assay was achieved in three stages: measurement of the absorption of ultraviolet light by hydrogen peroxide with a Beckman DU spectrophotometer and Gilford recording instrument; additional use of a digital voltmeter and IBM keypunch which simultaneously and automatically recorded optical density values on computer cards; and finally, incorporation of an autoanalyzer pump and flow-through quartz cuvettes.

To use the data on the computer cards, a program was developed employing Fortran 63 language for the CDC 1604 computer. The program finds the slope of a line (k), relating the value, log (S_1/S_2), to the reaction time. According to the program,

the computer calculates this slope for small time intervals. The activity of the enzyme, expressed in units per gram liver, is determined according to the equation: units = (2.303k/0.01155) × D.F. D.F. is the dilution factor converting the sample size to a gram of liver. The unit of activity is defined as the amount of enzyme required to convert one-half of the hydrogen peroxide present to its products at 0°C in sixty seconds.

The advantages of the automated assay include speed, reproducibility of sample uptake with the autoanalyzer, and reproducibility of results obtained.

Finally, the method was applied to a study of catalase activity in tissue subjected to the compound, AAF, for varying lengths of time. (Supported by the NSF through the University of Wisconsin Research Participation Program for High School Teachers and McArdle Laboratory for Cancer Research.)

338a

LOUIS E. DeLANNEY and K. V. PRAHLAD, Wabash College.

Influence of thymectomy and splenectomy on allografts of skin and tumor in histocompatible strains of the Mexican axolotl.

Thymectomy, splenectomy and allografting of integument or of a strain-specific lymphosarcoma in the Mexican axolotl was performed to explore temporal and/or obligatory involvement of thymus and spleen at constant 20°C. Vasodilation, epidermal slough and host replacement on allografts between black and white strains scored onset of rejection. One group was thymectomized at 58 days of age. Splenectomy was performed on groups 25 days and 47 days of age. At 318 days all—thymectomized, sham-thymectomized, splenectomized and unoperated controls—received allografts of skin from histoincompatible axolotls. All but thymectomized animals rejected allografts commencing at 26 days post-planting. Completely thymectomized animals showed delayed survival of allografts; rejection first appeared at 67 days and one remained normal to 167 days. Thymectomized animals differed from others by replacing graft tissue without vasodilation. None of the thymectomized animals showed runting.

Adult axolotls between 365-379 days of age were thymectomized, splenectomized or both thymectomized and splenectomized. Twenty-one days later they received skin allografts which were rejected. Splenectomized animals differed in showing vasodilation 4-5 days earlier than other hosts. Delay of rejection in thymectomized animals was noted but of uncertain significance.

A strain-specific lymphosarcoma could be passed to histoincompatible strains when thymectomy was performed at 58 days of age and the hosts raised past the normal tolerant period before planting tumor. Passage rather than host-cell transformation occurred as shown by subsequent tumor passage to non-thymectomized siblings (negative) and to tumor-strain-specific animals (positive). (Supported by grant GM 05619 from the USPHS.)

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T. JOHN LEPPI, The University of New Mexico.

Histochemical studies of carbohydrate-rich substances in the Pacific hagfish (*Polistotrema stouti*). (Introduced by A. J. Ladman)

Sections of hagfish skin were stained by single or combined basic dyes, by basic dyes in combination with the PAS method, or by certain of the diamine methods (Spicer, J. Histochem. Cytochem., 13:211, 1965). In addition, the effects of selective enzymatic digestions and certain chemical modifications on staining reactions were evaluated to distinguish between acid mucosubstances containing sulfate and/or sialic acid and neutral carbohydrates.

Of the five morphologically distinct cells in the highly specialized epidermis of hagfish skin, only two contain mucinous secretory products. They are (1) the small mucous cells which occupy the outer half of epidermis, and (2) the less numerous, larger mucous cells which are found in the basal half. The PAS reactivity of the mucins in both types of cells was diminished but not eliminated following diastase digestion for one hour at room temperature. There was also a reddish-purple wet metachromasia in the large and small mucous cells following exposure of sections to 0.02% azure A buffered to pH 1.5 with this metachromasia being reduced in dehydrated and mounted sections. Both types of mucous cells were strongly reactive toward the aldehyde fuchsin, high iron diamine and alcian blue (pH 1.0) reagents. On the basis of the elimination of all three different types of staining in both cell types by active methylation (4 hours at 60°C with acidified methanol), the carbohydrates in these mucosubstances are thought to contain sulfate. This type of prolonged methylation is necessary in the biochemical system to effect the hydrolytic removal of sulfate esters. Additional evidence for the presence of sulfate in the mucous cells of hagfish skin comes from the inability of demethylation of saponification with 1% KOH to restore staining in these cells. Digestion of sections with either purified sialidase or testicular hyaluronidase prior to staining did not affect any of the reactions. Similar histochemical studies are in progress on the slime glands of hagfish to elucidate further the chemical nature of mucus in lower forms.

339a

JOHN D. DECKER and VIKTOR HAMBURGER, Washington University.

Influence of the brain on the periodic motility of the chick embryo.

Motility of the chick embryo is spontaneous and periodic (Hamburger, Quart. Rev. Biol., 38, 1963; Hamburger and Balaban, Devel. Biol., 7, 1963). Total activity increases from 40% of the observation period at 9 days to over 80% on day 15. In embryos with cervical spinal gaps total activity is reduced by 10 to 20% (Hamburger, *et al.*, JEZ, 159, 1965).

The following experiments were designed to analyze the contribution of different brain parts for activity. Operations done on 1½ day old embryos consisted of: (a.) Extirpation of the mesencephalon. (b.) Extirpation of the metencephalon. The durations of the activity and inactivity phases were recorded at 9, 11, 13, 15, 17 days and the total

activity (in per cent of observation time) was calculated. The extent of the ablation was determined histologically. The data were analyzed statistically and compared with those for cervical spinal embryos.

Until 13 days, activity of the experimental embryos was as low as that of embryos with cervical spinal gaps. Hence fore- and/or midbrain add to activity during this period. Cerebellar contributions to activity were noted after 13 days and contributions of the medulla after 15 days. However, the cerebellum increased the activity to normal levels; the medulla alone raised the activity only slightly but significantly above that of cervical spinal embryos. (Supported by grant 52817 of the USPHS.)

340

CAROLINE N. HEBARD, University of Pennsylvania.

Ultrastructure of the cortical cytoplasm of unfertilized and first cleavage eggs of *Xenopus laevis*. (Introduced by R. C. Herold)

Unfertilized and first cleavage *Xenopus laevis* eggs were fixed for electron microscopy in 1.75% glutaraldehyde in collidine buffer (200-220 milliosmols), post-fixed in 2% osmium in collidine buffer (180 milliosmols), and embedded in Epon (Luft, J. Biophys. Biochem. Cytol., 9:409, 1961). Some eggs were centrifuged in a Misco angle-head centrifuge at forces from 500-5,000 × *g*, and were fixed as above. All eggs were oriented in agar blocks prior to post-fixation with osmium.

A yolk-free cortical layer was observed below the plasmalemma in first cleavage eggs. It consists of a yolk-free cytoplasmic band 0.5-5.0 microns in width, and a more peripheral dense layer 0.3-0.5 micron in width. The combined layer corresponds in width and position to that transplanted by Curtis (J. Emb. and Exptl. Morph., 8:163, 1960). The more peripheral dense layer is homogeneous in fine structure but varies in thickness and continuity over the surface of the egg, being more continuous and thinner on the dorsal and vegetal surfaces than on the ventral and animal surfaces. The dense layer is resistant to centrifugal forces of up to 5,000 × *g* and prevents cell inclusions from packing against the plasmalemma.

In unfertilized eggs, cortical granules lie beneath the plasmalemma but are not attached to it. There is no prominent dense layer, and after centrifugation, cell inclusions can penetrate to the plasmalemma. The cortical granules do not stratify under forces of 500-5,000 × *g*.

Possible functions of the dense layer in membrane contraction and permeability control are discussed.

340a

JOSEPH M. BRANHAM, Institute of Animal Genetics, Edinburgh.

Variation in velocity and swimming pattern of rabbit sperm.

Rabbit semen was diluted to about 10^6 sperm/ml in pH 7.6 phosphate buffered glucose-saline and observed horizontally in a chamber 4.8 mm^2 × 16 mm deep. About 60% of the progressing sperm were moving downwards. The maximum velocity of straight-down swimming sperm was two or three times greater than the velocity of slower ones. In a typical sample 3% of the sperm were still, 4% were twitching but not progressing, 35% swam in closed circles, 46% progressed helically and 12% swam straight with "normal" rotation. At pH 9.5 all swimming sperm moved in circles and at pH 6.0 all followed relatively straight paths. Circling sperm changed to straight progression when the pH was lowered to 6.0. Sperm flocculation was pronounced at low pH and reduced at higher pH.

The swimming behavior of sperm could reflect physiological or genetic characteristics significant to fertilization and development. When semen was layered over a 4 to 6% gelatin gradient which gelled while being centrifuged at 2000 RPM, more than 80% of the sedimenting sperm were trapped in a head-down position. The influence of active progression on sperm sedimentation is being investigated in hope of finding a method of separating sperm according to swimming ability. (Supported by grant HD-11,972-02 from the NIH.)

341

RICHARD MARSHALL, C. THOMAS CASKEY, and M. W. NIRENBERG, National Heart Institute, NIH.

Nucleotide sequences of RNA codons recognized by aminoacyl-sRNA from embryonic and adult *Xenopus laevis* tissues. (Introduced by Robert De Haan)

Nucleotide sequences and relative template activities of RNA codons were determined by stimulating the binding of aminoacyl-sRNA (AA-sRNA) from *Xenopus laevis* (South African clawed toad) and guinea pig liver to *E. coli* ribosomes. Nucleotide sequences recognized by *Xenopus* and guinea pig liver AA-sRNA were identical to those recognized by *E. coli* AA-sRNA. Thus the genetic code is clearly universal.

Although trinucleotides for the same amino acid usually differ in template activity, relative template activities found with amphibian and mammalian AA-sRNA were similar. However, metazoan sRNA differed markedly from *E. coli* sRNA in response to ten trinucleotides. Comparative studies then were undertaken with AA-sRNA from embryonic and adult tissues. *Xenopus* neurula Arg-, Asp-, Glu-, Gly-, Ile-, Lys-, Met-, Phe-, Ser-, Thr-, Tyr-, and Val-sRNA, and adult *Xenopus* skeletal muscle Arg-, Lys-, Met-, and Ser-sRNA preparations were tested in binding studies with trinucleotides and *E. coli* ribosomes. No differences were observed between *Xenopus* neurula, adult muscle, and liver AA-sRNA in nucleotide sequences recognized or in relative responses to degenerate trinucleotides. The results, however, do not exclude the possibility that alterations in codon recognition may influence cellular growth and differentiation.

341a

MAX HAMBURGH, LASLO NEBEL and GERALD GREENHOUSE, Albert Einstein College of Medicine and City College of New York.

Penetration and uptake of trypan blue in the yolk sac placenta of the mouse. (Introduced by J. Hagedoorn)

The possibility that the teratogenic action of the dye trypan blue in rodents is related to damage of the yolk sac placenta was investigated.

Pregnant female Swiss albino mice were injected with 0.4 ml of 1% solution of trypan blue on day 7½, 8½, 10½ and 11½ of gestation. Animals were sacrificed 1-6, 24, 48, 72 and 120 hours after injection and the localization of trypan blue granules was determined in histological sections.

Five hours after injection, the dye began to accumulate in the proximal yolk sac epithelium, and within 24 and 48 hours this cell layer was heavily loaded with dye. The dye never became detectable in any other membranes or the embryo proper. This pattern of dye distribution was the same whether injection was initiated at the 7½-8½ day stage of gestation or was delayed until the 10½-11½ day stage.

Histochemical tests for acid phosphatase revealed in the cells of the vitelline membrane from embryos of trypan blue-injected mothers very much lower activity than in the vitelline membrane from saline-injected controls.

The suggestion that loading with trypan blue affects other lysosomal enzymes of the yolk sac epithelium is being investigated. (Supported by Grants NB01716, NIH 5T1-GM-102, and 5 TOI HD00116 from NIH.)

342

HYMAN GUTHWIN and DANIEL FRIEDMAN, Hunter College of the City University of New York.

Mutual predatory behavior: *Amoeba* and *Pelomyxa* versus *Hydra*.

Amoeba proteus and *Pelomyxa* carolinensis exhibit aggressive behavior towards *Hydra*. The reverse was also observed. *Amoeba* commonly attacked *Hydra* tentacles. *Pelomyxa* occasionally attacked broadside but commonly attacked *Hydra* from the basal or oral ends. When *Pelomyxa* engulfed *Hydra* from the basal end, *Hydra* successfully paralyzed and ingested *Artemia salina* even when all but mouth and tentacles were within *Pelomyxa*. *Pelomyxa* was capable of severely constricting *Hydra* within the rim of *Pelomyxa*'s food cup. Cutting of *Hydra* was not observed.

When *Pelomyxa* engulfed *Hydra* tentacles, remaining free tentacles did not paralyze or retain *Artemia*. Engulfment involved mouth, hypostome and distal body. Generally, *Hydra* was released in time. Complete ingestion of *Hydra* by *Pelomyxa* was observed but *Hydra's* subsequent fate was not determined.

One instance of *Hydra* engulfing *Pelomyxa* was observed. The mouth did not gape prominently but the tentacular enfolding reaction occurred. Following total ingestion of *Pelomyxa* with characteristic *Hydra* body reactions, *Pelomyxa* was precipitously expelled. Mucilaginous particulate threads stretched from the mouth of *Hydra* to the body of *Pelomyxa*. *Pelomyxa* appeared unaffected. Within *Pelomyxa* was a partially digested brine shrimp.

Digestion of amoebae by *Hydra* and of *Hydra* by *Pelomyxa* remains to be ascertained, as does the possible adverse effect of the engagement of *Hydra* tentacles by *Amoeba* or *Pelomyxa* on the ability of *Hydra* to feed. *Hydra* eventually disappeared from the mixed culture whereas the initially fewer *Amoebae* and *Pelomyxae* thrived.

343

WILLIAM McENROE, Waltham Field Station.

Control of the "green +" and the "green —" behavioral type in the Two-Spotted Spider Mite (*Tetrynachus urticae* K.).

Two behavioral groups in the summer form of the adult female spider mite population are characterized by their differential spectral response at λ max. 380 and 530 mμ and their shift in gravitational response. These states are not two genetically distinct subgroups. The two stages are common to the adult population. The shift in behavioral response is correlated with the feeding activity and water stress of the animals. The above change in responses helps explain the behavior of these animals on fresh plants and on old blasted plants.

344

C. S. LIN, Huston-Tillotson College.

Nesting behavior of digger wasp *Cerceris morata*.

Cerceris morata Viereck and Cockerell is one of the largest species of the genus in North America, the average body length of the females is 20 mm. The field observations of the nesting behavior of this wasp were first made in late July, 1965 and July, 1966 on a wind-blown sand dune adjacent to Red River arm of Lake Texoma about 8 miles west of the University of Oklahoma Biological Station. Although over 30 nests were located by mid July, only 4-5 females were seen engaged in hunting and provisioning activities on any given day, presumably most of them spend very lengthy periods digging incredibly deep vertical burrows (2-3 meters) underground. The biology and prey selection of some *Cerceris* so far studied in this country including 12 species on hunt weevils and billbugs (Curculionidae), 5 species on leaf beatles (Chrysomelidae) and 2 species on metallic beetles (Buprestidae). *C. morata* hunts on a single species of darkling beetle, *Eleodes opaca* Say (Tenebrionidae) as larval food. (Supported by Grant-in-Aid from UOBS.)

345

NORMAN LIN, Ohio State University.

The establishment of a territory about the human observer by the cicada killer wasp *Sphecius speciosus* (hymenoptera sphecidae).

Sphecius was studied for nine generations (1956-1963, 1965) in the Parade Grounds in New York. On 10 (4.27%) of 234 days on which territoriality was observed, one or more males perched on the observer, chased passing males and returned usually to the same perch such as a hand or shoe. When the observer walked away, he was followed as far as 45 feet by the wasp which flew only inches from him. Each time the observer came to rest, perching and defense were resumed.

A perching day was usually followed by another perching day. Perching occurred in the same location, and on the same portion of the observer's anatomy, indicating the same wasps were involved.

Confirmation was made with two marked males.

While copulation was observed on only 19% of territorial days, it was observed on 70% of "human territorial days." Copulating pairs were observed within 27 feet of the "human territory holders" in 90% of the cases. One male pouncing on a copulating female, commenced perching on the observer when the pair left. Three males began perching on the observer located inches from a copulating pair.

Highly sexually motivated males seem to lose their ordinary fear response, and under these conditions may respond positively to the observer as a territorial perch. Successful perching presumably is rewarding, and trial and error learning probably occurs, accounting for repetition in the behavior one or more days later. One male showed evidence of one-trial learning following a delay of 24 hours.

346

MARY JANE WEST, University of Michigan.

The nature and determination of castes in Polistine wasps.

Adult *Polistes* females are of three functional categories: egglayers (queens), non-egglaying foragers (workers) and idle females. *Polistes fuscatus* queens observed in Michigan initiated the majority of new cells. Natural and experimental removal of queens resulted in a sharp decline of new cell addition and eventual colony disruption. Females which emerged when the queen was present became workers; females which emerged after queen removal did not forage. All marked females which mated and overwintered were of the latter (idle) category. Size and color differences between queens and workers are due to gradual seasonal change in brood characteristics; no discrete morphological difference between castes has been found. Marked overwintered siblings cooperated in nest founding. Sibling group formation results from orientation to the former nest site rather than recognition of former nest mates and depends on dominance-subordinance relationships between newcomers and resident females. Queen determination in *P. fuscatus* involves differential egg-eating, and cooperative nest enlargement continues during the period of competition among foundresses. However, in colonies of *P. canadensis* observed in Colombia (4°N. latitude) there was much aggression among foundresses and no new cell addition during this period. Only the established queen adds new cells. The mode of queen determination and consequently accelerated colony growth in *P. fuscatus* may be an adaptation to the shorter growing season of the temperate zone. Morphological caste differences in social wasps may also have originated as seasonal phenomena. The findings support the ideas of Hamilton (1964) regarding degree of relationship and the evolution of social behavior in insects. (Supported by NIH grants 5 TI GM 989-03 and 989-04 to the University of Michigan Department of Zoology, NSF GB-336 to the University of Michigan Museum of Zoology and the Edward C. Walker Scholarship.)

347

ILLAR MUUL, Walter Reed Army Institute of Research.

Photoperiodic influence on seasonal breeding cycles of *Glaucomys volans*.

The flying squirrel, *Glaucomys volans* has a bimodal breeding season in northern climates. In Michigan the largest numbers of litters were observed in April and May and again in August and September both in the wild and in the laboratory; none was observed in the winter. Experimental manipulations of photoperiod resulted in the delay of sexual activity (testicular descent) in males under a long photoperiod (15 hours) regime and a delay or absence of litters in females. Reversal of seasons through changes in photoperiod induced reproduction in winter (January) instead of summer. (Partially supported by grant GB-482 from NSF to Wm. Burt.)

348

G. EDGAR FOLK, JR., MARY A. FOLK, RICHARD C. SIMMONDS, and MAX C. BREWER, University of Iowa, Arctic Aeromedical Laboratory, and Arctic Research Laboratory.

A two-year study of winter lethargy in subarctic bears.

During the winter of 1964-1965, two grizzly and two black bears were studied in winter dens using the Iowa physiological radio-capsules (EKG and body temperature, long life, short range, implantable). The animals were maintained in isolated unprotected enclosures where they constructed dens of hay. Their wintering conditions closely simulated natural conditions. A condition of winter lethargy or dormancy was demonstrated; summer sleeping heart rates (40-50 b/m) were replaced by the winter resting heart rates of lethargy (8-12 b/m). During the winter of 1965-1966, the experiment was repeated on two grizzly bears. Again, winter resting heart rates were greatly reduced, down to 8 b/m. These results were compared with the data obtained by the same technique from wolves, wolverines, and Arctic foxes. Their sleeping heart rates go up in winter instead of down. (Supported by the NSF and by the Office of Naval Research.)

349

HERMAN KLEEREKOPER, McMaster University, Hamilton, Ontario.

A monitor of locomotion patterns of fish for direct computer analysis of orientation mechanisms.

Monitoring equipment was designed and built to obtain continuous data required for the construction of mathematical models of orientation of fish in a controlled environment, with and without sensory, particularly olfactory, stimulation.

Below the transparent bottom of a tank, measuring 15 ft × 15 ft × 3 ft deep, built of perspex supported on a steel frame, a square grid matrix of 2,500 photocells at 4 inch (10 cm) centers was mounted. The photosensitive surfaces of the cells, directed upward, received collimated light from a bank of low voltage bulbs. Each bulb is placed in the center of a fresnel lens below which a jet-black, aluminum honeycomb, 2 inches (5 cm) thick, is suspended. This lighting system avoids isolated vertical beams in the water and produces a uniformly lighted, collimated field.

The water supply is through perforated plates, placed in front of the four internal sides of the

tank. These plates were designed to produce laminar flow in any one of four transverse directions in the tank. Turbulent flow can be produced.

The 50 rows of 50 cells of the matrix form the X and Y axes of an electronic logics system and are continuously scanned. Thus, a cell, darkened by the presence of the fish in the optical field, is located and its address recorded on tape together with the time of the event. Direction, angular deflections, speed and diurnal rhythms form the basic data for computer analyses of locomotor patterns orientation mechanisms of the fish under investigation.

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WILLIAM J. L. SLADEN, Johns Hopkins University.

Social behavior of adult and juvenile Adelie Penguins. (Motion picture)

The film presents a preliminary report on a long-term population and behavior study of Adelie Penguins, *Pygoscelis adeliae,* in a rookery of 300,000 birds, and of the predator-prey relations with the South Polar Skua, *Catharacta maccormicki.*

During the past five years a total of over 24,500 Adelie chicks have been flipper-banded (Sladen and Penney, Bird Banding, 31:79, 1960) and/or web-punched at Cape Crozier, Ross Island, Antarctica, thus creating each year an increasing population of marked, known-age birds. The young depart to sea, returning to land in later seasons, first as juveniles, then as inexperienced breeders and finally as established breeders (Sladen, BioScience, 15:264, 1965). An estimated 7% of each of three age-groups (so far in our study in their second, third and fourth years) are returning to the rookery of their birth. A few three- and four-year-olds have laid eggs, but almost all were unsuccessful in rearing full-grown chicks.

The film compares juvenile non-breeding and first-time breeding behavior with that of the experienced breeders. The non-breeders wander around the rookery individually or in small groups. Their social interactions, which may at times be detrimental to the stability of the community, are illustrated in one example where a chick gets separated from the creche and is subsequently killed by skuas.

Also illustrated are hunting methods of the Leopard Seal, *Hydrurga leptonyx;* dramatic scenes on the beaches where Adelies negotiate ice-floes or heavy seas; and corralling and marking techniques. (Supported by grant GA-151 from Office of Antarctic Programs, NSF.)

PAPERS PRESENTED BY TITLE AND ABSTRACT ONLY

ANIMAL BEHAVIOR

351

DOUGLAS K. CANDLAND and T. JAMES MATTHEWS, Bucknell University.

Prediction of dominance rank from heart rate of the domestic fowl. (By title only)

Reports of physiological factors occurring in the establishment and maintenance of dominance orders in domestic fowl have not included direct recording of autonomic changes during social competition. In the present study, heart rate was recorded from adult white Leghorn roosters during pair comparison competition which matched each possible pair of birds from a six-bird flock against one another in competition for food. Each match consisted of a one-minute period in which the amount of time each bird controlled the food cup was recorded. This period was surrounded by two-minute, non-competitive exposure periods in which the birds could see, but not touch, one another. A three-minute period of visual isolation from one another occurred before and after each match. Heart rate was detected by subcutaneously implanted electrodes and conducted through direct leads to an oscilloscope rate monitoring device. To prevent electrode lead fouling during matches, birds were placed in individual compartments separated by a removable opaque wall for visual isolation and a screen partition which allowed visual exposure and food competition but maintained physical separation of the birds.

Birds high or low on the dominance order showed higher heart rates than birds in the middle dominance ranks. Heart rate was higher during exposure periods than isolation periods. Among birds which both won and lost contests, heart rate was higher when the match resulted in a win than a loss. (Supported by MH 06988-03 USPHS.)

352

DOUGLAS K. CANDLAND and DEREK B. TAYLOR, Bucknell University.

The aggression ratio: A measure of aggressiveness in domestic fowl. (By title only)

Indices of aggressiveness in domestic fowl are often based on the number of fights won; however, factors other than aggression contribute to the outcome (A. M. Guhl, in *Behavior of domestic animals,* E. S. E. Hafez, Ed., Wilkins & Williams, Baltimore, 1962). Presented here is the *aggression ratio* (A.R.), an index which includes information other than number of wins.

Six separately housed White Leghorn cockerels met in an initial paired encounter procedure daily for six days. Aggressive interactions were recorded by the method described by Guhl (Tech. Bull. Kansas Agric. exp. Sta., 1953, No. 73). On Day 7 the birds were moved to communal housing to form a flock and paired encounters were continued five days.

An A.R. was computed for each bird each test day with the formula

$$\text{A.R.} = \frac{a}{d + 1},$$

where a is the number of pecks and threats delivered by a bird, and d is the number of birds he dominates. The A.R. is the number of aggressive acts per bird that a bird directs against those he dominates.

Dominance relations were stable on Day 2 and remained unchanged after flock formation. Eliminating Day 1, the mean reliability of the A.R. before flock formation was $+ .917$ ($p = < .01$, $df = 4$), and after flock formation $+ .558$ ($p = > .05$, $df = 4$). Before flock formation a roughly linear relationship obtained between mean A.R.s and dominance rank; after flock formation an

inverted U-function appeared. Accordingly, the A.R. may be a useful technique for the description of aggressiveness and its correlation with other behaviors. (Supported by MH 06988-03 USPHS.)

353

SUSAN L. DONALDSON, A. J. HORVATH, MARY ANN ROSS and J. L. ALBRIGHT, Purdue University.

Dominance in young heifer calves as a function of breed difference over time and situation. (By title only)

Seventeen Holstein and 17 Holstein Red Danish Crossbred calves were raised from birth to the age of 10 weeks according to one of four feeding-rearing conditions: (1) Fed separately-reared separately; (2) fed separately-reared together; (3) fed together-reared separately; or (4) fed together-reared together. Two testing situations were conducted with each calf. In test #1, three calves from similar feeding-rearing conditions that had never been together were placed together. In test #2, one calf from each feeding-rearing condition was placed together. Dominance scores were calculated for each calf by scoring each subject in relation to each other subject. Student's *t* tests were computed to determine breed differences within groups and within tests. Data were further probed to determine reversals in position across time.

On test #1, purebred Holsteins that were fed together and reared together dominated Crossbreds from that same feeding-rearing condition. There were no other breed differences in any group or on any test. From all test situations and all groups, Holsteins dominated Crossbreds 91 times and Crossbreds dominated Holsteins 80 times. A total of 56 reversals of position of dominance between pairs of calves was observed.

These data suggest that breed differences in dominance can be significantly changed ($P<.05$) by appropriate feeding, rearing, and testing situations. It further confirms the observation of Hook *et al.* (Amer. Zool., 5:714, 1965) in that the dominance hierarchy among young calves is not so stable as among older cows. (Supported in part from a trust agreement between Purdue University and Normandy Farm, New Augusta, Indiana.)

354

WILLIAM C. GRANT, JR., Oxford University and Williams College.

Endocrine induced courtship in three species of European newts. (By title only)

Prolactin, the active principle which induces water drive in the spotted newt *Diemictylus viridescens,* was administered at various dose levels to three species of European newts (*Triturus cristatus, vulgaris* and *helveticus*) during their nonbreeding, terrestrial phase in the fall. Assumption of aquatic environments and partial development of secondary sexual characteristics was produced in all species. In water both sexes showed typical courtship patterns involving an alternating sequence of female advances with male, body spasms and tail fanning oriented toward the female. Prolactin is implicated as an important factor in eliciting sexual behavior independent of most gonadal influence as the gonads of both sexes continued in regression and males terminated courtship by simulated deposition of spermatophores. *T. helveticus,* the most aquatic species, responded to the lowest dose levels of prolactin (0.20 mg).

Quantitative analysis of behavioral sequences indicated that speed and intensity of male response was correlated with the timing of female advance. The total length of courtship was shortened relative to the per cent of total courtship time females spent in advancing although a too rapid advance not broken by periods of holding tended to disorganize male response. Previous work (Gauss stimuli to newt courtship. However, as complete 1961 etc.) has suggested the importance of olfactory stimuli to newt courtship. However, as complete sexual display was induced in males with the crudest type of female models during the present investigations it is concluded that at least in prolactin-treated animals visual stimuli are of equal importance. (Supported by Fellowship HD-29,500-01 and Grant HD 01164-09 from the USPHS.)

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NORMAN LIN, Ohio State University.

Standard and emergency courtship in the cicada killer wasp *Sphecius speciosus* (hymenoptera sphecidae). (By title only)

In standard courtship the male (mounted upon the female) may lash the female's antennae with his antennae or rub her eyes in up and down strokes with his forelegs. The above may occur in combination and in various sequences. Copulation usually follows 3-15 seconds after mounting, and in many cases, no courtship seems to occur.

Another courtship pattern occurred rarely. The male lashes the female's antennae—one at a time for about 15 seconds each—and alternates back and forth about 3 or 4 times. His left antenna lashes her left and his right lashes her right. Then he inserts his antennae between the female's, and spreads his antennae in a broad V. He then moves down the back of the female, his antennae pulling the female's antennae back over her head while he extrudes his genitalia and attempts copulation. In one case the male attempted standard courtship for 14 minutes, switched to "emergency" and on the second try, the female exposed her genitalia and copulation followed. Success was immediate on the other occasion of its use when a male switched to "emergency" after 10 seconds of standard courtship.

Courtship in *Sphecius* seems to increase sexual receptivity in the female. The existence of both courtship patterns may be explained as follows: "Standard" usually succeeds and can be performed more rapidly. Emergency courtship is perhaps a more potent releaser, but being of longer duration is called into play when "standard" fails. If this hypothesis is correct, subsidiary courtship patterns may be more widespread.

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Z. MICHAEL NAGY and JAMES R. MISANIN, Susquehanna University and Rutgers University.

Social preference in the guinea pig as a function of social rearing conditions and weaning age. (By title only)

To determine the effects of social rearing condi-

tions (being reared alone or with littermates) and age of separation from the mother upon later social preference, 120 Hartley guinea pigs were divided at birth into 10 groups of 6 males and 6 females. Five groups were separated from their littermates at birth (isolates); the other 5 remained with like-sex littermates throughout the experiment (communals). Within the isolate and communal groups, the mother was removed at 0, 4, 8, 16, and 30 days of age respectively for the 5 groups. At 135 ± 10 days of age, social preference was tested in a tilt-cage apparatus for 6 consecutive days, 15 minutes per day. One end of the tilt-cage contained another guinea pig behind a clear plexiglas barrier, while there was nothing at the other end. Time spent at either end and number of crossings were recorded on all days.

Social preference for another guinea pig was significantly greater ($p<.001$) for the communally reared animals than for those reared in isolation. Age of separation from the mother did not affect social preference for either group. Activity scores revealed that isolates were more active and displayed less "freezing" (no crossings) than communals, suggesting that the test situation was not as fear-provoking as for communally reared animals. (Supported by Grant M-1562, USPHS, to B. A. Campbell.)

BIOCHEMISTRY, CYTOLOGY and HISTOLOGY

357

JOYCE A. BARRETT and MARGARET A. KELSALL, Cleveland Psychiatric Institute.

Effects of aging on mast cells and arterial plaques in the hamster heart. (By title only)

Deposition of mucopolysaccharides in the coronary arteries and the aorta is considered an early stage of the atheromatous lesion (Moon and Rinehart, Circulation, 6:481, 1952). Mast cells, a source of the acid mucopolysaccharide heparin, which has lipemic and anticoagulant properties, are also implicated in atherosclerosis. The hearts of 15 male and 7 female Syrian hamsters, age 182 to 844 days, were fixed in Orth's fluid, serially sectioned at 16 or 30 micra, and stained with aqueous toluidine blue.

Heavy deposits of metachromatic substance and breakdown of the intimal elastic membrane occurred in the left coronary arteries and to a lesser extent in the right coronary arteries of all 6 animals, 534 to 844 days old. Scattered metachromatic granules, which may be precursors of these plaques, and increased metachromasia were noted in the coronary arteries of 14 of the 16 animals, 182 to 405 days old.

Mast cells are present in the adventitia of the left and right coronary arteries and along capillaries in the myocardium in both atria and ventricles of hamsters in all age groups. Mast cells did not occur in the intima, media, or in the adventitia of coronary arteries in areas of metachromatic plaques. This observation indicates that metachromatic granules of mast cells are probably not the direct source of mucopolysaccharide deposits in coronary arteries of old hamsters. (Supported by grants from The Heart Association of Northeastern Ohio and GRS 05563-03 USPHS.)

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WILLIAM H. BECKERT and BROTHER WALTER DOYLE, St. John's University.

Leukocyte culture and karyotype of *Bufo marinus*. (By title only)

The tropical toad, *Bufo marinus*, was chosen as the first in a series of investigations to elucidate the karyotypes of various Amphibia. Short term cultures of whole blood, leukocyte suspensions and bone marrow were utilized in the study. The culture medium was composed of fetal calf serum, tissue culture medium 199, and triple distilled water (22, 51 and 27 parts respectively). Penicillin (100 units/ml) and streptomycin (100 units/ml) were added to the medium which was distributed into one-half ounce glass prescription bottles in the amount of 4 ml per bottle. Most of the cultures received the mitotic stimulant, phytohemagglutinin (Difco, 0.015 ml/ml medium), and all were incubated at 25°C ±1°C for seven days. Colcemide was added to all cultures prior to hypotonic treatment, and slides were prepared by the air drying method.

Many good metaphase plates were obtained from all cultures, including those without phytohemagglutinin. These, we believe, are the first successful cultures of amphibian whole blood and plasma suspensions of leukocytes obtained without a mitotic stimulant.

The chromosome number of this species was found to be twenty-two. There are five large, four intermediate, and two small chromosome pairs, of which five are metacentric and six submetacentric. On one pair of the intermediate size chromosomes, which have been designated as numbers thirteen and fourteen, a satellite was detected on the shorter arm. In neither the male nor the female could a pair of sex chromosomes be distinguished. (Supported by Project B1 from St. John's University.)

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MARVIN HILL, The Creighton University.

Histotochemistry of phosphatase and dehydrogenase enzymes in the rat submandibular gland. (By title only)

Enzyme studies on the submandibular gland subserve the study of glandular function. Our results regarding the localization of alkaline phosphatase differ from previous reports in that activity was not found in both the nucleus and cytoplasm of acinar cells. Using sodium alpha naphthyl phosphate and Fast Blue RR, we find no nuclear localization and no activity in the acinar cells. We find this enzyme in capillary walls, the basement membrane of acini, in the basal cytoplasm of tubular cells and in the myoepithelial cells. This reaction may be used effectively in the demonstration of the myoepithelial cells of Boll which are prominent in the submandibular gland.

Using Burstone's procedure for acid phosphatase involving naphthol AS phosphate and Red-violet LB, the cytoplasm of the cells of the acini and the ducts show a positive reaction for this enzyme. The activity in the serous cells is greater than in the mucous cells. Cells of the ducts are more strongly positive than are the acinar cells, with the coloring being intense and uniform. The secretory tubules are non-reactive.

Using sodium succinate and nitro BT in the Nachlas method for succinic dehydrogenase, a slight diformazan deposition in the parenchymatous tissue is revealed. This is more evident in the serous than in the mucous components of the gland. By far the most intense reaction is in the intercalated and interlobular ducts. The reaction takes place in the mitochondria. This is the area of high transport activity. (Supported by Grant HD00180 National Institute of Child Health and Human Development.)

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ROY M. NUZZO, HELEN R. STRAUSSER and ROSE A. BUCSI, Rutgers—The State University.

Simultaneous disc electrophoresis of anionic and cationic substances. (By title only)

The polyacrylamide gel electrophoresis technique of Ornstein and Davis (Disc Electrophoresis, Dist. Prod. Ind., Rochester, N. Y., 1962) has been successfully used for separation of the many proteins found in serum and other biological fluids. The advantages of this method are partly offset by the necessity of performing dual trials of reversed polarity when the isoelectric points of some of the protein components of a mixture are unknown and when deviations from specific sample concentrations are desirable.

The following technique was devised and successfully employed to partially eliminate some of the above difficulties: A Shandon electrophoresis tank of the horizontal type was converted for tube use by means of a wooden support which was placed on the center bar of the electrophoresis apparatus. Polyacrylamide gels were prepared in tubes described by Ornstein and Davis, except that the stacking gel which usually contains the specimen was omitted. Two tubes were used for each specimen; these were connected to each other by means of a Y tube, cut to convenient length and fitted at two ends with short plastic couples into which the tubes containing the gels were connected. A buffer of desired pH and ionic strength was added to the open end of the Y tube. Tracer dye and desired amount of sample were then added. Then each set of two tubes was placed on the wooden support, the Y tube straddled the divider bar so that one tube served as the cathode the other the anode. We have used a constant current of 3 ma for each tube pair.

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JOHN D. TAYLOR, University of Arizona.

Electron microscopy of iridophores in hypophysectomized *Rana pipiens* larvae. (Introduced by Joseph T. Bagnara) (By title only)

Electron microscope studies of dorsal integument of hypophysectomized *Rana pipiens* larvae demonstrate that iridophores are of two types and are usually located in two areas of the dermis. The first type is small with thin arms and is usually found in contact with xanthophores which themselves are attached to the collagenous lamellae of the basement membrane by thin cytoplasmic filaments. In some cases these iridophores are attached directly to the collagenous lamellae by cytoplasmic filaments. The second type is larger with wide arms and is found above dermal melanophores. In both types empty spaces are found which are believed to represent sites of reflecting plates. It is also believed that these reflecting plates are lost during sectioning since silvery iridophores can be seen before sectioning. Reflecting plates are packed together in high concentrations in the first type of iridophore; while the second type of iridophore seems to have less packing with more cytoplasmic organelles. Crystalline structures of the reflecting places can be isolated by cytolization of iridophores and viewed by suspension on specimen grids. These studies reveal that they have a variety of shapes ranging from squares to octagons. Sections of iridophores indicate that sites of reflecting plates are approximately 0.18 μ thick; while isolation methods indicate that the majority of crystalline structures are approximately 1.9 μ wide and long. The largest crystalline structure observed was 5 μ long and 1 μ wide. Chemical analysis of these isolated crystalline structures indicate that they are composed of guanine, adenine and hypoxanthine. (Supported by NSF Grant GB-3681; Joseph T. Bagnara, Director.)

COMPARATIVE ENDOCRINOLOGY

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IAN P. CALLARD, Rutgers—The State University.

Testicular steroid synthesis in the snake, *Natrix sipedon pictiventris*. (By title only)

Natrix testicular tissue was incubated in 18-50 ml flasks (800 mg/flask) as described previously (Callard and Leathem, Proc. Soc. Expt. Biol. Med., 115:567, 1964). Each flask was treated separately. 16-^{3}H pregnenolone was added as precursor, and 4-^{14}C progesterone and testosterone were added prior to extraction for estimation of losses. On paper chromatography, three major peaks of activity corresponding to testosterone, pogesterone and pregnenolone were observed. Progesterone was identified by derivative formation and chromatography in one typical and one atypical system. After reduction, three major peaks were seen, corresponding to progesterone, 20 alpha and 20 beta hydroxy pregn-4-en-3-one. Aliquots of these three areas were found to have the same $^{3}H^{14}C$ ratio. Per cent conversion to progesterone was $1.85 \pm .16$. The testosterone area was also subjected to derivative formation. After oxidation, followed by reduction of the oxidation product (androstenedione), the $^{3}H^{14}C$ ratio remained constant in only 4/18 samples. In the remaining samples the radioactivity was too low to be measured with accuracy. Per cent conversion to testosterone in these four samples ranged from .005-.02. Histochemical localization of 3-beta hydroxy steroid dehydrogenase and comparison with histologic sections indicated the presence of the enzyme in the interstitial elements. (Supported by NSF Grant 1734 and USPH GM-835.)

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IAN P. CALLARD, Rutgers, the State University.

Conversion of acetate-1-C^{14} to progesterone by ovarian tissue of *Natrix sipedon pictiventris*. (By title only)

Ovarian tissue from *Natrix sipedon pictiventris* was incubated in 4-50 ml flasks as described previously (Callard and Leathem, Proc. Soc. Exp. Biol. Med., 115:567, 1964). 10 μc of acetate-1-C^{14} was

added to each flask as a precursor. Prior to extraction of the tissues and the incubate, H^3-pregnenolone was added to estimate procedure losses. The tissue was homogenized in the incubate and the resultant homogenate extracted. Steroid extracts were chromatographed on thin layer plates in systems: 1) 5% ethanol in methylene dichloride saturated with water and 2) 95% benzene/5% ethanol. Radioactive spots on the plates were located by exposure of the developed chromatoplate to Kodak "Royal Blue" X-ray film. The radioactive zone corresponding to standard progesterone was eluted from the silica gel and acetylated. On rechromatography of the acetate in system (2) a single radioactive spot was present migrating with progesterone. This material was eluted, reduced, and rechromatographed. Radioactive spots corresponding to progesterone, 20 alpha and 20 beta hydroxy pregn-4-en-3-one were located on autoradiography. On oxidation of the original progesterone material, no change was noted in the Rf of the compound. Quantitation of the progesterone zone in a Packard Liquid scintillation counter revealed 0.32% conversion of C^{14} acetate to progesterone by *Natrix* ovarian tissue. (Supported by NSF Grant 1734 and USPH GM835.)

EVOLUTION AND GENETICS

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MORRIS H. BASLOW and JAMES F. LENNEY, School of Medicine, University of Hawaii.

N-acetyl-L-histidine amidohydrolase activity from fish brain. (By title only)

The amino acid N-acetyl-L-histidine which is found in high concentration in teleost brain, but absent from mammalian brain (Zoologica 50:63-66, 1965) has been found to be deacetylated by a highly specific enzyme obtained from the brain of the skipjack tuna *Katsuwonus pelamis*.

The enzyme has been isolated from fresh tuna brain after homogenization in 0.05 M sodium phosphate buffer, dialysis, precipitation with 45% saturated ammonium sulfate and finally passage through a Sephadex G-100 column. An enzyme preparation 13.8 times more active than the crude homogenate has been isolated by these procedures. The pH optimum for the reaction was found to be 7.0 with the production of both acid and L-histidine. Maximum activity was obtained at substrate levels of 0.03 M and the enzyme preparation is stable to temperatures up to 35°C. A tentative molecular weight of 83,000 has been derived by gel filtration techniques.

The acylase has been found to be active against N-acetylhistidine and slightly active against N-acetylmethionine. N-acetylated derivatives of histamine, tryptophan, tyrosine, alanine, valine, glutamic acid, aspartic acid, cysteine, glycine and ornithine are not attacked.

Extracts from mouse and rat brain which are capable of deacetylating N-acetyl-L-aspartic acid were found to be inactive against N-acetyl-L-histidine which correlates with the absence of this amino acid from tissues of homeotherms and indicates that the enzyme activity may also be distributed along phylogenetic lines. (Supported by NSF grant GB-3607.)

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NEAL R. FOSTER, Academy of Natural Sciences of Philadelphia.

Structural defenses of aquatic animals against being swallowed whole by fishes. (By title only)

The majority of fishes (one of the largest and most significant groups of macroscopic predators in the aquatic ecosystem) swallow their prey whole, with a minimum of masticatory preliminaries. Teleonomically speaking, prey species such as various actively moving fishes, arthropods, and other animals rely upon specific structural or behavioral defenses to discourage predacious fishes from ingesting them. Those animals which are armed with sharp spines, serrae, ctenii, scutes, prickles, or tubercles usually have these structures pointing in a direction opposite from the direction in which the animal normally swims when it is fleeing a predator, for purely hydrodynamic reasons.

Since a predacious fish can seldom anticipate and "head off" the escape of its prey, the fish must instead pursue it, and the first portion of the prey which will be grasped in the predator's mouth will be that part which is nearest while the prey is fleeing. For this reason, the base of the tail of a fish such as a carangid (which swims "forward") may be the most heavily armored part of its body, while the head or chelae of a decapod crustacean such as a prawn or a crayfish (which flee "backward") is apt to be heavily armed with anteriorly pointing spines or serrae. Another evolutionary strategem is to increase greatly the width of some portion of the body such as the head (fishes) or the cephalothorax (many arthropods) and to arm the most exposed edge of this part with serrae or spines.

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W. F. HOLLANDER, Iowa State University (Ames).

Hydrocephalic-polydactyl, a recessive pleiotropic mutant in the mouse. (By title only)

Normal mice in a partially inbred stock derived from an X-rayed male in the Genetics Department's colony produced in 1964 some progeny of both sexes showing hydrocephaly and double hallux. Outcrosses of the normal parents to various other stocks gave only normal young. Linebreeding with progeny testing yielded a large number of abnormal offspring, with considerable variation in degree of hydrocephaly and of polydactyly. Sometimes one feature has occurred without the other, but they most commonly are associated. Also, most of the abnormal mice show a rabbit-like gait, both hindlegs tending to move in unison. With even moderate hydrocephaly, the mice have grown poorly and become emaciated, dying usually by two months. By contrast, young showing only double hallux usually have matured well. The males are consistently sterile, but most polydactyl females have given large litters and nursed them. In outcrosses only normal progeny have appeared.

It seems much less plausible to hypothesize two mutants with close linkage here than a single mutant syndrome with variable expressivity. Classing the progeny of segregating matings simply as normal : abnormal gives totals of 594 : 134. The deviation from 3:1 suggests incomplete penetrance, since little mortality occurs before classification.

The symbol *hpy* is proposed for the syndrome. Recombination with seven other marker loci has appeared free: *a, b, bt, Re, Fu, mi,* and *W*. (Supported by AEC Contract No. AT (11-1)-1382.)

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M. L. PETRAS and F. G. BIDDLE, University of Windsor, Ontario.

A new locus controlling the serum esterases in *Mus musculus*. (By title only)

The effects of this new locus, which controls the presence and absence of serum esterase *band VII* in the house mouse, *Mus musculus,* were studied using the techniques described by Petras (Proc. Natl. Acad. Sci. 50:112, 1963). Three inbred strains examined, C57Bl/10, C3H and CBA/J, possess *band VII* but numerous wild animals lack it. Results of five types of matings are consistent with a two-allele (with dominance), single autosomal locus mode of inheritance. That is: 1) offspring of the above inbred strains always possess *band VII*: 2) all 25 F_1 offspring, from matings of mice lacking *band VII* and C57Bl/10 animals, had *VII*: 3) matings of F_1 mice revealed 78 animals with *VII* present and 30 with *VII* absent; 4) backcrosses of F_1 mice on C57Bl/10 produced 43 offspring all of which had *VII,* and 5) backcrosses of F_1 mice on wild animals lacking *VII* produced 16 mice with *band VII* and 25 without.

This locus, which has been tentatively designated *Es-5,* is closely linked with *Es-1* on linkage group XVIII. An examination of several natural populations of mice in the Windsor area suggests the existence of a widespread polymorphism with both alleles being found in almost equal frequencies. (Supported in part by grants from the National Research Council of Canada and the Department of University Affairs, Ontario.)

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T. M. SONNEBORN, Indiana University.

A non-conformist genetic system in *Paramecium aurelia*. (By title only)

Until now, all examined syngens (i.e., 13 of the 14 known syngens) of *P. aurelia* have shown one or the other of two genetic systems for the determination and inheritance of mating types. One system (the A system) is characterized by independent caryonidal determination, the four caryonides of a synclone showing no correlation in their mating types. The other system (the B system) is characterized by a nucleus-dependent cytoplasmic factor which determines the caryonides: sister caryonides show high positive correlation of mating types, but co-conjugant clones show high negative correlation. The remaining syngen, syngen 13 (defined by Rafalko and Sonneborn, J. Protozool. 6, suppl., 30, 1959), is a non-conformist exhibiting neither the A nor the B genetic system. In it, mating types are determined and inherited as a simple pair of alternative Mendelian traits, all four caryonides of a synclone agreeing in mating type. Mating type XXV is recessive, mating type XXVI is dominant. Heterozygotes are predominantly type XXVI, but in at least some heterozygotes some cells go through brief transient phases as type XXV and this results in selfing. Thus far, selfing has never been observed in either homozygote. Without cell division, the type XXV member of selfing pairs can proceed quickly (within an hour or so) to a type XXVI phase. Both members of split pairs, if cultured, again produce predominantly type XXVI cultures that self. Whether the decisive "mating type locus" codes for mating type substances or operates indirectly, perhaps as a regulator locus, is under investigation. (Supported by Contract COO-235-32 of the AEC and by Grant E81-H of the American Cancer Society.)

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DOROTHEA J. WIDMAYER, Wellesley College.

Search for metagon activity for kappa's maintenance in stock 51m43 of *Paramecium aurelia*. (By title only).

Metagons, products of gene M of *Paramecium* which maintain symbiont mu, were described by Gibson and Beale (Genet. Res. 3:24, 1962). Metagon activity is characterized by a pattern of symbiont maintenance in which each descendant of animals becoming homozygous recessive maintains symbionts for seven generations and late generation cells containing symbionts produce progeny, at least one of which maintains symbionts. The theoretical importance of persisting gene products which confer phenotypes varying from genotypic expectation, prompted this search for evidence for metagons maintaining kappa, another cytoplasmic symbiont (See Sonneborn's review on kappa, Adv. in Virus Res. 6:229, 1959).

First fission products of homozygotes obtained by autogamy induced in heterozygotes (K/k) with mutant kappa 51m43 (Widmayer, Genetics 51:613, 1965) were separated. One half-clone served to identify sensitive lines; the other (reproducing three times daily) was studied for kappa's maintenance by either of two methods: (1) Fission products were exposed to killing kappa: if resistant (kappa indicated), cells divided and their progeny were tested. (2) After known fissions, half-clones were stained (Azur A) and examined microscopically for kappa.

Nineteen (19/36) sensitive lines (k/k) were studied for resistance. In seven, all animals were sensitive before fission six, and in all 19, *every* animal was sensitive before fission nine. *Every* animal of 22 of the 33 half-clones of genotype k/k stained before fission four lacked kappa. Thus in many lines mutant kappa is completely lost soon after gene replacement. If metagons are produced by gene K, they either are not stable in animals growing at three fissions per day or are incapable of maintaining mutant kappa. (Supported in part by Contract COO-235-31 of the AEC and grant E-81H of the American Cancer Society to T. M. Sonneborn, Indiana University, and by a Wellesley College Shell Faculty Research Award to the author.)

EXPERIMENTAL BIOLOGY

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IDELISA BONNELLY de CALVENTI, Instituto de Biologia Marina, Universidad Autonoma de Santo Domingo, Santo Domingo, Dominican Republic.

Molybdenum-copper interaction in the newt,

Notophthalmus viridescens: toxicity and pseudoerythroplastic anemia. (By title only)

Pseudoerythroplastic anemia specific for this newt was experimentally induced with intraperitoneal injection of 60 micrograms of copper (Calventi *et al.*, Anat. Rec. 137 (3):345, 1960) without affecting survival. In view of the undisclosed etiology of this dyscrasia, molybdenum was selected because of its known effect on copper absorption and retention by blood and internal organs in higher vertebrates.

Male newts (2.5 g) kept unfed for 3 weeks at room temperature, were injected intraperitoneally with 20, 40, 60, 80, 120, and 160 micrograms of molybdenum from a solution of ammonia molybdate (pH 4.7). During the first week all animals maintained their weight and activity without exhibiting skin and blood alterations that characterized newts injected with comparable amounts of copper. No microscopical changes were noted in the liver, kidney and spleen of newts sacrificed 48 hours after injecting 160 micrograms of molybdenum. When molybdenum treatment was repeated 6 days after the first injection, newts died in 2 days without exhibiting pseudoerythroplastic anemia. However, all newts developed anemia, and died in 48 hours, when molybdenum (40 micrograms) was given 1 hour after a non-lethal dose of copper (60 micrograms) that alone stimulated pseudoerythroplastid production.

The study suggested that in the newt molybdenum is less toxic than copper and it does not affect hemopoiesis. However, the combined effect of copper and molybdenum is more toxic, and pseudoerythroplastid formation is not suppressed.

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PO-CHUEN CHAN, L. IONE JOHNSON, FRANCIS C. MONETTE, JOSEPH LOBUE and ALBERT S. GORDON, New York University.

Early response of leukocytes from splenectomized rats to Phytohemagglutinin (PHA) stimulation. (By title only)

Pooled heparinized blood from 6-day post-splenectomized rats was incubated in PHA-P (Difco, 33 μg per 10 ml whole blood) for 30 minutes in an ice bath. Peripheral cultures from the same rats but without PHA-P served as controls. The blood samples were then centrifuged at 400 rpm for 10 minutes to obtain leukocyte-rich supernatants. These were then recentrifuged at 800 rpm for 10 minutes and the leukocyte buttons were resuspended with Medium 199 to a final concentration of 5×10^6 cells per ml. The cells were incubated in diffusion chambers as described by Johnson *et al.* (Brit. J. Haemat., in press) in the peritoneal cavities of rats splenectomized 6 days previously. Each host received four chambers. Similarly, leukocytes from normal rats were treated with PHA-P, or not exposed to this agent, and incubated in normal rats. The hosts were killed 18, 24, 48, 72, and 96 hours after receiving 2 injections of tritiated thymidine (20 μc intraperitoneally) at 60 and 30 minutes before sacrifice. Smears made of cultured cells were processed autoradiographically. Two thousand cells were enumerated per culture. By 18 hours of incubation, the PHA-stimulated leukocyte cultures from splenectomized rats exhibited an early blastogenesis and mitotic activity not observed in the PHA-treated leukocytes from normal rats. The labeling indices in the former group were also consistently higher during the first 48 hours of culture. Control cultures of non-PHA-stimulated leukocytes from normal and splenectomized rats showed the degenerative changes previously described by Johnson *et al.* (Supported by grant HE03357-10 and HE5645-02 from the National Heart Institute of the USPHS.)

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NICHOLAS D. DE PROSPO, Seton Hall University.

The role of the gonads and thyroid gland in quinoline-dye hepatocarcinogenesis. (By title only)

Having previously established that adrenalectomy and hypophysectomy alter the hepatocarcinogenicity of the dye quinoline-6-azo-p-dimethylaniline (Q6) (DeProspo, Bull. N. J. Acad. Sci. 9:38-39, 1964), an attempt was made to see if similar effects could be achieved by gonadectomy, thyroidectomy, the use of anti-thyroid agents, or the administration of sex steroids and thyroxine. Sprague-Dawley rats, averaging 125 grams in weight, were divided into nine experimental groups of ten animals each, with an additional ten males and ten females serving as "tumor controls." Tumors were induced in all rats by feeding them, *ad libitum*, a basic low protein, low riboflavin diet to which was added 0.03% Q6. There were three thyroid groups: thyroidectomized, thiouracil treated, and rats receiving daily ip injections of thyroxine. A group of gonadectomized males was injected with estradiol and a group of gonadectomized females was given testosterone. The two remaining groups of gonadectomized rats received no injections. Part of an intact male group received testosterone and the remainder were given diethylstilbesterol. An intact female group was similarly treated. After four months, all the rats were sacrificed and the liver tumor incidence was almost identical in all groups. This study indicates that liver tumors will develop when gonadectomized male and female rats are given Q6 and that sex steroids do not alter the carcinogenicity of the dye. Neither is there any difference in the susceptibility of both sexes. From the data obtained, there is no really convincing evidence that thyroid function is involved in the quinoline-dye initiation of liver tumors.

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IRA FOWLER, University of Kentucky, CHARLES E. MORRIS and ROYCE MONTGOMERY, University of North Carolina.

Rabbit lymphocyte transformation *in vitro* in experimental allergic encephalomyelitis. (By title only)

Human peripheral blood lymphocytes obtained from patients with multiple sclerosis and cultured three days in the presence of human cerebrospinal fluid (CSF) undergo increased per cent (10-15) transformation to a blastic cell (Fowler, *et al.*, New Eng. J. Med., in press). To investigate species specificity of antigens involved, experimental allergic encephalomyelitis was produced in six rabbits by multiple injections of rabbit spinal cord and Freund's adjuvant. One rabbit immunized with

CSF and four normal rabbits served as controls.

Duplicate tubes containing peripheral lymphocytes obtained from each were cultured in a series designed to contain its own controls. Each tube contained 3 ml of medium (T. C. 199 with 20 per cent chick embryonic extract and antibiotics) and approximately 1,500,000 lymphocytes to which was added either (1) CSF, (2) rabbit brain, or (3) no additional agents. Percentage transformation after three days was determined by counting 2,000 cells of each culture.

A transformation of approximately 10 per cent occurred in all cultures of lymphocytes from normal rabbits and in cultures without additives from immunized rabbits. With CSF added, lymphocytes from rabbits immunized with brain or CSF showed increased transformation (21 per cent). With brain added, transformation of lymphocytes from rabbits with encephalomyelitis was significantly greater (26 per cent) than that of the rabbit immunized with CSF (11 per cent).

Results suggest human CSF contains non-species-specific antigens to which rabbit lymphocytes are sensitized in experimental allergic encephalomyelitis. (Supported by GRSG-A3200 and NB06320-01 from USPHS.)

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SOPHIE JAKOWSKA and P. PAUL FAGUNDO, 27 West 96 St., New York.

Hepatotoxicity and fatty changes in the newt, *Notophthalmus viridescens*. (By title only)

Adult males collected in Connecticut in early summer (1.4-2.1 g B.wt.), kept unfed for 1 month at 15°C with 12-hr artificial daylight cycle, were given a single intragastric dose of 7.5 ml/kg of a miticide, 2-(p-tert-butylphenoxy) isopropyl 2-chloroethyl sulfite. The study was designed to determine the onset of hepatomegaly and increased liver fat. The latter was visualized directly with oil red O in sections prepared by a modified method utilizing propylene glycol and Carbowax, a polyethylene glycol polymer (Jakowska and Fagundo, unpublished).

Deaths occurred from 5 to 12 days. In sacrificed newts, liver weight increased after 1 day to 6.33% B.wt. (vs 3.5% B.wt. in controls), and to 7, 8-8.67, and 10% B.wt. after 5, 7, and 12 days, respectively. Only occasional hepatocytes with a few fat globules were seen 24 hrs after dosing; after 48 hrs larger numbers of globules appeared, especially in hepatocytes below the granulocytopoietic layer. Starting with 6 days practically all the hepatocytes contained increasing amounts of fat. In three pairs united in coelomic parabiosis the partners dosed once intragastrically with 5 ml/kg showed in 19 days numerous hepatocytes with confluent fat globules. The opposite parabionts, untreated, showed a lesser increase in liver fat. In selected livers from chronic dermal toxicity studies (Amer. Zool. 5:730, 1965) the amount and distribution of fat varied in proportion to liver weight, as it was the case in early changes following a single gastric dose. The pattern of fat distribution in all these treated newts differed, however, from that in fed and fasted untreated animals.

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SOPHIE JAKOWSKA and P. PAUL FAGUNDO, 27 West 96 St., New York.

Nutritional studies in newts, *Notophthalmus viridescens*. I. Dehydrated beef liver suspension. (By title only)

Captive newts are usually fed raw beef liver and individual consumption left to chance. Supportive intragastric feeding with known amounts of specific nutrients is desirable in long term toxicological and surgical experiments, especially after prolonged fasting and hibernation.

Adult males, collected in autumn in North Carolina, kept unfed for 3 months at 15°C with 12-hr artificial daylight cycle, averaged 2.7 g body weight. Under these conditions, dehydrated liver in aqueous suspension (25 mg/0.1 ml, equivalent to 95 mg fresh liver) was given intragastrically once a week for 11 successive weeks to 10 animals, 10 others constituting unfed controls. At one and 2 weeks after the last dose there was no difference in body weight of fed and fasted newts (average 2.33 g), but at the latter period fed newts showed a slight increase in the weight of the liver (3.54 vs. 3.02% B.wt.) and of the empty gastrointestinal tract (3.86 vs. 3.63% B.wt.), and a significant increase in spleen weight (1.17 vs. 0.65% B.wt.). Fat bodies were absent in both groups.

Peripheral blood of fed newts showed active regeneration, consistent with actively hemopoietic spleen and perihepatic layer; this was associated with thickening of duodenal submucosa. In fasted newts older erythrocytes predominated, the spleen was inactive, the perihepatic layer deduced and disorganized.

Although gradual weight loss was not prevented in these newts fed dehydrated liver suspension, the findings suggested metabolic improvement.

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RAOUL MICHEL MAY and JACQUES BOUCHARD, University of Paris.

Effect of tobacco tars on five-day-old chick embryos. (By title only)

Five-day-old chick embryos were treated with tobacco tars dissolved in carbowax (polydiethylene glycol, both lipo- and hydrosoluble), and with pure carbowax.

Histological studies on the 10th day of development show that the tobacco tars bring about an important mortality, frequent coelosomies and numerous haemorrhages, while pure carbowax has a similar action, but much less marked.

Besides, with tobacco tars abnormal mitoses are very numerous and accompanied by dystrophy of mesenchymatous elements: 34.9% abnormal metaphases against 15.5% with carbowax alone. We observed lesions of mitotic spindles, with partial migration of chromosomes four times (17.7%) more often in embryos treated with tobacco tars dissolved in carbowax than in those treated with pure carbowax (4.1%). In such cases chromosomes are often expulsed, and more often still migrate independently of each other in the spindle. Tobacco tars thus have a deleterious effect on the spindles of dividing cells in chick embryos.

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E. A. MIRAND, R. MILANOVICH, E. DYWINSKI, and A. BULBA, Roswell Park Memorial Institute and State University of New York at Buffalo.

Depressant effect of hypertransfusion and transfusion on murine and human erythropoietin levels. (By title only)

Hyperoxia, acute starvation, and hypertransfusion of washed red blood cells to a mouse are an effective depressant of erythropoietin and erythropoiesis. Administration of 2.5 ml of isologous washed red blood cells intraperitoneally in a period of 3 days increases the hematocrit to 70-75%. At this time, reticulocytes are at 0.0% and bone marrow and spleen erythropoiesis ceases. This state can be maintained indefinitely by red cell transfusions. Thus, the hypertransfused mouse is a sensitive assay for erythropoietin. In a patient with myeloblastic leukemia, 24-hour urine samples were collected on 3 consecutive days. The patient had hematocrits and hemoglobins in the range of 28-32% and 9-10 grams. The urine sample from day 1, concentrated by carbowax 1:10, showed high levels of erythropoietin as judged by 16-17% Fe^{59} uptake in hypertransfused mice. After transfusion with a pint of whole blood on day 1, the erythropoietin level in the urine on day 2 decreased to 5.9% Fe^{59} uptake. An additional pint of blood given on day 3 further reduced the erythropoietin level to 0.78% in the day 3 24-hour urine sample. Evidence is provided that like hypertransfusion of red blood cells to a mouse, transfusion of whole blood to patients can drastically reduce the output of erythropoietin. Moreover, although the patient was still anemic (hematocrit 33%) after transfusion, the erythropoietin level had been reduced. This response suggests that factors other than red cell volume alone are involved in the production and release of erythropoietin. (Supported by USPHS grants AI-04506-06, CA-07745 and FR00262-01, the American Cancer Society, and the John A. Hartford Foundation, Inc.)

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E. A. MIRAND, C. REILLY, and R. MILANOVICH, Roswell Park Memorial Institute, University of Arizona, and State University of New York at Buffalo.

A murine virus producing polycythemic hypervolemia. (By title only)

An attempt was made to detect the presence or absence of erythropoietin in HaM/ICR Swiss mice infected with a polycythemic strain of Friend virus which produces a syndrome similar to polycythemia vera of humans. Repeated bleeding of polycythemic (hematocrit level $\geq$70) mice was done to create normal or anemic levels. Bleeding was performed via jugular vein at 24 hour intervals. Some animals required as many as 5 bleedings, and in this group an average of 4.2 cc of blood per mouse was withdrawn. Similar test animals bled only 4 times had an average of 4.0 cc of blood removed. Although the values were not accurate estimates of the total blood volume, the amount of blood removed far exceeded normal levels for a mouse. Using I^{131} labelled albumin (RISA) it was noted that a progressive increase in plasma volume accompanied the increase in erythroid elements. Plasma volume had increased 2.5% at 7 days post-infection; at 21 days 28.3% and at 45 days 48%. Total red cell volume (determined by Cr^{51} labelling of erythrocytes) increased 2 times normal at 21 days and 3 to 4 times normal at 45 days post-infection. These data suggest that the Mirand strain of Friend virus (1961-66) produces a polycythemic hypervolemia along with other characteristics of Friend Disease. Moreover, significant levels of erythropoietin were not detected in this viral induced polycythemia. The question remains as to whether erythropoietin is involved in this type of polycythemia as well as in polycythemia vera in humans. (Supported by USPHS grants AI-04506-055, CA-07745 and FR00262-01, the American Cancer Society, and the John A. Hartford Foundation, Inc.)

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E. A. MIRAND, L. WEISS, and J. JIVIDEN, Roswell Park Memorial Institute and State Universtiy of New York at Buffalo.

An immunological study on Friend Virus Disease. (By title only)

Friend Virus Disease (FVD) is associated with malignant reticulum cell proliferation, hypervolemic polycythemia, and a pronounced erythroblastosis in some strains of adult mice, as well as progressive hepatosplenomegaly. A study with formalinized vaccine was carried out to determine if this vaccine would prevent infection, increase the latent period or delay death of the infected animals receiving the vaccine. Ha/ICR Swiss male weanlings were injected i.p. with a 0.2 ml 10% cell-free splenic filtrate from FV infected animals. The formalinized vaccine was made by diluting the filtrate with formalin 1:100 and aged for 21 days. 0.25 ml was injected i.p. into the same mice at intervals ranging from 72 hrs pre-virus to 72 hrs post-virus injection. Protection occurred in the pre-virus groups and up to 6 hours post-virus. Plasma from those animals free from FV infection for over one year was used for *in vivo* neutralization test. The plasma was mixed (1:1) with a 10% FV cell-free splenic filtrate in dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. 0.2 ml of this mixture was injected i.p. into Ha/ICR Swiss female weanlings. The ID_{50} titers (Kärber Method) are as follows: virus and normal plasma (control group) 10^{-5}/ml; vaccine only plasma, $10^{-4.5}$/ml, 24 hr pre-virus plasma, $10^{-3.7}$/ml; 0 hr plasma, $10^{-3.5}$/ml; 2 hr post-virus plasma, $10^{-4.1}$/ml and 6 hr post-virus plasma, $10^{-3.1}$/ml. One can conclude that delayed death was observed in the low dilution groups and complete protection was achieved in the 10^{-4} group as compared with 100% infection in the controls. (Supported by NSF GW-564, AI-04506-05, and CA-07745.)

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GEORGE D. RUGGIERI, S.J., and ROSS F. NIGRELLI, New York Aquarium.

Effects of extracts of the sea star, *Acanthaster planci*, on the developing sea urchin.

A saponin-like substance was extracted from the whole sea star, *Acanthaster planci*, using the procedure described by Yasumoto, *et al.* (Bull. Jap.

Soc. Scientific Fisheries, 30:357, 1964). This extract was tested on sperm motility and pre-fertilized and 2-cell stages of the sea urchin *Arbacia punctulata*.

Sperms were immediately immobilized by concentrations of 50 ppm. Concentrations of 10 ppm caused a noticeable decrease in sperm motility.

Unfertilized eggs exposed to concentrations of 50 ppm for 2 hours, washed and transferred to sea water and fertilized, underwent early cleavage stages: these were abnormal and did not develop further. Unfertilized eggs exposed to concentrations of 10 ppm and fertilized after 2 hours did not raise fertilization membranes and underwent nuclear division without cytoplasmic division. Animalized larvae were obtained when eggs were treated prior to fertilization with concentrations of 50 ppm for ½ hour and 10 ppm for 1 hour.

Two-cell stages exposed to concentrations of 50 ppm for 5 minutes resulted in animalized larvae. Treatment for ½ hour at this concentration caused an arrest in development at late cleavage stage. Exposure of 2-cell stages to concentrations of 10 ppm for 1-2 hours produced animalized larvae. Continuous exposure of 2-cell stages to concentrations of 5 ppm arrested development at late cleavage. Treatment with this concentration for 2 hours resulted in 95% normal plutei and 5% animalized larvae. (Supported by a grant from the John A. Hartford Foundation, Inc. to the New York Zoological Society.)

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GEORGE D. RUGGIERI, S.J., and ROSS F. NIGRELLI, Osborn Laboratories of Marine Sciences, New York Aquarium.

Animalization of *Arbacia punctulata* larvae by extracts of the sea cucumber, *Holothuria edulis*. (By title only)

Animalization of sea urchin larvae has been obtained with toxins extracted from a wide variety of echinoderms (Ruggieri, G. D., Toxicon 3:157-162, 1965). The balance maintained by a double gradient in the sea urchin egg is shifted resulting in a hyper-development of the ectoderm at the expense of the entomesoderm.

A saponin-like toxin has also been extracted from the Hawaiian sea cucumber, *Holothuria edulis*. This extract is a powerful cytotoxic agent. Unfertilized eggs and various cleavage stages were arrested in development and severely cytolyzed by relatively weak concentrations (continuous exposure to concentrations of 1 ppm). Fragmentation of the developing embryo was obtained when 2-cell stages were exposed to concentrations of 1 ppm for 1 hour.

Sea urchin sperms were immediately immobilized by concentrations of 1 ppm and motility was markedly decreased at concentrations of 0.5 ppm.

Unfertilized eggs, exposed to concentrations of 1 ppm for 1 hour and 0.5 ppm for 2 hours and then transferred to sea water and fertilized, resulted in animalized lavae.

Animalized larvae were obtained when 2-cell stages were exposed to concentrations of 5 ppm for 5 minutes, 1 ppm for ½ hour and 0.5 ppm for over 3 hours. Continuous exposure of 2-cell stages to concentrations of 0.1 ppm resulted in 75% normal plutei and 25% animalized larvae. (Supported by a grant om the John A. Hartford Foundation, Inc. to the New York Zoological Society.)

PROTOZOOLOGY

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E. FAURÉ-FREMIET and J. ANDRÉ, Collège de France, Paris, and Faculté des Sciences d'Orsay.

Ultrastructural study of the ciliate *Pseudomicrothorax dubius* Maupas and its systematic and evolutionary implications. (Introduced by J. O. Corliss) (By title only)

The electron microscope has revealed interesting structural aspects in the morphology of *Pseudomicrothorax dubius* Maupas which are of significance in the comparative morphology, evolution, and systematics of a sizeable group of rather enigmatic holotrich ciliates. Such a study has allowed acquisition of data beyond those obtainable in the light microscopic work carried out several years ago by Corliss, using silver impregnated material, who emphasized possible hymenostome affinities for *Pseudomicrothorax*. Three characteristics studied in some detail definitely appear to relate this curious organism to *Leptopharynx* in the family Microthoracidae; but the exact taxonomic position of that entire family must remain uncertain until further ultrastructural investigations can be carried out on members of the probably related cyrtophorine gymnostome family Nassulidae. Certain hymenostome species may also be implicated.

The principal structures examined were the following:

(1) The "nasse" in the oral area, a structure equipped with simple, asymmetric nematodesmas. Such "trichites" are also known for certain Nassulidae and for the *Leptopharynx* species studied by Prelle.

(2) The non-toxic trichocysts, which are of the fusiform type and possess apical caps deployed into four arms opening like an umbrella on their discharge. Kahl long ago described similar structures for the Microthoracidae.

(3) The sub-pellicular membrane, which lends rigidity to the ribbed body surface of the ciliate. This *lamina corticali*· is ca. 120-150 mμ in thickness in *Pseudomicrothorax*; a similar membrane is thicker in several Nassulidae, but slightly thinner (50-100 mμ) in *Leptopharynx*.

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DAVID McLAUGHLIN, ALICE PLATTE, and HAROLD E. FINLEY, Howard University, Washington, D.C.

Induced division synchrony in *Telotrochidium henneguyi*. (By title only)

Standard temperature cycling was applied to our laboratory stock of *T. henneguyi* to induce synchronous cell division. The peritrichs were cultivated in tela broth as described by Finley and McLaughlin, *J. Protozool.*, 12:41, 1965. The stock normally attained maximum log growth 48 hr after initial inoculation; it was further characterized by a doubling of the population in 4.19 hr at a rate of 0.24 generation/hr.

Cells were allowed to grow 18 to 24 hr (lag phase) and then subjected to different heat shocks in the range 23°-60°, with the result that 38°

was chosen for synchronization. At the end of the fourth synchronizing 30/10 cycle the division maximum of experimental cells was 23.2%, in contrast to no division in comparable control cells maintained at 28° constant temperature for the same duration as experimental cells. (Supported by grant 6/7410-3427 from the NSF.)

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OTTO H. SCHERBAUM, JEAN HENZE, and JOHN E. BYFIELD, University of California, Los Angeles.

Effect of temperature shifts on lipid metabolism in synchronized *Tetrahymena*. (By title only)

The effects of single and multiple temperature shifts on the incorporation of C^{14} glycerol into TCA insoluble, chloroform/methanol soluble material (including the bulk of structural lipids) have been studied in a heat-synchronized ciliate (*Tetrahymena*). Single shifts to temperatures 5-7°C above the optimum value result in increased rates of incorporation in the strains studied. The temperature shifts paralleled those needed for the induction of synchronous cell division in the respective strains. When returned to the optimum temperature after thirty minutes, a reduction in rate is seen suggesting a possible temperature-related feedback control. Since protein synthesis is known to be reduced during similar shifts these cells experience a dissocation of growth parameters (protein and lipid) during such shifts. When studied throughout the cycle leading to the induction of synchronous division a net increase in accumulation of lipid occurs resulting in a significant excess (per cell) at the end of the heat treatment. Actinomycin D can prevent the subsequent synchronous division but has no effect on glycerol incorporation which occurs linearly during the recovery period and synchronous division. Presumably, therefore, the vital mRNA fractions depleted during the heat treatment and synthesized and translated during the recovery period do not appear to be tightly linked to the controls of lipid biosynthesis operative in these cells. (Supported by NONR-233-71.)

RADIATION BIOLOGY

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DON J. KARR, Oregon Technical Institute, and A. L. SODERWALL, University of Oregon.

The effects of X-irradiation on the seventh and eighth day placental tissue of the golden hamster. *Mesocricetus auratus* Waterhouse. (By title only)

Pregnant hamsters were exposed to 100r whole body X-irradiation at 72 hours developmental age (post ovulation). The embryo at this time of development is in the morula stage (9-16 cells) and has just entered the upper uterine cavity. One could postulate any damage would be to the embryos and not to the decidua as no implantation sites have been as yet indicated. Animals were sacrificed at days seven and eight developmental age. Macroscopic examination gave no evidence that reabsorption had begun at sites where fetal mortality might be occurring. About 50% resorption evidence can normally be seen at day nine. Normal histological techniques were applied. Proper controls were prepared. The decidua, fetal placentae, and embryos were examined to see if some criterion could be established in the tissue that would predict forthcoming fetal mortality and consequent resorption.

At day seven, two of fourteen embryos were in advanced stages of degeneration with leukocytes packed into the area around the site of resorption. There were no other evidences of damage in the remaining embryos.

At day eight, one of sixteen embryos was nearly resorbed, while eight other sites with no evidence of resorption had extremely high concentrations of leukocytes (mostly neutrophils) packed in capillaries and vessels mesometrial to the embryo. No noticeable damage was visible in these embryos nor in the fetal placental tissues. The remaining seven embryos and tissues appeared normal throughout.

The sites of extreme leukocyte concentrations are being investigated to see if they could be used as evidence that fetal mortality is occurring. (Supported by Grant 50-4600 with the USAEC.)

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ROSAMOND KILLEBREW, Louisiana State University.

Morphological changes in *Dugesia tigrina* regenerated parts due to Cobalt 60 irradiation. (By title only)

Cobalt 60 irradiation of posterior parts of *Dugesia tigrina* produced greater diversity of forms in groups exposed to the largest doses. Irradiation in low doses accelerated regeneration of heads by one day over controls. Planarians undergoing accelerated regeneration lived as long as the controls, 5 months.

The doses ranged from low level at 1600 R to 9600 R, lethal. Previous investigators in this area used much larger doses on the whole animal. In this study the heads were removed before irradiation of the parts.

The classical descriptive method for head forms devised by C. M. Child (Head forms and head frequencies in planarian reconstitution. In W. C. Child, Patterns and problems of development, University of Chicago Press, Chicago) was used in this investigation.

From 84 posterior portions of *D. tigrina* given 1600 R there were regenerated 78 normal heads, 2 tetratophthalmic and an X, or unclassified form.

At 3200 R, 43 normal heads were regenerated, 18 tetratophthalmic, 16 acephalic, and 7 X forms.

At 6400 R only 17 normal heads were produced, 22 tetratophthalamic, 12 tetratomorphic, 10 anophthalmic, 14 acephalic and 9 X forms.

The 9600 R dose was lethal to the total population.

In the control group 72 normal heads were regenerated, 2 acephalic, 10 deteriorated. Since the irradiated portions were free of parasites, it is expected that the control group suffered from parasitic deterioration.

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EVELYN KIVY-ROSENBERG and BENJAMIN W. ZWEIFACH, New York University Medical Center.

Vascular reactivity of the microcirculation of young

rats following X-irradiation *in utero*. (By title only)

A good parameter of microcirculatory sufficiency is the reactivity or responsiveness of the muscular vessels. This was tested by direct microscopic observations of the circulation in the exteriorized mesocecum of rats (under anaesthesia) as described by Zweifach (Ergeb. Anat. Entwicklungsgeschichte 35:175, 1956). The reactivity or constrictor response was measured by determining the lowest quantity of topically applied epinephrine (i.e., epinephrine threshold concentration or E.T.C.) which stopped the flow through capillary side branches.

Studies were done on 328 young rats of both sexes between ages 15-42 days. These included offspring of 78 pregnants which had received 100 R at 10 ± .5 days of gestation, 9 which had received 200 R at 15 ± .5 days of gestation and 71 unirradiated pregnants. At ages 15-21 days, controls were less responsive to epinephrine than the offspring of pregnants which had received either 100 R or 200 R: i.e., the E.T.C. for over 67% of the controls was 0.5 μg or more, whereas over 60% of those which had received 100 R *in utero* and over 75% of those which had received 200 R *in utero* had an E.T.C. which was below 0.5 μg (some as low as 0.2 μg). With increasing age (from 22 to 42 days) there was a gradual shift toward the control E.T.C. range. It appears that prenatally irradiated individuals that continued to live and grow, approached levels of vascular reactivity of controls of parallel age. (Supported by Grant H-4298 (C_2) from USPHS and Contract AT (30-1) 1680 from USAEC.)

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M. L. MANDELL and E. L. COOPER, University of California, Los Angeles.

Allograft survival and irradiation in *Rana pipiens*. (By title only)

The effects of Co-60 upon the median survival time (MST) of skin allografts were studied in the leopard frog, *Rana pipiens*. Twenty-four frogs received 1000 r of whole-body radiation and were grafted five days later. The MST of their allografts was 22 ± 4.5 days. There was a significant difference between the MST of this group and normal controls (MST: 13.5 ± 0.6 days). The reaction rate curves of these two groups were also found to deviate significantly. A third group of twenty frogs received 3000 r of sub-total radiation; the left tibio-fibula being shielded in order to provide undamaged bone marrow. These animals also received allografts five days after exposure. The MST for their grafts was 17.5 ± 0.8 days. This group of marrow protected frogs rejected its grafts significantly faster than the previous experimental group (MST: 22 ± 4.5 days) even though the marrow protected animals were exposed to three times the gamma radiation. Though able to reject their grafts faster than the unshielded groups, these frogs were unable to survive the lethal effects of the radiation as long as the whole body irradiated group. A final experimental group of twenty-five frogs received 1000 r of whole-body radiation, but was grafted ten days after exposure. The results from this group are not yet conclusive; however, these animals appear to be rejecting their grafts more rapidly than their companion group, which was grafted five days after exposure. (Supported by Grant AM 09030 from the USPHS.)

COMPARATIVE ENDOCRINOLOGY

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J. N. BALL, M. R. GIDDINGS, and M. P. HANCOCK, University of Sheffield, England.

Pituitary influence on hepatic glycogen stores in the teleost *Poecilia latipinna*. (By title only)

Liver glycogen (as % liver wet weight) was determined in adult female *P. latipinna* by Montgomery's (1957) method. Fish were routinely fed Aronson's mixture and kept in 1/3 sea water at 25 ± 1°C, with 9 h illumination per day. In *experiment A*, intact fish (8) had 2.57 ± 0.61% glycogen, while 23-day hypophysectomized fish (7) had 9.02 ± 1.49%. Intact fish starved for 9 days (8) had 1.38 ± 0.28%, and starved hypophysectomized fish (7) had 7.39 ± 1.68%. Thus hypophysectomy elevates glycogen stores ($p<0.01$), even in starved fish ($p<0.01$). In *experiment B*, pituitary hormones were injected into hypophysectomized fish on alternate days for 2 weeks, starting 14 days after hypophysectomy. Glycogen figures were: intact controls (11), 3.23 ± 0.82%; hypophysectomized fish with saline (9), 8.73 ± 0.58%; with growth hormone (0.01 IU/g, 9 fish) 9.58 ± 1.18%; with prolactin (0.01 IU, 11 fish), 8.99 ± 0.89%; with TSH (0.006 IU, 10 fish), 7.45 ± 0.77%; and with ACTH (0.75 IU, 8 fish), 6.70 ± 0.57%. Only ACTH reduced glycogen ($p<0.05$).

In *experiment C*, in which the fish were intensively fed, intact fish (12) had 2.92 ± 0.51% glycogen; 21-day hypophysectomized fish (15) had 13.62 ± 1.72%; and fish in which the pituitary gland had been autotransplanted to the axial musculature 21 days previously had 7.92 ± 0.82%, significantly less than the hypophysectomized group ($p<0.01$). Thus the autotransplanted pituitary continues to secrete the factor, perhaps mainly ACTH, that opposes excessive storage of glycogen, though unable to reduce this storage to normal levels. (Supported by a grant from the SRC.)

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INA C. BRADEN and C. DONNELL TURNER, Duquesne University.

Effects of early administration of steroid hormones on later size and behavior of male and female rats. (By title only)

Testosterone propionate (1.75 or 2.00 mg) was administered to 14 female pups and estradiol dipropionate (100 μg) to 13 males, subcutaneously in sesame oil on the third and sixth days after birth. Controls received sesame oil (9 females, 7 males) or nothing (5 females, 9 males). After weaning the animals were given Purina lab chow *ad lib* with supplemental fruit or vegetables and vitamins. At ages 4 to 14 weeks the estrogenized males were markedly smaller than male controls in live measures of body weight, head width and length, tail length and body length. The group of androgenized females was slightly larger than the group of control females on all but the lateral head measure. Behavioral tests at maturity indicated that control females were most active of all

groups in a revolving drum and were least hesitant to leave their home cage to explore an unfamiliar open arena. Normal males were less timid than estrogenized males in the same situation. Further behavioral tests are in progress.

These results agree generally with those of Swanson (J. Endocrin., 34:vi, 1965) who found that androgen treatment of neonatal female hamsters was followed by reduced activity and increased emergence latencies. The data are also in accord with those of Swanson and Ten Bosch (J. Endocrin., 26:197, 1963) concerning effects of neonatal androgen treatment on growth of rats. Thus the present experiment confirms and elaborates present knowledge that treatment with hormones, neonatally, affects both growth and behavior of rats. (Supported by grant HD 99597-04 from USPHS.)

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ROBERT R. CARDELL, JR., Harvard University.

Alternations in the endoplasmic reticulum of rat liver cells following hypophysectomy and prolonged growth hormone administration. (By title only)

Hypophysectomy impairs the ability of the rat hepatocyte to synthesize proteins and to maintain its glycogen stores. When, however, growth hormone is administered after pituitary ablation the capacity of the liver cell to incorporate amino acids into proteins is enhanced and its ability to store glycogen is restored. In the present experiments, liver samples from hypophysectomized rats (4-120 days after the operation) and hypophysectomized animals injected intraperitoneally daily with 2 mg of crystalline growth hormone (NIH-GH-S6) for 10 days were fixed in glutaraldehyde-osmium and examined by electron microscopy.

The rough endoplasmic reticulum in liver cells from hypophysectomized animals shows dilated cisternae which contain a finely fibrous material and moderately electron-opaque granules approximately 500Å in diameter. The pattern of the rough ER is more disorganized than in control liver cells and the stacks of parallel cisternae characteristic of control liver cells are encountered rarely in hepatocytes from hypophysectomized rats. Growth hormone administration restores the normal appearance of the rough ER.

The smooth endoplasmic reticulum normally is present in the glycogen rich areas of the cytoplasm of hepatocytes from control animals, and the tubular or vesicular elements of this system have an intimate relationship with the glycogen particles. After hypophysectomy the glycogen particles are absent in most liver cells and greatly reduced in quantity in others. The smooth ER is absent from those cells devoid of glycogen and appears in those containing glycogen particles. Growth hormone administration restores the glycogen and smooth ER to the hepatocyte.

The results of this study show that the hormonal inbalance of hypophysectomy alters the form and undoubtedly the function of both the smooth and rough endoplasmic reticulum. Growth hormone administration restores these subcellular systems to their normal appearances. (Supported by Grant GM 06637 from the USPHS.)

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WALTER CHAVIN and YU MIN CHEN, Wayne State University.

Evolution of integumental tyrosinase activity in vertebrates and vertebrate melanomas. (By title only)

The comparative biochemical aspects of tyrosinase activity in vertebrate skin and melanomas were studied in an evolutionary series. The animals used were *Eptatretus stoutii, Petromyzon marinus, Ginglymostoma cirratum, Urolophus halleri, Rhinobatus productus, Platyrhinoidis triseriatus, Acipenser fulvescens, Polypterus senegalus, Calamoichthys calbaricus, Amia calva, Lepidosteus osseus, Carassius auratus, Anguilla rostrata, Protopterus aethiopicus, Necturus maculosus, Rana pipiens, Rana catesbiana, Anolis carolinensis, Columba livia, Rattus norvegicus* and *Homo sapiens*. The abnormal pigment cells studied included the hybrid swordtail melanoma (Sd) and amelanotic melanoma, the B-16 and Harding-Passey mouse melanomas. The tyrosinase radiometric assay was utilized (Chen and Chavin, Anal. Biochem., 13:234, 1965). The study included the anatomic and subcellular enzyme distribution, substrate carboxyl incorporation, D- and L-tyrosine utilization, etc.

The dorsal integument generally contained higher tyrosinase levels than the ventral integument. The amniote enzyme was confined to the particulate fraction whereas that of the anamniotes was limited to the particulate fraction only or to both fractions. Substrate carboxyl incorporation occurred from 4 to 39% and was species specific. Six species used D-tyrosine at levels of 5 to 16% of the L-tyrosine converted. An increase in tyrosinase activity during storage at 0-4°C occurred in the garpike enzyme preparations.

Extremely high tyrosinase activity occurred in all vertebrate melanomas or amelanotic melanomas. The substrate carboxyl incorporation into melanin in these abnormal pigment cells varied from 3 to 34%. (Supported by USPHS Grant Ca 07273-03 from the National Cancer Institute.)

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WENDY B. FAIN and MAC E. HADLEY, Brown University.

In vitro response of melanophores of *Fundulus heteroclitus* to melatonin, adrenaline, and noradrenaline.

Melatonin is considered the most potent lightening agent of amphibian dermal melanophores, reported to be 10^6 times as effective as noradrenaline. Whether melatonin exerts a similar action on dermal melanophores of other poikilothermic vertebrates has not been determined.

The *in vitro* response of *Fundulus* melanophores to melatonin was investigated and compared to other melanin granule aggregating agents. Melanin granules within melanophores of isolated scales become dispersed when placed in amphibian Ringer's solution. Owing to this response of *Fundulus* melanophores, it has been possible to test the effects of aggregating agents in a medium uncomplicated by the presence of dispersing agents. Scales from each fish tested were removed and placed in Ringer's solution and in Ringer's solu-

tion containing either adrenaline, noradrenaline, or melatonin. Adrenaline and noradrenaline in concentrations as low as 1 μg/ml brought about rapid melanin granule aggregation within melanophores. The effective concentrations of these catecholamines are comparable to published data from studies on frog skin. Melatonin (100 μg/ml, and higher), in contrast, was totally ineffective in bringing about melanin granule aggregation. This result contrasts strikingly with the amply documented action of melatonin on amphibian melanophores.

Embryonic and larval melanophores of *Fundulus* become aggregated, *in vivo*, when treated with beef pineal extracts; the melanophores of adults do not (Wyman, 1924; McCord and Allen, 1917). Developmental changes in melanophore control appear to be reflected in these differential responses of larval and adult melanophores to melatonin. Loss of sensitivity to melatonin may relate to the developmental acquisition of nervous innervation mediated by a catecholamine. (Supported by Grants No. CA-06097-05 and GM-00582-05 from the USPHS.)

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HARVEY H. FEDER, JOHN A. RESKO and ROBERT W. GOY, Oregon Regional Primate Research Center.

Progestin levels in peripheral plasma of guinea pigs during the estrous cycle. (By title only)

Progesterone and its 20-hydroxy epimers were estimated in peripheral plasma of virgin adult female guinea pigs by utilizing gas-chromatography (J. Clin. Endocr. 25:1625). Estrus was defined as the period during which the lordosis response could be elicited.

The following progesterone levels were found (μg/100 ml plasma): 0.003 (1 day post-estrus), 0.030 (2 days), 0.131 (3 days), 0.223 (5 days), 0.222 (7 days), 0.248 (9 days), 0.220 (11 days), 0.042 (13 days). Additionally, progesterone was detected during estrus, but not at its termination. The first six figures each represent means of three determinations. Remaining data are based on two samples for each group. When no chromatographic peak appeared a value of 0 was used to calculate the mean. 20 α-hydroxy-Δ^4-pregnen-3-one was found in one of two plasma samples collected 11 days following estrus. None of 10 samples collected one to nine days post-estrus contained either of the 20-hydroxy epimers.

We conclude that: a) progesterone is the chief progestin in guinea pig peripheral plasma; this contrasts with ovarian vein data from rats in which 20 α-hydroxy-Δ^4-pregnen-3-one predominates (Steroids 2:119); b) high progesterone levels during days 5-11 may interfere with estrogen conditioning of neural tissues mediating sexual behavior (Gen. Comp. Endocr. 6:267); and c) in addition to this possible inhibitory effect the presence of progesterone during estrus suggests a facilitatory action for sexual behavior. (Supported by NICHD Fellowship 1 F2 HD-29, 006 and Grants FR-00163 and MH-08634.)

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ANTHONY J. GALLO and NICHOLAS D. DE PROSPO, Seton Hall University.

Isoquinoline Azo-dye carcinogenesis and the hypophysis. (By title only)

The present study was initiated to determine whether hypophysectomy would alter the carcinogenicity of the isoquinoline azo-dye, isoquinoline-*N*-oxide-5-azo-*p*-dimethylaniline, and to ascertain whether these effects are related to possible pituitary-adrenal function. Forty male Sprague-Dawley rats were hypophysectomized. Following the operation they were fed regular Purina Rat Chow for two weeks. Eight rats died during this period, failing to survive the trauma of the hypophysectomy. The remaining thirty-two rats were equally divided into two experimental groups, with half of them receiving ACTHAR gel every 48 hours and the others given no hormone therapy. Ten rats were selected as tumor controls. A basic low protein, low riboflavin diet containing 0.03% dye was used and was fed *ad libitum*. Body weights were recorded at weekly intervals and laparotomies were performed periodically to study tumor progress. Six experimental animals died within the initial five weeks of the experiment. At the end of four months all surviving rats were sacrificed and their livers examined. All controls exhibited hepatomas, whereas the hypophysectomized group receiving no hormones were characterized by normal livers. The group receiving the ACTHAR gel exhibited mildly cirrhotic livers without the formation of distinct tumors. The results obtained in this experiment indicate that hypophysectomy effectively inhibited the formation of hepatomas in rats fed a basic diet containing the isoquinoline azo-dye. Since the ACTHAR gel partially restored the hepatocarcinogenicity of the dye in the hypophysectomized rats, adrenal function may also be implied to be involved.

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JULIUS S. GREENSTEIN, Duquesne University.

Some effects of long-term daily administration of cortisone acetate to adult rats. (By title only)

Adult albino rats of both sexes were subjected to daily subcutaneous injections of 2.5 mg cortisone acetate for a period of one month. The animals appeared to enjoy a brief period of exhilaration and hyperactivity soon after each injection. Blood samples were taken by heart puncture at the time of sacrifice and routine histopathologic studies were made with special emphasis on the liver, thyroid, and adrenals.

Treated animals experienced a weight loss of approximately 10 per cent as compared to their normal litter mates. Sections of liver revealed evidence of mild fatty changes, focal areas of moderate sinusoidal dilation, glycogen infiltration, and the parenchymatous cells bordering the dilated sinusoids gave evidence of pyknosis and karyolysis. The thyroid gland reflected altered metabolic activity with enlarged follicles composed of columnar cells, basal nuclei, and were largely devoid of luminal colloid. Adrenal tissue sections were characterized by reduction in the overall size of the cortex associated with obvious hypoplastic changes, especially in the zona fasciculata. A mean blood sugar value of 172 mg per cent was obtained prior to necropsy suggesting a mild, persistent hyperglycemia, a result which is consistent with an abnormal rate of gluconeogenesis from protein.

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CHARLES S. NICOLL, University of California, Berkeley.

Prolactin secretion by tetrapod adenohypophyses (AP) *in vitro*. (By title only)

AP of young female guinea pigs, and of male and female pigeons, Mallard ducks and Diamondback terrapin (*Malaclemys terrestres*) were incubated in organ culture for 10 days in synthetic medium 199. AP of homiotherms were incubated at 36°C and those of turtle at 26°C. Medium was changed at 2-day intervals and assayed for prolactin in pigeons. Guinea pig AP secreted prolactin *in vitro* at a constantly high rate and total prolactin production was 175 mU/mg tissue. Duck and pigeon AP secreted very little prolactin and levels in medium decreased with time. Total prolactin secreted by pigeon and duck AP were 17 and 10 mU/mg, respectively. Turtle glands secreted prolactin at a low but constant level and total amount secreted was 27 mU per mg AP. Survival of the cultured AP was uniformly excellent.

These results indicate that guinea pig AP can actively secrete prolactin autonomously, but avian glands cannot. Accordingly, prolactin secretion by guinea pig AP is probably controlled by hypothalamic inhibition while that of the avian species is dependent upon a hypothalamic stimulatory factor. Results with turtle AP are less readily interpretable because they were incubated at a lower temperature. However, they did secrete more prolactin than either of the avian species and showed no reduction in prolactin output with time. This may indicate that turtle AP secretes prolactin autonomously; therefore, the hormone is probably controlled by hypothalamic inhibition.

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TETSUO NOUMURA, University of Tokyo.

Steroid biosynthesis by the gonads of chick embryos. (By title only)

The sex organs of chick embryos are asymmetrical in structures and in functions. The left gonads have more cortical nature, while the right ones more medullary. It was of interest, therefore, to examine whether or not there were any differences in gonadal steroid biosynthesis between the left and the right gonads.

Gonads were removed from 8- to 21-day chick embryos. Fifty mg of the left and the right testes and ovaries were minced, respectively, and placed separately in incubation flasks which contained: 1.5 ml Hanks' medium, 12.5 μc tritiated precursor steroids, and co-factors. The incubations were carried out at 40°C for 3 hours in an atmosphere of 95% O_2-5% CO_2 in a Dubnoff metabolic incubator. After incubation, tracer amounts of ^{14}C-labeled steroids were added to the flasks to permit correction for losses during the purification procedures, and the steroids were extracted and purified by different paper and thin-layer chromatographic systems.

The left and right ovaries of 8-day embryos may convert progesterone into estrogens in areas of estrone and estradiol, and dehydroepiandrosterone into estrogens in areas of estrone, estradiol, 16-oxoestradiol, 16-epiestriol, and estriol. Testes and ovaries of 12-day embryos can convert progesterone into phenolic steroids, especially polar ones in estriol and 16-epiestriol areas. In 15-day embryos, more phenolic steroids are produced from progesterone and 4-androstene-3,17-dione by the left gonads than by the right ones, and in the females than in the males. Testicular tissues from 18-day embryos metabolize progesterone to much testosterone, and the right ovaries of 21-day embryos, just before hatching, may not produce estrogenic steroids from progesterone and pregnenolone.

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MADELEINE OLIVEREAU, Institut Océanographique de Paris.

Modifications of the "prolactin cells" in sea water eels. (By title only)

It has been shown that hypophysectomized eels can withstand changes of salinity (Fontaine *et al.*, 1949); however, ovine prolactin administration induces modifications of plasma electrolytes in the eel (Chartier and Olivereau, 1965). The secretion of a "prolactin-like hormone" by the erythrosinophils (eta cells) of the rostral pars distalis has been suggested in some teleosts (Ball and Olivereau, 1964). What changes may occur in these cells after transference from freshwater to seawater? Eels were kept for 15 minutes to 6 months in seawater. In freshwater, eta cells are grouped in elongate or spherical follicles; these tall cells (18-32 μ) look active, with a well developed ergastoplasmic reticulum at the cell periphery, a round nucleus, a prominent nucleolus; basal granules are abundant. After transfer into seawater, these cells gradually became flat and less active, the ergastoplasmic reticulum was no longer visible, nuclei were smaller and darker, nucleoli were indistinct, granulations disappeared. After 6 months, follicles were less numerous, with empty cavities and all cells were chromophobic. In contrast TSH cells appeared well granulated. These results suggest that the production of a "prolactin-like hormone" is not needed in seawater eels. The inactivity of eta cells in seawater is in agreement with their stimulation in the *in situ* or grafted pituitary of *Fundulus* and *Poecilia* transferred into freshwater (Pickford, Ball, and Olivereau . . .) and their overactivity in *Salmo salar* penetrating into freshwater at the beginning of the spawning migration (Olivereau, 1954). (Supported by the CNRS.)

399

ROBERT ORTMAN and DAINA BOTYRIUS, City College of New York.

Bioassay of segments of the pars distalis of male *Rana pipiens* for ovulation-inducing hormone. (By title only)

The gamma cells of *Rana temporaria* (van Oordt, 1963) are homologous to the purple cells of *Rana pipiens* (Ortman, 1961). Both cells are virtually restricted to the rostral region of the pars distalis and lie next to the portal vessels. Accordingly, a transection of the pars distalis at the midpoint will provide a segment of anterior lobe tissue almost free of these cells and a second segment containing the bulk of them. Van Oordt has suggested, on the basis of correlative studies, that the gamma cells produce luteinizing hormone.

Male *Rana pipiens* served as donors. Half-lobes

were isolated under acetone and acetone-treated for 24 hours. Winter *Rana pipiens* were given a mean total dose of 1.52 mg of half-lobes suspended in Ringer's fluid by 2 daily intraperitoneal injections. The wet weight of the eggs stripped was the measured response. Control (uninjected or Ringer-injected) frogs did not ovulate.

The weight (grams) of the eggs obtained from individual females receiving rostral half-lobes taken from males sacrificed at different seasons were as follows: March (14.6, 2.3), June (5.5, 0.0, 4.6), July (12.2, 9.5), August (14.5, 6.7, 18.0). The mean weight was 8.79 grams. The weight of the eggs from recipients receiving the caudal half-lobes were: March (1.0, 10.0), June (0.0, 0.0, 0.0), July (10.1, 11.6), August (6.4, 13.4, 16.5). The mean weight was 6.9 grams.

The data are not regarded as offering any strong support for van Oordt's hypothesis. (Supported by USPHS grant No. AM 7204-03.)

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RUSSEL J. REITER, RALPH J. HESTER, and MARY A. MULLER, U.S. Army Edgewood Arsenal, Md.

Action of FSH and LH on the reproductive organs of blinded female hamsters. (By title only)

Pinealectomy prevents gonadal atrophy in dark-exposed or blinded hamsters. Theoretically, pineal substances interfere with the normal functioning of the pituitary-gonadal axis, i.e., by limiting the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the adenohypophysis, by inhibiting the action of these hormones at the target organ level, or both. In an attempt to partially resolve this problem the following experiment was conducted.

Thirty adult female hamsters were divided into 4 groups: 1) untreated; 2) blinded; 3) blinded and pinealectomized; 4) blinded and given FSH (0.5 mg/day) and LH (0.5 mg/day) for 10 consecutive days prior to necropsy. All animals were killed after 9 weeks of treatment. The endocrine and reproductive organs were weighed and expressed as mg/100 g body weight (mg%).

Ovarian weights of groups 1-3 were similar (18.9-20.4 mg%); however, ovaries of blinded hamsters lacked antral follicles and contained only occasional corpora lutea. Gonads of the blinded animals treated with FSH-LH were markedly enlarged (46.1 mg%) and contained numerous corpora lutea. Uteri of the blinded animals were atrophic weighing 63 mg% compared with a mean uterine weight 272 mg% for untreated animals. Uteri of blinded-pinealectomized animals averaged 283 mg%. Uteri of blinded animals given FSH-LH weighed 123 mg% (significantly heavier than those of blinded animals). The findings show that the ovaries and uteri of blinded animals are responsive to gonadotrophic hormone stimulation even in the presence of antigonadotrophic factors of pineal origin. The data indicate that pineal substances may act at the hypothalamo-pituitary level to modify reproductive functions.

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J. A. RESKO, H. H. FEDER, and R. W. GOY, Oregon Regional Primate Research Center.

Androgen concentrations in rat plasma and testis as a function of age. (By title only)

Testosterone and androstenedione from pools of peripheral plasma and testis of male rats were estimated using gas-liquid chromatography. Androstenedione was reduced to testosterone with sodium borohydride before quantification. Plasma concentrations of testosterone (μg/100 ml plasma) found at various postnatal time periods were: day 1 (0.027), 5 (not detectable), 10 (not detectable), 30 (0.018), 90 (0.202), 120 (0.118). Androstenedione was found only at 90 days (0.011 μg) and at 120 days (0.053 μg).

Testosterone was extracted from testis (μg/g tissue) in the following concentrations: on day 1 (0.463), 5 (0.134), 10 (0.122), 30 (0.005), 60 (0.014), 9 (0.111). Androstenedione levels for the same time periods were: 0.131, 0.043, not detectable, not detectable, 0.005, not measured and 0.015 μg.

The presence of small quantities of testosterone in the plasma of the day-old rat is consistent with current views on the role of testosterone in the establishment of a male-type nervous system (Endocrinology 65:369; 75:627). This hormone appears to decline with age and is again detectable at 30 days, perhaps indicating the onset of puberty. More androgen was extractable from testis (days 1-5) than from plasma. The opposite was found on days 30-120. The reversal of this relationship with age suggests trophic release at the latter time periods but not during the pubertal period. (Supported by Grants MH-08634 and FR-00163 and NICHD Fellowship 1 F2 HD-29, 006.)

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GILBERT A. RINARD and SAMUEL L. LEONARD, Cornell University.

Protection of phosphorylase *a* activity in rat uterine homogenates by estrogen, *in vivo* and EDTA, *in vitro*. (By title only)

Previous reports indicated that estradiol-17β, *in vivo*, and the chelating agent, EDTA (ethylenediaminetetraacetic acid) *in vivo* or *in vitro*, (i.e., in the homogenate), increased rat uterine phosphorylase *a* activity (Endocrinology 63:853, 1958; ibid. 76:491, 1965). In those studies, uteri were excised, trimmed, frozen with solid CO_2, homogenized in 0.1 M NaF and assayed. Subsequent experiments indicate: 1) Tissue manipulation before freezing converts inactive phosphorylase *b* (which requires AMP for activity) to the active, *a* form. 2) Following homogenization, phosphorylase *a* activity decreases *in vitro*, following first order kinetics (in respect to phosphorylase *a* activity) under assay conditions (0.05 M NaF, 0.014 M glucose-1-phosphate, 0.5% glycogen). Addition of AMP, 8.3×10^{-4} M final concentration, elicits full phosphorylase *t* (total) activity when phosphorylase *a* activity has become negligible. The apparent activation effects of estrogen and EDTA now appear to be protective effects against *in vitro* loss of activity. Estrogen, *in vivo*, increases the *in vitro* half-life of uterine phosphorylase *a* activity four-fold. EDTA, *in vitro*, completely prevents loss of activity, making the assay linear with time. Initial phosphorylase *a* activities in uterine homogenates from estrogen-primed or spayed rats are similar, calculated from kinetic data or assayed with EDTA present. When

rat uteri, frozen before manipulation, are homogenized in EDTA-NaF, EDTA has two effects on phosphorylase: 1) prevention of phosphorylase *b* to *a* conversion, the classical effect, 2) prevention of loss of phosphorylase *a* activity, a new effect compared to other known phosphorylase systems. (Supported by NIH Grant AM 04965-05.)

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ROBERT C. THOMMES and JOHN A. LAMPING, De Paul University.

Endocrine control of electrolyte distribution in the chick embryo. I. Blood sodium and potassium. (By title only)

The effect of hypophysectomy upon the blood levels of sodium and potassium has been studied in the developing chick embryo.

Animals were hypophysectomized at 36 to 40 hours' incubation by the "partial decapitation" method of Fugo (1940). Blood samples were collected at 24-hour intervals from day 10.5 through 16.5. Sodium and potassium levels in plasma of normal and hypophysectomized White Leghorn chick embryos were determined by means of a Beckman DU spectrophotometer with flame attachment.

Normal plasma sodium levels range from 183.2 mEq/l on day 10.5 to 171.0 on day 16.5 of incubation. Potassium levels ranged from 4.59 mEq/l on day 10.5 to 4.68 on day 16.5. Hypophysectomy by means of surgical decapitation had no statistically significant effect upon sodium or potassium in the blood of developing chick embryos. The blood of pituoprivic chick embryos showed no change in sodium and potassium ratios when compared to normals at any of the stages studied. (Supported by grant HD 01475 from the USPHS.)

404

C. DONNELL TURNER and RICHARD E. FALVO, Duquesne University.

In vivo production of androgen by the polyvesicular ovaries of androgen-sterilized rats. (By title only)

Weisz and Lloyd (Endocrinology, 77:735, 1965) reported that the ovaries of androgen-sterilized rats (ASR) can convert labeled progesterone to androgens and estrogens. We made extensive histological studies of these ovaries and, through the use of transplanted seminal vesicles and ventral prostates, determined their ability to secrete androgen *in vivo*. These male accessories were transplanted within the periovarian sacs or beneath the kidney capsules of ASR and compared with comparable grafts persisting in ovariectomized hosts.

The epithelial cells of prepuberal seminal vesicles became tall columnar and contained characteristic secretory granules after persisting for 15-20 days within androgen-sterilized hosts, but grafts within castrated hosts gave no evidence of androgen stimulation. After 15 days, some of the grafts in ASR contained localized areas of epithelial metaplasia, and this suggested an estrogen effect. Most uniform results were observed in the grafts which grew to the surface of the ovary. Essentially similar results were obtained with transplanted fragments of ventral prostates. Accessory tissues maintained in Millipore filter chambers provided confirmatory evidence, but the epithelia survived less well than in well-vascularized grafts. It is concluded that the ovaries of adult ASR release enough androgen *in vivo* to support secretion in transplanted male accessories, and probably enough estrogen to affect them.

The ovaries of ASR are characterized by a high rate of follicular atresia. The granulosal remnants of atretic follicles persist in the interfollicular areas as cords and irregular masses of intertsitial-cell-like deposits. It is likely that this histopathology correlates with the abnormal pattern of steroidogenesis. (Supported by Grant HD 00597-04 from the USPHS.)

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JOSEPH THOMAS VELARDO, BARBARA A. KASPROW and WAYNE A. KRUEGER, Institute for the Study of Human Reproduction, John Carroll University, and Saint Ann Hospital, Cleveland, Ohio.

Comparative analysis of the growth phase of intact and ovariectomized rats receiving estrogen. (By title only)

Previously, it was reported that estradiol-17β programming in ovariectomized, adult albino rats did not induce significant body weight changes as compared with non-estrogenized, ovariectomized controls (Velardo *et al.*, Amer. Zool. 4(4):419, 1964). The method of estradiol-programming was consonant with physiological amounts of estradiol, i.e., dosages and timing in regard to its uterotropic effect. These investigations were extended with two modifications: (a) adult, *intact* albino rats were used, and (b) pellets containing estradiol-17β-benzoate were implanted.

Experimentally, 130 adult albino, female rats, 52 days of age, and weighing approximately 170 grams were subcutaneously implanted with pellets containing 10.0 mg estradiol benzoate. An additional 130 animals of same age and approximately similar body weight *not implanted* with estrogen pellets served as concomitant controls. Body weights of animals in both groups were determined daily. Necropsies were performed weekly from 1-10 weeks and 12 and 16 weeks after pellets were implanted, each group containing 8-10 rats. Parallel controls not implanted with estradiol benzoate pellets were similarly necropsied.

Comparatively, the estrogenized animals obtained weight increases that were much less than those of the non-implanted controls. Body weight determinations revealed that the non-estrogenized rats gained (a) 16.2 g more than the estrogenized series at the end of the first week; (b) 28 g per week from second through fourth week; (c) 40 g per week from fifth through tenth week; and (d) thereafter, 53 and 65 g after 12 and 16 weeks, respectively. (Supported by USPHS Grant No. 00147-05.)

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JOSEPH THOMAS VELARDO, BARBARA A. KASPROW, and WAYNE A. KRUEGER, Institute for the Study of Human Reproduction, John Carroll University and Saint Ann Hospital, University Heights, Cleveland, Ohio.

Response of endocrine organs of adult rats to estradiol benzoate pellet implants for different periods of time. (By title only)

Currently, numerous hormone, time-exposure studies are in process with a view toward elucidating effects of sex steroid hormones and related metabolites at cellular and subcellular levels of endocrine glands and associated structures.

Experimentally, intact, adult albino rats, 52 days old, weighing approximately 170 g, were utilized for these experiments; one group of 120 rats was subcutaneously implanted with pellets containing 10.0 mg estradiol benzoate; an additional 120 rats, of similar history, but not implanted, served as concomitant controls. The two major groups were sub-divided, 12 sub-groups each, with 8-10 rats per sub-group; body weights were determined daily; necropsies of experimentals and controls were performed weekly, 1-10, and at end of 12 and 16 weeks.

Gravimetric and statistical comparisons revealed that animals containing estrogen pellets had (a) body weight increases that were less than controls, commencing one week after estrogenization (at 16 weeks: estrogenized animals weighed 266 $\pm$ 5 g, controls 320 $\pm$ 6 g); (b) ovaries indicating initial impeded growth during 1st through 3rd week, thereafter showing inhibition (at 16 weeks: ovaries of estrogenized rats weighed 61.8 $\pm$ 4.1 mg, controls 115.8 $\pm$ 6.5 mg); (c) uteri that were heavier than controls (1st week, estrogenized = 393.1 $\pm$ 15.4 mg, controls 292.8 $\pm$ 31.6 mg; 16th week, estrogenized = 596.8 $\pm$ 20.3, controls 481.9 $\pm$ 35.3 mg); (d) pituitaries that were 2-3 times heavier than controls from weeks four through sixteen; and (e) thyroids and adrenals that were not markedly different from those of controls. (Supported by USPHS Grant No. HD 00147-05.)

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ALLYN J. WATERMAN and BRENT BUTCHER, Williams College.

Correlation between locomotor and thyroid daily activity in mice. (By title only)

Following acclimatization to their cages and to a 12-hour photoperiod, male $B_6D_2F_1$ mice (Jackson Laboratory) were tested for running activity in tilting-type activity cages, during fall and early winter. Movements were recorded electrically as blips on an Esterline-Angus Channeler, and data transposed into graphic form by plotting traverses per hour. Thyroid activity was measured by 12-hour uptakes of I-131, expressed as a percentage of the injected tracer dose (2 microcuries) at the end of tests of the running activity, and by histological technique. This preliminary study was repeated on mice exposed to continuous diffused light (50-55 foot candles) for six days preceding the test.

With onset of darkness at 8 p.m. there occurred increased locomotor activity (12-hour photoperiod), with a peak around 10-11 p.m., followed by a decline until 8 a.m.; also a sharp increase of I-131 uptake at 8 p.m. (ave., 8.99 $\pm$ 4.0% of injected dose.) Uptake values: 8 a.m., 2.88 $\pm$ 1.3%; 2 p.m., 2.32 $\pm$ 0.96%; 2 a.m., 3.49 $\pm$ 0.96%. With onset of increased locomotor activity at 8 p.m. there was a corresponding elevation of I-131 uptake (activity) of the thyroid. This was also reflected in height of follicular cells.

Exposure to continuous diffused light shifted activity rhythm 180 degrees from the 12-hour photoperiod. Activity peaked at 9-10 a.m. High level of thyroid activity appeared at 8 a.m. (8.57 $\pm$ 2.4%) in contrast to 2.70 $\pm$ 0.81% to 3.80 $\pm$ 1.2% at the other three periods of the day. The histological picture reflected the shift. (Supported by Grant GB1591 from the NSF.)

408

DANIEL C. WILHOFT, Rutgers University.

The effect of thyroidectomy on the preferred body temperature of a lizard. (By title only)

Previous work with the lizard, *Sceloporus cyanogenys,* has shown that animals maintained in a thermal gradient wherein thermoregulatory behavior took place responded to thyroxine injections with an increase and to thiourea injections with a decrease in oxygen consumption. Further experiments were carried out to ascertain whether a change in lizard preferred body temperature would accompany the decease in oxygen consumption caused by thyroidectomy.

Male *Sceloporus cyanogenys* received raily injections of 0.9% saline containing 0.3 mg of thiourea per gram of body weight. Body temperature of lizards maintained within a thermal gradient, range 12°-60°C, were recorded from appropriate Yellow Springs Instrument thermistors inserted deep in the intestine before and after thyroidectomy. Mean recorded preferred body temperature of this species before thyroidectomy was found to be 32.8 $\pm$ 0.47°C.

After 7 days of thiourea injections mean recorded body temperature of 8 lizards was 29.1 $\pm$ 0.68°C; after 21 days 28.5 $\pm$ 1.06°C. After 7 day of thiourea injections 11 lizards received daily saline injections containing 0.2 gamma of L-thyroxine per gram of body weight in addition to the thiourea. After 7 days of administering thyroxine to thyroidectomized lizards mean recorded body temperature was 32.3 $\pm$ 0.81°C; after 14 days 31.9 $\pm$ 0.40°C. Four of the 11 lizards were continued on thiourea without thyroxine, after 7 days mean recorded body temperature was 30.6 $\pm$ 0.95°C suggesting a gradual return to the pretreatment mean recorded temperatue of 32.8°C.

These data suggest that thyroidectomy in this lizard causes not only a decrease in oxygen consumption but a concomitant adjustment in themoregulatory behavior and physiology resulting in a lower body temperature. Such an adjustment might be of survival value in preventing lizards from attaining the preferred body temperature when thyroxine is not available to sustain the higher level of physiological activity usually associated with the preferred body temperature. Further experiments will investigate this hypothesis. (Supported by Grant AM-08710 from the USPHS.)

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IRVING ZUCKER, Oregon Regional Primate Research Center.

Progesterone in the experimental control of behavioral sex cycles in the female rat. (Introduced by Charles H. Phoenix) (By title only)

Sexually mature female Sprague-Dawley rats were maintained on an artificial light cycle that provided 12 hours of illumination/24-hour period (midnight to noon). A manual stimulation technique was used to ascertain the existence of be-

havioral estrus; this permitted classification of females as having 4-day or 5-day cycles.

Injections of 2 mg of progesterone to 4-day rats at noon on days 1, 2 or 3 of the cycle delayed the next occurrence of behavioral estrus by 1-2 days in 20/20 rats. Treatment on day 4 of the cycle was without effect in 7/8 animals.

Injections of 2 mg of progesterone on days 1 or 2 of the 5-day cycle delayed behavioral estrus in only 3/16 rats. However, injections on day 3 were effective in delaying behavioral heat by 1-3 days in 17/17 animals. Treatment with progesterone at 2 PM on day 4 of the cycle advanced heat by one day in 8/8 rats; injections on day 5 did not affect the interval to the next occurrence of behavioral heat in 8/8 females.

Fourteen 4-day rats were treated with progesterone on day 1 of the cycle (this transforms the 4-day cycle into an artificial 5-day cycle). These animals were then given a second injection of progesterone on day 4; 9/14 rats displayed heat on day 4. Thus the second injection of progesterone transformed the artificial 5-day cycle back into a 4-day cycle, and thereby cancelled the delaying action of the initial progesterone treatment in the majority of the animals.

The experiments suggest a biphasic effect of progesterone on the induction of behavioral receptivity. (Supported by Grants MH 08634 and FR 00163.)

COMPARATIVE PHYSIOLOGY

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THOMAS K. AKERS and DONALD A. MONDA, University of North Dakota.

Phosphorus levels in working smooth muscle of *Ciona intestinalis*. (By title only)

Longitudinal smooth muscle strips from the body wall of *Ciona intestinalis* isolated and suspended in a cooled (10°C) perfusion chamber were washed 10 seconds out of every minute for 20 minutes with fresh sea water. The muscle was electrically stimulated and contractions measured by a linear transducer coupled to a galvanometer recorder. Lever mass and after-load for each preparation was the same. The total muscle work could be calculated. Following this procedure, the muscles were treated by the method of Fiske and Subarrow (J. Biol. Chem. 66:375, 1925) for total and inorganic phosphate levels.

It was found that the inorganic phosphate levels increased with increasing work while the total phosphate decreased with increasing work. The results of 10 experiments show that inorganic phosphate is related to muscle shortening as follows: $Y = 5.73 + 0.237X$, r 0.8454, where Y is inorganic phosphate (mg%) and X is % shortening. The total phosphate varied inversely to muscle shortening according to the equation: $Y = 88.36 - 0.719X$, r 0.4409, where Y is total phosphorous in mg/g wet weight; and X is % shortening.

These preliminary experiments indicate that high energy phosphate bonds of arginine phosphagen or perhaps ATP are being broken to yield energy for the muscle work. Further biochemical data will be required to elucidate this. (Supported in part by Summer Medical Fellowship from Lederle Laboratories.)

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THOMAS H. CLEWE and WILLIAM M. DuVALL, Vanderbilt University.

Observations on frequency of ejaculation of squirrel monkeys, *Saimiri sciureus*. (By title only)

Adult male squirrel monkeys were allowed access to adult "test" females for a limited observed time daily from January 4 to July 31 of their second year in captivity. Between observation periods the males were immediately adjacent to the females in wire cages. Two males were caged together and tested with the same six females; each of two other males was caged with two adult females and tested with two additional females. Each male was allowed access to his test females daily in the early afternoon for 20 to 30 minutes. Tests were also done 5 mornings each week from March 23 to June 1; the interval between morning and afternoon tests was varied to determine the duration of the post-ejaculatory interval.

The shortest interval between ejaculations for any male was 183 minutes. Each of the four males proved capable of a second ejaculation in less than 3½ hours. Individual males ejaculated as often as four times in two days and series of daily ejaculations up to six days long occurred. The two males caged with females were less sexually active on the whole during the test periods than the males with more limited access to females. Many observation periods contained much mounting and thrusting which did not culminate in ejaculation.

The post-ejaculatory intervals observed in these squirrel monkeys were considerably longer than those reported for the rhesus monkeys observed by Kuehn and Young (Am. Zool. 5:687, 1965). (Supported by Grant HD-00673 from USPHS.)

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MILTON FINGERMAN, ERNEST F. COUCH, and EDWARD W. STOOL, Tulane University and Marine Biological Laboratory.

Circadian rhythm of red pigment dispersion in intact and eyestalkless fiddler crabs, *Uca pugilator*. (By title only)

Intact fiddler crabs, *Uca pugilator*, collected in the region of Woods Hole, Massachusetts, during June 1966 exhibited the usual rhythm of pigment dispersion whereby the chromatophoric pigments were more dispersed by day than by night. The rhythm of red pigment dispersion was circadian as has been shown previously for the black pigment. Furthermore, eyestalkless crabs showed a circadian rhythm of red pigment dispersion, but the melanin in eyestalkless crabs remained fully concentrated at all times. The circadian nature of the rhythm was revealed by observation of isolated individuals maintained under a constant illumination of 4.9 meter-candles. The red pigment of eyestalkless crabs was at all hours less dispersed than that of intact crabs under this intensity of illumination. The time of maximal dispersion of the black pigment in the intact crabs was usually different from that of the red pigment, thereby suggesting that each pigment was under the control of a different rhythmical center. The amplitude of the rhythm of red pigment dispersion in the eyestalkless crabs averaged one-third of that in the intact individu-

als. Furthermore, the amplitude of the rhythm in the eyestalkless crabs was greatest the first day after the eyestalks had been removed and then decreased progressively until finally the red pigment showed no significant fluctuation between day ond night. (Supported by Grant GB-5236 from the NSF.)

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DAVID L. HAMMERMAN, Long Island University.

Lingual premetamorphic papillae in larval anurans: a neurological study. (By title only)

The existence of a larval taste perceiving apparatus has been postulated in ranid tadpoles (Helff and Mellicker, 1941; Hammerman, 1964). Premetamorphic papillae have been suggested as such sensory receptor structures because of their location and relationship to the developing lingual organ's dorsal surface. The degeneration or reincorporation of premetamorphic papillae into the surface of the developing tongue at a time when lingual taste buds are differentiating (Hammerman, 1959 and 1960) provides some support for this hypothesis.

The lingual premetamorphic papillae of *Rana catesbeiana* and *R. clamitans* tadpoles were examined microscopically. Ehrlich's thionin stain, urea utilized because of their preferential neurological silver nitrate stain and methylene blue stain were staining. Serial sections cut at 7 microns were used in these studies.

A fine network of fibers was observed by the techniques employed. These fibers and their branches were observed to leave the central core of the premetamorphic papillae, penetrate the basement membrane and travel through the surrounding epithelial layers. Some free nerve endings were seen between adjacent epithelial cells. Some of the fibrils extend to columnar cells in the papillary epithelium. These latter cells were generally located at the bluntly-pointed distal portions of the papillae.

The presence of such a network with each of the staining methods utilized supports the original hypothesis. Additional physiological and behavioral studies are in progress to confirm the hypothesis that premetamorphic papillae are the taste receptors in frog larvae. (Supported by a grant from The Committee on Research of LIU.)

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DAVID L. HAMMERMAN and ALICE GOLDFELD, Long Island University.

Taste cell proliferation in *Rana pipiens*. (By title only)

A study has been made to extend the information accumulated regarding the dynamic flux of cells in the taste sense organ in *Rana pipiens*. The effects of varying dosages of colchicine on adult frog tongue tissue were evaluated. At a dosage of 0.5 ml of a 1.0% colchicine solution, c-mitoses first appear 4½ days after administration of the drug. The mitotic index (0.0151 ± 0.0027) and the turnover time (308.7 ± 57 days) of the taste cells were determined.

Frequently metaphase arrest brought about by colchicine causes changes in metabolism and the degeneration of the cell. At first c-mitoses accumulate and then decline, accompanied by a rise in degenerated cells.

Tritiated thymidine (0.5 mc/ml, Schwarz Bio-Research) was injected intraperitoneally (0.05 ml per organism) into another group of animals. Slides were prepared for autoradiographic studies. Slides made from the taste bud tissue exposed to tritiated thymidine for 4½ days showed grains above cells at the center of the taste bud's end disc. Labeled cells from material exposed for 5½ and 6½ days were seen midway between the center and the periphery of the taste bud. After 7½ days of exposure to tritiated thymidine, the isotopically labeled nuclei were observed in the peripheral cells ot the taste bud.

The results are in agreement with work done on mammalian taste cells (Beidler *et al.*, 1960). Differences in timing can be attributed to differences in the metabolic rates when comparing a poikilothermic with a homeothermic organism.

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O. W. HENSON, JR., Yale University.

Comparative physiology of middle ear muscle activity during echolocation in bats. (By title only)

Brief (1-5 msec) FM sonar signals are emitted by many species of bats during echolocation. In *Tadarida* it has been shown that the tympanic muscles contract prior to the emission of these pulses, a maximum state of contraction is reached just as pulse emission begins, and relaxation occurs over the duration of the signal (Henson, J. Physiol. 180:871-887, 1965).

Rhinolophidae, Hipposideridae and *Chilonycteris parnelli* (Phyllostomidae) employ sonar signals characterized by pure tones of relatively long durations (6-50 msec); each pulse, however, is terminated by a brief FM sweep. Measurements of middle ear muscle activity in *Chilonycteris* by the same techniques previously used for *Tadarida* have shown that muscle contractions begin almost simultaneously with the onset of pulse emission; a maximum state of contraction is attained prior to the FM sweep and muscle relaxation occurs during the FM sweep. Thus, in both *Tadarida* and *Chilonycteris* muscle contractions protect the ear from the intense outgoing signal and, by relaxing during the FM sweep, permit FM echo energy to be transferred to the cochlea more efficiently than the preceding pulse energy. In *Chilonycteris* pure tone pulse-echo overlap normally occurs; pulse-echo masking and the contracting middle ear muscles reduce sensitivity during periods of overlap. This, and other evidence (Henson, Anat. Rec. 154:356, 1966) suggest that the FM sweep is an important part of the pulse. (Supported by Grant MH 5430-05 from the USPHS.)

414

F. R. HUNTER, Centro Experimental de Estudios Superiores, Barquisimeto, Venezuela.

Evidence for facilitated diffusion of anions in erythrocytes. (By title only)

Using a densimeter technique the effect of tannic acid and *n*-butyl alcohol on the rate of anion exchange with human and rabbit erythrocytes was studied. Cells were equilibrated in NH_4Cl and the rate of shrinking was measured when these cells

were placed in a NaCl solution containing different concentrations of bicarbonate and of tannic acid or butanol. At all bicarbonate concentrations the other two substances markedly decreased the rate of shrinking as NH_3 left the cells and chloride inside was exchanged for bicarbonate outside. Since it has previously been postulated that marked inhibition by tannic acid and butanol indicates facilitated diffusion (J.C.C.P., 65:299, 1965) it is suggested that the present data indicate that facilitated diffusion is involved in this exchange of bicarbonate for chloride ions.

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F. R. HUNTER, Centro Experimental de Estudios Superiores, Barquisimeto, Venezuela.

An analysis of the "catalytic" effect of bicarbonate on the permeability of erythrocytes to ammonium chloride. (By title only)

Using a densimeter technique, the effect of increasing concentrations of $NaHCO_3$ on the rate at which NH_4Cl leaves human and rabbit erythrocytes was measured. When half-times for shrinking were plotted against reciprocals of bicarbonate concentration the data could best be fitted by two straight lines. The x-intercept for the high bicarbonate concentrations gave a value of the same order of magnitude as the K_m for carbonic anhydrase. The x-intercept for the low bicarbonate concentrations was smaller by at least two orders of magnitude. The break occurred at approximately 4×10^{-4} M $NaHCO_3$. Measurements were made at 31°, 35° and 40°C. Arrhenius plots gave a μ value at higher bicarbonate concentrations very similar to that of carbonic anhydrase. The value for lower concentrations of bicarbonate was low between 40-35° but was much higher for the interval 35-31°. These data might suggest that carbonic anhydrase has more importance in this anion exchange at higher than at lower bicarbonate concentrations (cf. Jacobs *et al.*, J. Gen. Physiol. 25:539, 1942).

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JOSEPH C. C. HWANG, Chinese University of Honk Kong.

Effect of pH on oxygen equilibrium curves in Asiatic horse-shoe crabs *Tachypleus tridentatus*. (By title only)

The experimental animals were obtained from mudbanks of Tolu Harbour in Hong Kong. Blood samples were collected from the gill books. After clotting the blood was centrifuged at 3500 rpm for ten minutes. The supernatant fluid was stored with chloroform overnite in refrigerators. The pH determined under this condition was 7.2. The absorption spectrum for the hemocyanin was determined. Two absorption peaks were seen, one at 385 mμ and the other at 580 mμ. Oxygen equilibrium curves at pH 7.2 were determined by calorimetric method at 580 mμ. The hemocyanin was considered to be saturated with oxygen after bubbling air through the serum for five minutes, the equivalent oxygen tension being 158 mm Hg. Complete deoxygenation was obtained by addition of sodium hydrosulphite. Intermediate range of oxygenation was effected through a high vacuum pump. A sigmoid curve was obtained. When the hemocyanin was 90% saturated with oxygen, the oxygen tension was at 30 $\pm$ 1.2 mm Hg; and 10% saturation at 5 $\pm$ 0.4 mm Hg. 80% saturation was obtained at 17 $\pm$ 0.5 mm Hg, and 20% saturation at 6.5 $\pm$ 0.3 mm. 50% saturation was obtained at 11 $\pm$ 0.8 mm Hg. The effect of pH on this displacement of the oxygen equilibrium curves was studied by using buffers.

In the range from pH 5 to 9.5 the affinity of hemocyanin for oxygen was lowest around pH 8.5, at 50% saturation, the oxygen tension being around 18 mm. Above pH 8.5 the affinity increases, and at pH 9.5 the 50% saturation tension was around 11 mm. At pH 5.2 the affinity was high, 50% saturation tension being around 7 mm. The overall working range of the oxygen tension was low (5 mm to 20 mm Hg) as compared with terrestrial and active marine animals. This was correlated with the animal's habitat where the oxygen tension was also low. (Supported by a grant from the Research Institute of Science & Technology, Chinese University of Hong Kong.)

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THEODORE L. JAHN, University of California, Los Angeles.

Anodal vs cathodal contraction of protoplasm. (By title only)

When the protoplasm of protozoan cells responds to electrical stimulation by contraction, contraction always starts at the *anodal* end, and the cell membranes of protozoa do not have self-propagating depolarizing action potentials.

If we assume that contraction of the cytoplasmic protein is caused by an increased association of calcium with the protein, as in muscle, these facts can easily be explained. Another necessary assumption is that all of the anionic sites on the protein have associated counterions, in accordance with the theories of Debye-Hückel, Ling, and Eisenman.

On the basis of ionic electrical mobilities it can be predicted for cells with non-excitable membranes that calcium 1) is removed from both the inside and the outside of the membrane at the cathodal end and is added to both sides of the anodal end, 2) is added to cytoplasmic protein throughout the cell, but more rapidly at the anodal end, and 3) is made available for addition to cytoplasmic protein at the cathodal end, as it is released from the membrane. In a cell with an excitable membrane the above will be true, and furthermore 4) the action current will release larger quantities of calcium than those resulting directly from the stimulus. For these reasons it is predictable that in cells with a non-excitable membrane, contraction should start at the anodal end, whereas in cells with excitable membranes, it should start at the cathodal end, and that is exactly what occurs.

418

V. F. LINDEMAN, Syracuse University.

5′ Nucleotidase activity in brown and white fat of the white rat. (By title only)

Studies were made upon mature white rats maintained at a constant temperature of 78 degrees within the animal quarters. Brown fat was obtained from the region between the scapula at

the nape of the neck and epididymal fat pads were used for white fat. Adenosine 5′ phosphoric acid was employed as the basic substrate and crude homogenates containing 100 mg tissue in 0.25 m sucrose provided the enzyme source.

The pH optimum was determined for each enzyme preparation and was found to be 7.5 for white and 8.4 for brown fat. The substrate specificity for white and brown fat respectively, when compared to adenosine 5′ monophosphoric acid of optimal concentrations were as follows: 5′ UMP 97 and 89 per cent, 5′ CMP 89 and 90 per cent, 5′ GMP 42 and 56 per cent, deoxy 5′ AMP 18 and 38 per cent, 2′ AMP 0 and 33 per cent, sodium beta glycerophosphate 0 and 0 per cent.

The specific activity of 5′ nucleotidase (substrate 5′ AMP) was 116.5 for white and 10.25 for brown fat (per microgram P hydrolyzed/mg/protein/hour).

The enzyme in the white fat was found to have a greater thermostability than that of the brown. After 30 minutes of continuous exposure of the homogenates to 60 degrees C the white fat retained 44 per cent of its activity and brown 13 per cent. After 1 hour exposure the brown fat was completely inactivated but the white retained 24 per cent of original activity.

419

JOSEPH E. McCARTHY and F. P. CONTE, Oregon State University.

Determination of the volume of vascular and extravascular fluids in the Pacific hagfish, *Eptatretus stoutii* (Lockington). (By title only)

The partitioning of body fluids in the Pacific hagfish was investigated using several experimental techniques. Direct measurement of the blood volume was obtained through the simultaneous use of tagged red blood cells and plasma proteins labeled with either Evans Blue Dye or H^3-polyglycyl amino acid residues. Red cells obtained from donor animals were incubated with L-methionine-methyl-C^{14} in a physiological saline for two hours. The labeled cells were washed three times and injected either into the anterior or posterior subcutaneous sinus of experimental animals. Blood samples were collected over a period of 104 hours through an indwelling catheter in the posterior subcutaneous sinus. Dilution curves were obtained. Fluid volume calculations were based upon values extrapolated to "zero" time. The packed red cell volume for five animals was 5.5 $\pm$ 0.8 per cent of B.W.

Another group of animals (5) was used to estimate the extracellular fluid volume, as measured by inulin-carboxyl-C^{14}. The average value of 26.7 $\pm$ 4.3 per cent of B.W. was obtained. The interstitial fluid, being the difference between the extracellular fluid volume and the total blood volume, was 6.1 $\pm$ 4.9 per cent of B.W.

Total body water was determined following the dehydration of individual animals to a constant weight at 104 $\pm$ 1°C. The value for five animals was 74.6 $\pm$ 0.9 per cent B.W.

420

WILLIAM W. MILLER III, Stanford University.

Experimental epilepsy in the rat. (By title only)

A chronic epileptic state was induced in rats weighing approximately 60 g, individually housed, and maintained on a choline deficient diet supplemented with ethylaminoethanol (EAE). Controls were fed a similar diet supplemented with choline. Rats receiving the choline deficient diet plus EAE ceased to gain weight after approximately 60 days. The first neurological disturbances, hypersensitivity to light and sound, were noted soon after the cessation of weight gain. The hypersensitive condition was accompanied by tremors that were accentuated by volitional movements. Routine handling of the rats precipitated some convulsive seizures.

To determine the degree of hypersensitivity in EAE fed rats, six animals were injected with Metrazol (pentamethylenetetrazole) and compared with controls receiving similar injections. All rats fed the diet supplemented with EAE showed a highly significant lower threshold to Metrazol than the controls. Electroencephalographic records revealed wave patterns commonly associated with epilepsy in the rats with neurological symptoms.

Clinical examination of the blood and urine revealed no abnormalities except for a minor negative nitrogen balance in animals that had been on the diet for long periods. Histological studies of the heart, liver, intestines, pancreas, spleen, and brain showed no detectable abnormalities. However, slides of testes demonstrated spermatogenesis had ceased and no spermatozoa were present.

Use of a choline deficient diet supplemented with EAE to produce a chronic epileptic condition in the rat should be of value in neurophysiologic, biochemical, and pharmacological investigations.

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LARRY C. OGLESBY, FREDERICK F. HARTLINE, and KERMIT EDWARDS, Reed College.

Some osmotic responses of the sipunculid *Themiste dyscritum*. (By title only)

Themiste (formerly *Dendrostomum*) *dyscritum*, a common sipunculid worm on the open coast of Oregon, was used in an investigation of several aspects of osmotic regulation. There was complete survival in the laboratory (T = 12°C) in salinities from 120% to 50% of sea water (100% = 560 mM Cl/l). Survival was limited to several weeks in 40% sea water. Osmotic pressure (measured with a Mechrolab vapor pressure osmometer) of centrifuged coelomic fluid of fully acclimated animals was within 2% of that of the medium over this entire salinity range. In high salinities, the chloride concentration (measured by direct titration with a Cotlove chloridometer) of centrifuged coelomic fluid was also within about 2% of the chloride concentration of the external medium. However, in 70% and 50% sea water, the coelomic fluid chloride concentration dropped to 80% and 73% of the external value. This suggests the addition to the coelomic fluid of osmotically active particles during the sojourn in low salinity. Worms were capable of a limited amount of volume regulation, but only after imposition of a moderate osmotic stress. There was much individual variation in ability to regulate body volume. (Supported by grant GB-4429 from the NSF.)

422

HOWARD ROTHSTEIN, JOSEPH FORTIN and

MIHIR BAGCHI, University of Vermont.

Anomalous stimulation of thymidine incorporation by actinomycin D. (By title only)

In past studies (Rothstein, Reddan and Weinsieder, Exp. Cell Res. 37:2, 440, 1965; Rothstein, Lauder and Weinsieder, Nature 206:1267, 1965) it has been shown that either injury to or isolation of the bullfrog lens gives rise to pronounced hyperplasia in the epithelial cell population. Antecedent to this hyperplasia, in both explanted and wounded tissues, are found periods characterized by cellular incorporation of H^3 uridine (RNA synthesis) and H^3 thymidine (DNA synthesis) (Rothstein, Fortin and Youngerman, Exp. Cell Res. In press). When uridine uptake is partially suppressed by administration of the antibiotic actinomycin D the impending rounds of DNA synthesis and mitosis are both obliterated. This is only true however when the drug is supplied to the system prior to 36 hours after isolation or *in vivo* injury. Generally, when given after 36 hours the level of thymidine uptake at the usual peak time (48-52 hours) is not drastically affected. We wish to report now, however, that there is a period after wounding during which actinomycin actually stimulates the uptake of thymidine at 52 hours. This occurs at 36 hours post-injury. The reasons for such an effect are certainly not apparent. Papaconstantinou has shown that actinomycin can stimulate protein synthesis in calf lens fibers (Biochim. et Biophys. Acta 114:428, 1966). Whether this bears any relation to the present finding is not certain A careful study of the problem is now underway and a fuller discussion will be published shortly. (Supported by grant NB-05425-02 from the USPHS.)

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RANDALL M. SNEIDER, GEORGE R. SIGGINS, and HERBERT J. BERMAN, Boston University.

Comparison of electroanesthesia and MS-222 on vasomotor activity in the retrolingual membrane of the frog (*Rana pipiens*). (By title only)

Since chemical anesthetics may suppress peripheral vasomotor activity, the effects of electronarcosis and the relatively innocuous MS-222 on vasomotion in the same retrolingual membrane were compared. Observations were made first during light chemical anesthesia and later during electroanesthesia induced by passage of a complex sine wave of 100 and 10,000 cps at 2.5 to 12 ma between cloaco-nasal electrodes. The per cent of spontaneously motile arterioles was the same under both types of anesthesia but vasomotion was more rapid during electronarcosis. A cycle there averaged 13 seconds with the constrictor phase lasting 9 seconds, compared to 25 and 10 seconds, respectively, under MS-222 anesthesia. The heart rate at onset of electroanesthesia averaged 81 bpm (range 51-104) and at termination 52 bpm (34-64), in contrast to the steadier rate of 72 (60-82) bpm under chemical anesthesia. Stasis was 3 times more prevalent during electronarcosis. The minimum stimulus delivered to a retrolingual nerve that would induce local vasodilation under electronarcosis and MS-222 anesthesia averaged, respectively, .103 ($\pm$ .024 S.D.) and .045 ($\pm$ .031) ma applied for .01 msec; the corresponding thresholds for vasoconstriction were .37 ($\pm$ .04) and .31 ($\pm$ .09) ma applied for 1 msec. Electroanesthesia as applied here offered no definite advantage over MS-222 in the study of the frog's microcirculation. (Supported by Grant HE-902 and Contract DA-49-193-MD-2696.)

424

P. B. VAN WEEL and L. H. CORREA, University of Hawaii.

Electrophysiological responses in the antennula of *Thalamita* and *Procambarus* to changes in the environment. (By title only)

In a previous communication van Weel and Christofferson (1965) reported on electrophysiological responses in the antennula of poorly osmoregulating crabs to diluted sea water, the first response being obtained with 95% and 90% sea water. It was suggested (van Weel and Christofferson, in print) that osmo-perception was located in these appendages. Experiments were carried out to determine reactions to different sea water concentrations in a good osmo-regulator, *Thalamita crenata,* and in the crayfish, *Procambarus clarkii.* In *Thalamita* 125% sea water did not evoke a response. The first weak increase in activity occurred with 65-70% sea water. A better reaction was obtained with 50% sea water, and fresh water caused a very strong response. The crayfish antennula showed a marked activity with fresh water. 5-15% sea water caused as a rule a definite suppression of activity, but the latter was increased upon application of 50% and 100% sea water, although this remained as a rule lower than in fresh water.

Lowering the pH of sea water (HCl) to $\pm$ pH 6.0 caused but a slight increase in activity of rather short duration in *Thalamita.* At pH $<$ 6.0 the activity became definitely higher. CO_2 as an acidifying agent, did not produce different results. At the high pH 9.0 a rather weak increase in activity was noted. The crayfish antennula showed stronger activities, both at high and low pH's. Again CO_2 had no effect different from that by HCl.

Feedback into the antennula was again noted in both species. That of the crayfish was so strong that it often completely masked the responses to different salt concentrations and pH. Amputated antennulae had therefore to be used to cut out this feedback.

425

DANIEL C. WILHOFT and EDWARD O. REITER, Rutgers University.

The effect of photoperiod on the oxygen consumption of a lizard. (By title only)

Although daily photoperiod has been shown to affect feeding, growth and the reproductive physiology of reptiles, little has been reported of its influence on metabolism. Between September 9 and December 21, 1965, adult *Anolis carolinensis* collected near New Orleans, were acclimated for 21 days to one of three photoperiod conditions: 28°C-16 L. (hours daily photoperiod), 28°C-8 L. and a control group at 28°C in continuous dark. Oxygen consumption was measured at 28°C by the Warburg-Barcroft method of constant volume respirometry utilizing special 250 ml vessels.

Lizards acclimated to 28°C-16 L. averaged .197 ± .014 ml O_2/g body wt/hr while animals acclimated to 28°C-8 L. averaged .237 ± .007 ml O_2/g body wt/hr. Control animals averaged .198 ± .017 ml O_2/g body wt/hr. Each group contained at least 20 animals.

The 8 L. photoperiod resulted in a significantly ($P<.05$) higher oxygen consumption whereas similar values for the 16 L. and control animals were evident. These results suggest that illumination significantly influences the oxygen consumption of this species of lizard maintained at a constant temperature of 28°C. It is possible that the premature exposure of the 8 L. group to near winter light conditions caused a compensatory adjustment which may have adaptive significance in preparing the animals for the light conditions of winter. Future studies will compare spring animals with fall animals under similar temperature and light conditions in an effort to understand the effect of light on the metabolism of reptiles. (Supported by grant AM-08710 from the USPHS.)

DEVELOPMENTAL BIOLOGY

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M. JEAN ALLEN, Wilson College, and Kristineberg Zoological Station, Sweden.

Nucleic acid synthesis in the developing oocytes of the budding form of the syllid, *Autolytus edwarsi* (Class Polychaeta). (By title only)

The stocks or budding form of *Autolytus edwarsi* with their linear chains of successively differentiating stolons were immersed for periods varying from two minutes to twenty hours in 2.5 μc/ml of uridine-H^3 (specific activity 0.90 C/mM). Autoradiographs of serial sections were stained with azure B at pH 4. Incorporation of uridine-H^3 (as evidenced by overlying silver grains) was in structures shown cytochemically to contain RNA (control sections extracted with RNase did not stain for RNA and had very few silver grains). Developing oocytes of stolons which had been treated with uridine-H^3 for two to fifteen minutes show incorporation of the radioisotope primarily over nucleoli. Autoradiographs of eggs exposed to uridine-H^3 for thirty minutes show grains over the nucleolus and over the rest of the nucleus. Oocytes exposed to the radioisotope for two hours show grains over the cytoplasm as well as over the nucleolus and the rest of the nucleus. Further increase in length of exposure to uridine-H^3 resulted in an increased number of silver grains over the cytoplasm, especially in the case of younger eggs. Results indicate that RNA is synthesized (or accumulated) mainly in the nucleolus of the developing oocytes of *A. edwarsi* from which it migrates to the rest of the nucleus and then to the cytoplasm. Results thus far suggest that RNA synthesis is greater in younger eggs; i.e., in oocytes that have not completed their growth period.

Results to date indicate that oocytes studied (mainly older ones) do not incorporate thymidine-H^3 indicating that no significant DNA synthesis was occurring. (Supported by Grants G-19958 and GB-2853 from the NSF.)

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M. JEAN ALLEN, Wilson College, and Institute of Marine Biology, University of Puerto Rico.

Observations on nucleic acid and protein synthesis following actinomycin D and radioisotope treatment of developing stages of the syllid, *Autolytus fasciatus* (Class Polychaeta). (By title only)

After treatment of early cleavage stages of *Autolytus fasciatus* with uridine-H^3, autoradiographs show silver grains (absent in controls pretreated with RNase) over the mitotic apparatus of blastomeres, indicating synthesis of RNA. Autoradiographs demonstrate that RNA is being synthesized during gastrulation, particularly in interphase nuclei, and in the differentiating germ layers of trochophores and post-trochophores.

Protein synthesis, evidenced by incorporation of phenylalanine-H^3, is occurring during gastrulation and in trochophore and later larval stages, particularly in nuclei and cytoplasmic areas rich in RNA (autoradiographs stained with azure B were compared with those predigested with RNase).

DNA synthesis is occurring during cleavage and gastrulation and in trochophore larval stages as evidenced by incorporation of thymidine-H^3 into nuclei (silver grains are absent in sections pretreated with DNase).

Treatment with actinomycin D (100 μg/ml) for 12 to 18 hours inhibits cleavage and gastrulation and prevents further differentiation. At the trochophore stage, actinomycin D (100 μg/ml) for 18 hours results in dedifferentiation of the pharynx and inhibits further differentiation. Autoradiographs of these actinomycin-D-treated stages as compared with controls show that RNA synthesis was inhibited during gastrulation and in post-trochophore stages. However, some autoradiographs also demonstrate inhibition of protein synthesis following a 13-hour treatment of gastrula stages, and inhibition of DNA synthesis following an 18-hour treatment begun at cleavage. Experiments are in progress using actinomycin for shorter periods and at other concentrations in an attempt to demonstrate the effects of specific inhibition of DNA-primed RNA synthesis on the development of *Autolytus fasciatus*. (Supported by Grant GB-2853 from the NSF.)

428

LUCENA J. BARTH and LESTER G. BARTH, Marine Biological Laboratory, Woods Hole, Massachusetts.

Evidence for a diffusible inhibitor of cellular differentiation in hybrid embryos. (By title only)

Cells from the lethal hybrid cross *Rana pipiens* ♀ × *Rana sylvatica* ♂ survive and differentiate into nerve, melanocytes, cardiac and striated muscle, ciliated epithelium, etc. (Develop. Biol., 13: 95-111, 1966). Since the same cells left within the hybrid embryo die without differentiating, it was proposed that diffusible inhibitory products are made by the genetically incompatible nucleus and cytoplasm. The present experiments provide evidence for the inhibitor hypothesis.

Media collected at several intervals from dishes containing bisected hybrid gastrulae retard healing of diploid *Rana pipiens* cells. Media collected so

as to conserve perivitelline and blastocoele fluids as well as diffusible cell products, reduce the proportion of cells that differentiate and in some experiments kill the cells.

Using appearance of heartbeat as the criterion for differentiation, the critical mass of hybrid cells below which differentiation occurs was found to lie somewhere between half the gastrula and the entire gastrula. Differentiation of functional cardiac muscle as well as other cell types invariably occurs in aggregates of approximately 100 cells prepared from the dorsolateral marginal zone. Now it can be added that fragments approximately 1/12, 1/6, 1/8, 1/4 and even 1/2 the gastrula will form a heart if the dorsal region is included. Presumably above this critical mass inhibitory gene products accumulate that result in the block to development.

Hybrid cell surfaces differ from normal so that dissociated cells do not cross aggregate but sort out according to nuclear type. (Supported by grant HD 00701-02 from the USPHS.)

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W. E. BERG and K. M. KILGORE, University of California, Berkeley.

Effects of nucleic acid extracts on development of half embryos of the sea urchin. (By title only)

Nucleic acids were extracted from developmental stages of the sea urchin, *Lytechinus anamesus*, by the cold phenol procedure. Embryos were homogenized in 2.5% dodecyl sodium sulfate in 0.05 M Tris buffer and mixed with an equal volume of cold phenol. After centrifugation the nucleic acids in the aqueous phase were purified by repeated precipitation with ethanol. The final precipitate was dissolved in buffered saline and diluted with sea water. Extracts were prepared from early and late stages of embryos strongly vegetalized by 0.03 M LiCl. Varying amounts of these extracts were injected by means of a micropipette into the blastocoel of animal half embryos previously isolated at the 8-cell stage. In another series of experiments animal half embryos were reared in different concentrations of the above extracts. Development of the animal half embryos was arrested, retarded, or abnormal in degrees dependent upon the amount injected or the external concentrations of the extract. Vegetalization of animal half embryos occurred occasionally in the experimental cultures; however, in no instance could it be considered a specific effect of the extracts.

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ALEXANDER CENSOR and V. F. LINDEMAN, Syracuse University.

Collagen turnover under age delaying levels of food intake restriction in mice. (By title only)

In an attempt to account for the retarded aging of animals and collagen fibrils, both of which occur during restricted feeding, a study of the turnover and synthesis of soluble and insoluble collagen in the tail tendons of female A/HeJ mice was carried out. Mice injected with L-glycine-C^{14} or L-proline-C^{14} were subjected to *ad libitum* feeding, constant intake restriction (20 to 43 per cent below *ad libitum;* 2.3 g/day), and 20 days of 2-day intermittent feeding and fasting cycle.

No statistically significant differences in turnover rate were found under either constantly restricted food intake or intermittent intake. In *ad libitum* mice, from 42 to 66 days of age, the ratio of soluble/insoluble collagen increased from .553 to 1.12. The ratio of total tail tendon collagen/body weight decreased (2.18 to 1.91 mg/g) in this period while the total collagen/tail tendons of one tail increased slightly (32.2 to 35.9 mg). Constant restriction inhibited net synthesis of both soluble and insoluble collagen, resulted in 18 per cent higher ratio of soluble/insoluble collagen, and caused a 6 per cent increase in ratio of total tail tendon collagen/body weight. The intermittent fasting and feeding caused a 5 per cent reduction in tail tendon soluble collagen but did not affect the soluble collagen/body weight ratio.

431

NICHOLAS COHEN, University of California, Los Angeles.

The role of the thymus in the development of transplantation immunity in the salamander, *Ambystoma tigrinum*. (By title only)

A comprehensive study of the development of immunologic responsiveness in the Urodela is in progress. This preliminary report deals with the role of the salamander thymus in the maturation of transplantation immunity.

Fifteen *Ambystoma tigrinum* larvae (9.1 to 11.7 cm long) were totally thymectomized 36 days post-hatching. Eighteen control larvae (same age and size) were sham-thymectomized. All hosts were maintained at 23-24°C.

Controls: Complete melanophore destruction (zero end point) was observed in 67% of the control allografts with a Median Survival Time of 24.5 ± 2.9 (S.D.) days. A zero end point for the remaining six allografts could not be determined accurately since the migration of host melanophores over a partially destroyed graft eventually made it impossible to distinguish between viable donor and host melanophores. A 50% end point determination for all control grafts was 21 ± 6.3 (S.D.) days.

Experimentals: Thymectomized larvae metamorphosed normally and were not runted. Of the fifteen allografts examined, 52% are presently fully viable 100 days after transplantation. A ninth graft was fully viable when its host died 30 days post-grafting. None of the remaining six allografts exhibited a zero end point. Rather, they were characterized by approximately 10-50% destruction before host replacement of graft melanophores or host death occurred. In all but one graft, comparable destruction occurred significantly earlier in the controls.

Therefore, thymectomy of *Ambystoma* larvae 36 days post-hatching greatly prolonged allograft survival. Moreover, no concomitant "runting" syndrome (associated with thymectomy of neonatal mammals) occurred. (Supported by USPHS Grants 1 F2 CA-30,748 and HD-01252.)

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BRUCE B. CRILEY, University of Illinois.

Development of the chick embryo *in ovo* following complete removal of the vitelline membrane over

the blastodisc. (Introduced by Ray L. Watterson) (By title only)

Experimental embryologists have apparently presumed that chick embryos would die or develop abnormally following complete removal of the vitelline membrane over the blastodisc *in ovo*. The presence of this membrane is a great obstacle in experimenting *in ovo* on early stages (up to 48 hours of incubation). This membrane readily takes up vital dyes thereby obscuring the embryo and making accurate staging and major operational procedures difficult. Large slits in this membrane result in high post-operative abnormalities and mortality, presumably due to desiccation (loss of sub-vitelline fluid). Careful observations by the author, however, indicated that the vitelline membrane itself is responsible for these effects, in that slits in this membrane (particularly those one-fourth the length of the developing embryo or larger) subsequently enlarge and the vitelline membrane adheres to and tears the embryo as its loose edges recede peripherally. After the entire embryo and overlying vitelline membrane are stained with neutral red dye contained in an agar disc, complete removal of the membrane over the entire blastodisc is easily accomplished by poking a hole in the membrane, adding a few drops of warm 0.7% saline solution in order to elevate it, and tearing away the elevated, stained membrane with a watchmaker forceps. The entire stained embryo is thus completely accessible for operational procedures and very accurate staging. This technique has been used successfully for various operational procedures on hundreds of chick embryos incubated 18-48 hours, and survival has never been less than 80% to day 6, when experiments were terminated.

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JOSEPH C. DANIEL, JR., University of Colorado.

Fusion of blastomeres when rabbit ova are cultured in an alkaline medium. (By title only)

At first observation, most rabbit ova retained in alkaline medium do not appear to cleave *in vitro*. Time-lapse cinematographs of these cultured ova show that in some cases cleavage does occur but the resultant blastomeres fuse immediately back together again, consequently giving the impression that no change has occurred. The fusion of blastomeres has been observed in different specimens at the first, second, and third cleavages when the pH of the culture medium was about 8.3. A second cleavage of the fusion-blastomere after its first abortive attempt has not been observed, when retained at the same pH, although the cytoplasmic movements of the cell, observable in the time-lapse pictures, and presumably an indication of viability, may continue for 12 hours or more. (Supported by Grant GB-4401 from the NSF.)

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RICHARD A. FENNELL and LYMAN B. CRITTENDEN, Michigan State University and Regional Poultry Research Laboratory.

Some histochemical observations on lymphatic tissues of inbred Rous sarcoma virus resistant and susceptible 20-day embryos. (By title only)

Embryos of White Leghorn chickens (inbreeding coefficient exceeds 95%) with various degrees of resistance to Rous sarcoma virus were used for experimentation, e.g., line 6 susceptible, line 7 resistant and line 15I variable in response to the virus (Waters and Burmester, J. Natl. Cancer Inst., 27:665, 1961). Localization of dehydrogenases, reductases and hydrolases was made by the procedures of Pearse (Histochemistry, Theoretical and Applied, 1961). From 0.05 to 0.3 ml of 1-50 dilution (W/V) of tissue homogenates were used for colorimetric tests. Tetrazolium salts were used as acceptors for electrons. Purple formazan (reduced tetrazolium) was extracted with ethyl acetate. Tetrazolium-cobalt mixtures plus DPNH and phenazine methosulfate were used for the calibration curve.

Lactate dehydrogenase reactions were highest in 15I and lower in bursae of lines 6 and 7, e.g., homogenates (0.3 ml) reduced 0.064, 0.049 and 0.045 mg of tetrazolium, respectively, in 30 minutes. Likewise DPN diaphorase, malate (DPN), isocitrate and succinic dehydrogenase reactions were more intense in line 15I bursae. Reactions were essentially the same in the spleen and thymus of all lines. Alcohol, β-hydroxybutyrate and glucose-6-phosphate were weak or absent in all lymphatic tissues. Acid phosphatase activity was highest in line 15I and lower in 6 and 7 bursae.

Similar patterns of distribution of oxidative enzymes existed in sections of tissue. Intensity of enzyme reactions paralleled that observed colorimetrically. Enzyme activity in 15I bursae was intense in the epithelium and outlines of follicles were distinctly shown by deposits of formazan. In many instances, 4 rows of follicles were identified in each bursal fold whereas in lines 6 and 7 the number was usually 2. Leucine aminopeptidase activity was intense in the spleen of all embryos. The lamina propria mucosae of all bursae reacted intensely for leucocyte alkaline phosphatase.

Two lactate isozymes were identified in all tissues. The largest number of protein bands identified by disc electrophoresis was found in lymphatic tissue of line 7 embryos. (Supported by grants from USDA, Poultry Research Branch, Animal Husbandry Division and the All College Research Fund, MSU.)

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BEATRICE B. GARBER, E. J. KOLLAR, and A. A. MOSCONA, University of Chicago.

Age-dependent differences in the development of chimeric aggregates of embryonic mouse and chick skin cells. (By title only)

Cell suspensions were prepared by trypsinization of skin from 13- to 16-day mouse embryos (MS) and 8-day chick embryos (CS). Cells from the following tissues were commingled, co-aggregated by rotation, and the development of their aggregates was studied in CAM grafts: (1) MS with CS, (2) M epidermis with CS, (3) M dermis with CS. Control aggregates of embryonic CS cells consistently formed skin and feathers; aggregates of MS cells formed skin and hair. In all bispecific co-aggregated with mouse epidermis or dermis cells formed; however, they differed strikingly with the age of cells at the time of co-aggregation. CS cells co-aggregated with mouse epidermis or dermis cells

from embryos *earlier* than 14 days formed skin and feathers which included mouse cells in their structure; in these combinations no hair developed. In aggregates of CS cells with MS cells from embryos *later* than 14 days, hair follicles developed abundantly; however, feather development was completely suppressed; bispecific skin sheets were common but hair follicles did not include chick cells.

Reconstruction of tissues in heterotypic aggregates involves sorting-out and histogenetic grouping of cells. Our findings suggest that the sorting-out properties and morphogenetic "recognition" of embryonic cells dissociated from the same tissue change with age or state of differentiation, hence, that contact behavior of cells is related to other aspects of cellular differentiation. Thus, in these bispecific co-aggregates, early embryonic MS cells tended to be included in the developing chick integumental structures, and hair formation was suppressed; on the other hand, older MS cells pursued their characteristic course of development and suppressed feather formation. (Supported by Grants HD-01253-06 from the USPHS and G-23852 from the NSF.)

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CHARLES W. GIBLEY, JR. and JEFFREY P. CHANG, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

Fine structure of tubule cells in the functional pronephros of *Rana pipiens*. (By title only)

Proximal tubule: The tubule consists of a single layer of high cuboidal or columnar epithelial cells resting on a thin, undulating basement layer. The luminal surface is covered with a characteristic brush border observed as a series of evaginations of the apical cell surface. Pinocytotic vesicles and vacuoles are beneath the microvilli. The nucleus is located near the basement layer. The plasma membrane at the base of the cell invaginates and follows an irregular course into the apical cytoplasm. These basal infoldings compartmentalize the mitochondria which vary in shape and contain abundant cristae. The endoplasmic reticulum is sparse and studded with ribosomes. The boundaries of adjacent epithelial cells are characterized at their apices by a junctional complex—the desmosome.

Distal tubule: The distal tubule cell is easily distinguishable by its shorter microvilli, central nucleus, and lack of pinocytotic vesicles and vacuoles. The infoldings of the plasma membrane are more complex than in the proximal cell. The endoplasmic reticulum is better organized and contains attached ribosomes. Mitochondria are present in greater abundance. The desmosomes and arrangement of the Golgi resemble that observed on the proximal tubule cells.

These observations suggest a similarity in fine structure between the functional embryonic kidneys in frog and chick (Gibley and Chang, '66, this meeting), and adult kidneys. (Supported by a grant from the Pennsylvania Heart Association and by NSF Institutional Grant Number GU-1499.)

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RICHARD W. GLADE, DAVID W. SONNEBORN, and HOWARD ROTHSTEIN, University of Vermont.

Effect of actinomycin D injected at various periods after forelimb amputation in *Triturus viridescens*.

Actinomycin D, an antibiotic which strongly inhibits DNA-primed RNA synthesis, has been injected intraperitoneally at various times after amputation into newts, *Triturus viridescens*, at a concentration of 5 μg/g wt. This level was found to inhibit limb regeneration when administered one day prior to amputation. A very high mortality occurred in all groups receiving the antibiotic. However, of the survivors, those receiving actinomycin one day after amputation failed to regenerate, whereas those receiving the drug 2, 4, 8, 16, or 21 days after amputation did regenerate (16 of 70 cases).

Regeneration was retarded in experimental animals and was typically preceded by a phase characterized by hemorrhage under the epithelial cap.

It seems reasonable to postulate that stable messenger RNA, normally produced within the first 48 hours (as a result of the stimulus of amputation), was inhibited by the actinomycin. These results, however, are not inconsistent with a more indirect action of the drug.

The actinomycin D used in these studies was kindly furnished by the Merck Sharp and Dohme Laboratories, Rahway, New Jersey. (Supported by NSF Institutional Grant GU-1505.)

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LOUIS A. HANSBOROUGH, E. AWATEFE, and DOLORES BUTLER, Howard University.

Innervation of grafted mouse limb buds by the chick embryo. (By title only)

Fore- and hind-limb buds of 9-day albino mouse embryos were grafted to the right wing bud of 3-day White Leghorn embryos. Wings with grafted mouse limb buds were removed from the host daily from 1 to 9 days after transplantation. Some were fixed in Bouin's fluid, prepared for sectioning, and the sections cut at 10 microns and stained in Delafield's hematoxylin and eosin. The rest were fixed, sectioned and stained according to Morse's modification of Bodian's technique.

Examination of the prepared slides showed that in the early stages of development of host wing and graft there is extensive migration of the graft cells into the wing. They differentiate into epidermis, cartilage and myoblasts. Later stages show further differentiation into mouse skin, bone and muscle bundles. All grafts examined during the first four days after grafting show no nerve fibers. Nerve fibers appear in the graft on the fifth day after tansplantation and can be traced to their origin in the brachial nerve of the host. Innervation is more extensive on succeeding days up to the ninth day.

These results give evidence of the presence of a nerve growth-promoting substance which "attracts" growing nerve fibers into the end organ which produces it. Such a substance may be produced by the grafted mouse limb bud and its nonspecificity is evident.

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LOUIS A. HANSBOROUGH and ISAAC J. POWELL, Howard University.

Initial function of the transplanted mouse thyroid in the chick embryo. (By title only)

Thyroid glands of the 15-day albino mouse fetus were transplanted to the chorioallantoic membrane of the 9-day White Leghorn embryo. At daily intervals from 1 to 10 days after transplantation some of the glands were removed from the host membrane and fixed in Bouin's fluid and prepared for sectioning according to the usual paraffin method. Sections were cut at 7 microns and stained in Harris' hematoxylin and eosin. On the seventh through the tenth days after transplantation the remainder of the hosts were injected through a large allantoic vein with 1 ml of radioactive iodine (I^{131}) with an activity of 50 microcuries. After 24 hours the grafted glands were removed and from these autoradiographs were prepared.

The results show that the mouse thyroids were vascularized within 2 days after transplantation and contained a few empty follicles in the cortex. The thyroids of from 18 to 21 days (3 to 6 days after transplantation) showed an increase in number and in size of definitive follicles, the majority of which were still located in the cortex. Those in the medulla were less numerous and less well developed. Colloid began to accumulate in the larger follicles of the 22-day-old glands (7 days after transplantation) and increased in amount along with an increase in the number of definitive follicles during the succeeding days. Autoradiographs were also obtained from 22-day-old and older glands. The initial functioning hereby indicated, showed a delay of about 3 to 4 days when it is compared to that of the intact gland, which has been demonstrated elsewhere.

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ROLF E. HUFF and SAMUEL A. RAMIREZ, Texas Technological College.

Cytological changes in artificially activated eggs of *Rana pipiens*. (By title only)

This report is a sequel to a recent study (Huff and Preston, Texas J. Sci. Vol. 17, 1965) which demonstrated that artificially activated eggs of *Rana pipiens* produce a mitogenic agent, the second factor, capable of initiating cleavage in other unfertilized frog eggs. The aim of the present study was to examine artificially activated eggs for cytological changes that might accompany second factor production. Eggs of *Rana pipiens* were subjected to electrical shock or cortical puncture. At various periods of time after activation eggs were fixed, sectioned, and stained.

Although the eggs remain uncleaved, nuclear replication occurs as early as 10 hours after activation, and nuclei are gradually distributed throughout the eggs. Progressive vesiculation begins after 18 hours and eventually fills the animal hemisphere with vacuoles. The eggs contain many cytasters during the period in which they also produce the second factor.

Numerous studies have shown that successful parthenogenetic treatment of sea urchin eggs leads to cytaster formation. The present study raises the possibility that the main difference between parthenogenesis in sea urchins and frogs is the time required for activated eggs of these two animals to produce cytasters capable of forming cleavage-spindles. In sea urchin eggs cytaster formation occurs shortly after activation and leads to normal cleavage. In frog eggs cytaster formation is delayed until after the eggs have lost their capacity to cleave. Extracts of such eggs induce cytaster formation and cleavage in other unfertilized frog eggs. The same basic mechanism probably initiates parthenogenesis in both types of eggs.

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A. A. HUMPHRIES, JR. and WILTON M. WORKMAN, Emory University.

Sialic acid in the oviducts of the newt, *Triturus viridescens*. (By title only)

Cytochemical evidence from previous work (Humphries, Develop. Biol., 13:214, 1966) suggested that parts of the jelly capsule of the egg of *Triturus viridescens* contain sialic acid. Preliminary assays of the sialic acid content of oviducal segments, eggs and jelly of *Triturus viridescens* have been made, using the thiobarbituric acid method of Warren (J. Biol. Chem., 234:1971, 1959). Most, if not all, of the sialic acid of the mature egg with capsule, taken from the posterior part of the oviduct, is derived from the oviducts and is in the capsule itself. Regions B and D of the oviduct, which secrete jelly layers J2 and J4, respectively, are rich in sialic acid, while the remaining three secreting regions (A, C, and E) have little or none. Regions B and D differ in that there is a sizeable amount of free sialic acid in D, whereas in region B the sialic acid is almost all bound. We have not yet established clearly the situation with regard to the jelly layers themselves, but these findings, plus cytochemical observations, suggest that jelly layers J2 and J4 contain sialic acid. Cytochemical findings suggest that some sialic acid from layer J4 may ultimately accumulate in layer J5. (Supported by grant GM-09878 from the USPHS.)

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JOHN M. MALLETTE and BEATRICE CHAN YUEN MAN, Tennessee A & I State University.

Some effects of maternal intake of nicotine on fetal mice. (By title only)

Nicotine, in .05 mg/ml concentrations was given to pregnant CF1 mice; controls were given distilled water. All animals were fed mouse Breeder chow and daily food and water intake were recorded. Chronic exposure to nicotine is associated with a decrease of water and food intake and consequently decrease in increment of weight gained during pregnancy. From the results obtained the following effects were noted: (1) absence of orbital structures in over 90% of the embryos born, (2) absence of the gall bladder in approximately 60% of the animals born, (3) decrease in the number of animals per litter in all of the experimental groups. (Supported by Grant GY-182 from the NSF.)

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RICHARD L. MILLER, Oregon State University.

Gel filtration of the chemotactants of the hydroids *Campanularia, Tubularia* and *Gonothyrea*. (By title only)

Alcohol extracts of the female gonangia of *Campanularia calceolifera,* of the male and female hydranths of *Tubularia crocea,* and of male and female colonies of *Gonothyrea clarkii* have, in each instance, yielded active solutions that attract the sperm of the homotypic species. There is no cross-reactivity between the extract of the athecate *Tubularia* and the sperm of *C. calceolifera* or *G. clarkii*; however, cross-reactivity exists between the two thecates.

A Bio-gel P2 column 0.9 × 54.2 cm (void volume, 25 ml; total volume, 55 ml) was prepared and distilled water samples of the active extracts of *C. calceolifera* and *T. crocea* passed through the column. Both samples appeared in the second void volume, the *Campanularia* peak at the 35th ml and the *Tubularia* peak at the 48th ml. If both chemotactants were mixed prior to chromatography, almost complete separation was obtained.

Compounds of known molecular weight were used to bracket the peaks of chemotactic activity and on this basis rough estimates of 600 MW for the *Campanularia* chemotactant and 240 MW for the *Tubularia* chemotactant have been made.

Extracts of female colonies of *G. clarkii* were run on a similar column (0.9 cm × 55 cm; void volume, 23 ml; total volume, 49 ml) and these yielded two rounded peaks of activity, one at 30 ml and the second at 39 or 40 ml. The trough was at the 35th ml. A comparative run of the *C. calceolifera* extract gave the usual single sharp peak. Assay of both runs was done with *C. calceolifera* sperm. The fractions of the first *Gonothyrea* peak were combined, evaporated to 1 ml, and rechromatographed on the same column. This gave a single low peak at 31 ml with some loss of activity. The second peak was treated in the same way and yielded a single active peak at the 39th ml with a similar loss of activity. This indicates that ther are two sperm attracting substances produced by *Gonothyrea* colonies, though whether one or both is involved in the actual attraction of sperm to the female medusoid is as yet unknown. It is of interest, however, that a contact guidance phenomenon is coupled with chemotaxis during fertilization in *Gonothyrea clarkii.* (Supported by an NIH postdoctoral fellowship.)

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OLIN E. NELSEN, University of Pennsylvania.

Some features relative to the behavior of the cortical layer during blastulation and gastrulation in *Rana pipiens.* (By title only)

The cortical layer is established quickly following the 28-32 cell stage as the complex blastula supersedes the simple blastula. It maintains its integrity throughout later blastulation and gastrulation, and also contributes cells inwardly. The inner cells organize themselves in relation to this layer thereby creating architectural design in blastocoelic development. As the pregastrulative changes transform the late complex blastula into a gastrulating mechanism the cortical layer above the floor level of the blastocoel, i.e., the animal-pole cortical layer, may be regarded as the *superficial epiblast* and the vegetal-pole cortical layer as the *superficial hypoblast.*

During gastrulation the chorda-mesoderm moves inwardly through the involution passageway provided by the area between the gastrular cleavage or groove and the blastoporal lip composed of superficial hypoblast. It thus passes below the lip and not over the lip. The invaginating superficial hypoblast ultimately comes to line the primitive enteron other than stomodaeal and proctodaeal portions which are composed of superficial epiblast. Later, also, the latter will surface internally the primitive neural tube as well as give origin to the precocious periderm.

The cortical layer of cells at the time of its origin apparently inherits substance from the various areas of the cortical region of the fertilized egg. Hence, it probably is a bearer of guidance information to various parts of the developing blastula; and, altered by pregastrular change, it may aid, informatively, the processes of gastrulation where cellular groups organize in relation to it.

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OLIN E. NELSEN, University of Pennsylvania.

Effects of increased partial pressures of O_2 upon activities relating to organization in the early embryo of *Rana pipiens.* (By title only)

Fertilized eggs were exposed in chambers to an air-oxygen mixture containing increased partial pressures of O_2 approximating 2.2 and 3.2 atmospheres at a temperature of 4°C-5°C. Controls similarly exposed in air under comparable pressures. Exposed eggs removed to room temperature. Unless controls presented 90% development or better experiment discarded. Exposure to 2.2 atmospheres of O_2 results similar to controls, but 3.2 atmospheres produced adverse effects. Exposure to 3.2 atmospheres for 14 hours from 20 minutes after insemination to near 1st cleavage premitted 63% to escape late blastular block but same exposure during 1st and 2nd cleavages allowed 12% escapees. Exposure for 24 hours to 3.2 atmospheres of O_2 from insemination to 1st and 2nd cleavages produced arrest in 89%; during 1st to 4th cleavages 96%; during middle to late blastula arrest in rare instances only. Arrested blastulae sectioned demonstrated great majority to be late blastulae, few in pregastrular condition. Escapees developed degrees of gastrular block, abnormal and few normal larvae.

Theoretical: Cortico-endoplasmic change following insemination establishes developmental direction and blastular morphogenetic potential sufficient to organize to late blastula. Pregastrular morphogenetic potential is added, particularly to crescentic area, during early cleavages under aegis of competent genes from both pronuclei. Genes induced to act by species-specific, animal-pole ooplasm. Pregastrular morphogenetic potential changes during blastulation and effects pregastrular transformation of late blastula into gastrulating mechanism. Theory applicable to hybrid crosses, haploid syndrome, nuclear transplantation.

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C. EDWARD QUINN and ROBERT L. CRISTOFARO, Manhattan College.

A loss of antigenicity during pupation of *Tenebrio molitor.* (By title only)

Tissue homogenates (1.0%) were prepared from 1, 3, 6, 9, 11, and 14 day old *Tenebrio molitor*

pupae. Each homogenate was used to evoke antibody formation in a different pair of adult male rabbits. Antisera from the rabbits were collected and Ouchterlony agar gel diffusions were used to indicate the specific antigenicity of each homogenate. All possible antigen vs. antiserum diffusions were carried out.

The test results indicated a striking lack of antigenicity on the ninth day of the 14-day pupal period, although antiserum to the 9-day homogenate crossreacted normally with all other homogenates. This is interpreted as indicating extensive degradation of proteins during the ninth day of pupal development. (Supported by Grant GE-8149 from the NSF.)

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WILLIE M. REAMS, JR., University of Richmond and the Medical College of Virginia.

Pigment cell densities and the emigration of melanoblasts from the skin in the chick embryo. (By title only)

Since various spotting patterns have been attributed to the available number of pigment cells, an attempt was made to ascertain the effects of pigment cell densities on the colonization of the leg musculature in the chick embryo. Various dosages of melanoblasts from Silver Campine embryos were inoculated into various sites in the ectoderm of the hind limb bud of White Leghorn host embryos. Three hundred hosts were recovered at 13 to 18 days of incubation and examined. The extent of pigmentation of the inoculated limb skin was proportional to the size of the inoculum.

When an inoculum was sufficient to establish a pigment cell density of 400/mm² in the skin of the leg prior to day 13 of incubation, the subjacent muscle possessed melanocytes. In cases where a small inoculum was received, the critical density was not attained until after day 13 and the muscle remained free of melanocytes. A thorough study of the operants indicates that in normal embryogenesis, the emigration of melanoblasts from the skin is dependent upon an adequate number of initial precursor pigment cells from the neural crest to establish a critical population density in the skin prior to the structural restriction of pigment cell migration by the advancing differentiation of the tissue substrate. (Assisted by grants from the Virginia Academy of Science and the University of Richmond.)

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FRANK SETO, University of Oklahoma.

Variations in embryonic splenomegaly in chick embryos as the function of donor age. (By title only)

The capacity of immunologically competent tissues to initiate splenomegaly in chick embryo recipients by the graft-versus-host reaction increases with the age of donor birds (Solomon, J. Embryol. exp. Morph., 9:355, 1961). A systematic reinvestigation was made to quantitate the growth of this capacity in chickens. Chick embryos of 13-14 days of incubation, which exhibit the greatest splenomegaly, (Isacson, Yale J. Biol. Med. 32.209, 1959), were used to assay the capacity of blood, spleen and thymus cell suspensions from various aged homologous donors.

Each of 7 game and 6 white leghorn chicken donors was bled by cardiac puncture at weekly intervals for 8 weeks and at 2-4 week intervals for the next 12 weeks. An equivalent of 0.1 ml of whole blood was injected intravenously into each host embryo, which was sacrificed 5-6 days later to obtain organ weights. The donors differed in the degree of splenomegaly elicited in host embryos, but in most donors the relative change with age in this capacity followed a similar pattern for the first 10 weeks. Rather than a progressive increase with age, blood from white leghorn donors at the 3rd and 6th week produced significantly greater enlargement than at the 4th and 8th week. Thereafter the capacity increased progressively in older birds. The splenomegaly capacity of game bird blood was consistently greater, also showed decreased effectiveness at the 4th and 8th weeks, but in general increased progressively with donor age. Preliminary tests of spleen and thymus cell suspensions showed a similar trend. (Supported by Grant E-409 from the American Cancer Society.)

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GARY E. WISE, University of California, Berkeley.

An ultrastructural comparison of expanded and punctate melanophores in the Pacific Coast Treefrog, *Hyla regilla*. (Introduced by Jane A. Westfall) (By title only)

Expanded melanophores of normal tadpoles and punctate melanophores of tadpoles rendered albino by previous hypophysectomy were examined with the electron microscope. An expanded melanophore usually possesses several long branching processes radiating from the perikaryon. Each process or arm contains membrane-bound pigment granules which appear to lie predominantly just under the cell membrane. The bases of the arms are characterized by a concentration of mitochondria, microtubules, and rough endoplasmic reticulum. Furthermore, this is the only site of the Golgi apparatus, indicating that the base of each process may b the synthetic center for pigment granules. Microtubules are present in the distal parts of the processes as well as basally but it is not clear whether they facilitate the migration of pigment granules.

In the punctate melanophore, all of the pigment granules and organelles are concentrated centrally adjacent to the nucleus and only remnants of the long processes remain, namely, evacuated processes devoid of organelles and containing little, if any, cytoplasm between the apposed cell membranes.

It is postulated that melanin granule migration in melanophores involves the movement of pigment and other organelles within the cell processes. The processes are not withdrawn like pseudopodia. However, since in the punctate state the process consists only of apposed cell membranes, pressure of some type (water uptake perhaps) appears necessary to separate these membranes to accommodate the pigment granules during melanin dispersion. (Supported by a grant from the USPHS to Richard M. Eakin.)

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MARIA de ISSEKUTZ WOLSKY, JACQUES MONTEIL and ALEXANDER WOLSKY, Manhattanville College and Fordham University.

Tail regeneration in the newt *Triturus (Diemictylus) viridescens* under the influence of RNA preparations from adult tissues of the same species. (By title only)

Gross RNA preparations were made with the method of Kirby (J. Biochem. 64:405, 1956) from two organs, liver and tail. The former is composed mostly of a type of cells, which is not found in the tail, the latter containing mostly elements of the skin, muscles and connective tissue including blood vessels, spinal cord, nerves and skeletal tissue. The preparations were dried, dissolved in amphibian Ringer (0.1 mg per cent) and injected (in 3 to 5 day intervals in 0.5 ml doses) into specimens, the tail of which was amputated half way between tip and base on the day of the first injection. Fifteen days after amputation the regenerates were removed, fixed in Bouin's and compared with controls, which were injected simultaneously with Ringer. Specimens receiving liver RNA produced poorer regenerations than the controls, those receiving tail RNA exceeded the controls in regeneration (average length of regenerates: control 0.73 mm; liver RNA 0.58 mm; tail RNA 0.95 mm). These preliminary results seem to agree with findings of Wolsky and Fogarty (Nature, 195:621, 1962) and Skowron, Rzehak and Maron (Folia Biol. 11:259, 1963) who found increased regeneration after autografting to regenerating newts blastema tissue from a previous regeneration. It seems now possible that the active factor in these grafts was tissue-specific RNA but further tests will be made with further purified preparations for a definitive answer. (Supported by a grant from the McNaughton Foundation.)

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RALPH HOLT CHENEY and CARL CASKEY SPIEDEL, Brooklyn College of the City University of New York and University of Virginia.

Trichloroacetic acid effects on gametes and zygotes of *Arbacia punctulata*. (By title only)

Gametes and zygotes of *Arbacia* were subjected to various concentrations of trichloroacetic acid, *a general protein inhibitor*. A concentration of 0.0003 M trichloroacetic acid was suitable for significant comparisons of abnormalities induced in the resultant progeny following treatments of (1) *eggs alone*, (2) *sperm alone*, and (3) *zygotes*. Progeny that developed from *treated eggs* fertilized by *normal sperm* (#1 above) were *still without skeleton* after two days and remained so. In sharp contrast progeny that developed from *normal eggs* fertilized by *treated sperm* (#2 above) *formed skeletal material*. Such skeletal material was usually disordered, resulting in the differentiation of many abnormal plutei after 2 or 3 days that were either *armless, short-armed,* or *one-armed*. Progeny that developed from *treated preblastular 6-hour-old zygotes* in #3 also formed skeletal material which was arranged in more orderly fashion than in #2.

Thus, in #1 the abnormal individuals were induced by trichloroacetic acid injury to *both* nucleus and cytoplasm of eggs. In #2 they were induced by injury to *only* sperm nucleus. Therefore, in #1 genetic and cytoplasmic constituents were affected; in #2 only genetic constituents. Accordingly, we conclude that these treatments at the molecular level probably differentially modified underlying biochemical mechanisms responsible for normal development of skeleton.

Other significant abnormal features included surface roughening, noted in #1 but not in #2, and various irregularities of motion, autotomy, and pigment distribution. Abnormalities induced by trichloroacetic acid were recorded by cinephotomicrography. (Supported by Grant RH 00325-02 to C.C.S. from USPHS.)

INVERTEBRATE ZOOLOGY

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RICHARD M. EAKIN and JEAN L. BRANDENBURGER, University of California, Berkeley.

Fine structure of antennal receptors in peripatus (Onychophora). (By title only)

Electron microscopy of the tips of the antennae of two Panamanian species of peripatus (*Macroperipatus geayi* and *Epiperipatus braziliensis*) revealed at least two kinds of sensory organs: bulbs and pegs. The former are ovoid bodies situated on the sides of grooves on the surface of the antennal tip. Each body consists of an outer layer of lightly pigmented epithelial cells and a core of sensory cells. The distal ends of the sensory cells possess many long microvilli (0.1 μ in diameter) and remarkable cilia, the shafts of which divide and subdivide several times. The terminal branches have about the same diameter as that of the microvilli. Basally each cilium possesses the typical 9 plus 0 pattern of doublets of microtubules. The microtubules arise from a kinetosome (centriole) proximally and pass distally into the subdivisions of the cilium. Numerous mitochondria lie close to the kinetosome from the lower pole of which extend several slender striated rootlets. Microvilli and ciliary branches project into a space at the outer end of the organ which is capped by a thin loose membrane. The sensory elements (presumably ciliary branches and perhaps microvilli) of the bulbs are thought to be chemoreceptors.

The pegs, of several types perhaps, are conical projections from the surface of the antennae. Each peg is covered with a thick cuticle, is slightly constricted at its base, and contains ciliary shafts and microvilli. The presence of pores through the cuticle of some pegs suggests that they are chemoreceptors; others may function as mechanoreceptors. (Supported by a grant-in-aid of research from the USPHS.)

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JULIUS FELDMESSER and KENNETH J. MAY, U. S. Department of Agriculture, Beltsville, Maryland.

An *in vitro* method for increasing saprophagous nematode populations. (By title only)

Quart containers, 18 cm high and 9 cm wide, containing 875-900 ml of aerated tapwater, are inoculated with approximately 100 larval or adult nematodes. These mixtures are enriched at the time of inoculation, and weekly thereafter, by 15

ml per container of a 5% commercial dehydrated potato suspension in water. Excess liquid is drawn off at regular intervals.

Each container is fitted with a short exhaust tube and an aeration tube that extends to the bottom of the vessel: both pass through a rubber stopper. The aeration tube connects to a manifold leading from a 1/50 hp air-pump with a 1510 cm per minute capacity. The manifold accommodates from 10-15 aeration tubes. Aeration is continuous and provides the low oxygen tensions, judged necessary by Santmyer (Proc. Helminthol. Soc. Wash. 23:30, 1956), that enable nematodes to live at all levels in the mixture.

Population increases *in vitro* follow general patterns described for other organisms, with initial stationary and lag phases, followed by periods of logarithmic growth and decline. Highest populations occur after three months. Aliquot samples from the mixtures showed that top, middle, and bottom areas of the vessels may support from 675 to 1000 nematodes per ml of fluid. Populations may, therefore, exceed 800,000 per container.

We grew the following genera successfully. *Alaimus, Panagrellus, Acrobeles, Acrobeloides, Rhabditis, Pelodera, Panagrolaimus,* and *Dorylaimus.* An attempt to grow *Wilsonema* was unsuccessful.

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A. GEORGE NACE III, Cornell University.

Histology of anterior digestive tract of *Thyone briareus.* (Introduced by John M. Anderson) (By title only)

Detailed histological study of the anterior part of the digestive tract of *Thyone briareus* reveals previously undescribed features, including the presence of two differentiated segments between stomach and intestine. Cuticle lines the digestive lumen from upper pharynx through the first of the differentiated segments. The tissue layers of the anterior gut wall (epithelium, muscle, and nerve layer) are supported in a spongy connective-tissue framework. Groups of cells of the lining epithelium of pharynx and stomach occupy connective-tissue compartments of specific design. Mucous glands are localized in the lining of pharynx and esophagus only. Morula cells (Endean, 1958, QJMS 99) occur in varying concentrations in all layers. From the variously thickened inner connective tissue layer extend partitions which form compartments containing clumps of epithelial cells. The inner connective tissue layer is also threaded by weakly-developed bands constituting an inner longitudinal muscle layer. Next to this lies the well-developed circular muscle layer, with fibers organized in slot-like compartments. A second longitudinal muscle layer, outside the circular muscles, occurs only in the stomach. A nerve layer, partially supported by connective tissue, surrounds the outer muscle coat and is covered by flagellated peritoneum. The second of the differentiated post-stomach segments is peculiar in that it lacks both cuticle and inner longitudinal muscle layer; its epithelial lining, without connective-tissue compartments, covers two connective-tissue sheets bounding a hemal space, occasionally distended by large cellular clumps. Weakly developed circular and longitudinal muscle fibers form a network outside the hemal space. The nerve layer and peritoneum are continuous with those of adjoining regions.

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HERBERT C. PERKINS and BERNARD E. SKUD, U.S. Bureau of Commercial Fisheries Biological Laboratory, Boothbay Harbor, Maine.

Body proportions and maturity of female lobsters. (By title only)

Six hundred female lobsters from the North Atlantic continental slope were measured to determine whether changes in body proportions could be used to indicate maturity. The width of the second abdominal segment was compared with the length of the carapace. The carapaces ranged from 48-197 mm; the shortest carapace of an egg-bearing female was 88 mm. The width of the abdominal segments ranged from 27-139 mm: in the smallest egg-bearing female it was 58 mm. All lobsters with a carapace length less than 88 mm had abdominal widths less than 58 mm and only 2.1% of the lobsters with a carapace measure longer than 88 mm had abdominal widths less than 59 mm. When plotted on probability paper, the measurements of the abdominal segments formed a sigmoid curve, indicating a bimodal distribution. The inflection of this curve was between 55 mm and 60 mm, closely corresponding to the size of this segment in the smallest egg-bearing female. Thus, the inflection represents the increased width of the abdominal segment as females approach and attain maturity. A similar analysis of inshore lobsters showed that the abdominal width was usually less than that of offshore individuals for a given carapace length, and that maturity occurred at somewhat larger carapace sizes in the coastal population. This difference, along with other morphometric craracters, may serve as a means of differentiating the two stocks.

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ANNE MARIE STEWART, Tufts University.

Ultrastructure of the eyes in three freshwater triclad flatworms. (Introduced by Chester Roys) (By title only)

The eye structure of three freshwater triclad flatworms was investigated with the electron microscope. The eyes of *Procotyla fluviatilis, Phagocata gracilis,* and *Dugesia tigrina* consist of aligned photoreceptor cells shielded by a cup formed of pigment cells. Light can enter the eyes only through a rather narrow cone whole long axis is parallel to that of the photoreceptor cells. The photoreceptor cells are divided into three regions: an outer segment, consisting of microvilli; a middle segment packed with mitochondria and smooth endoplasmic reticulum and an inner segment which contains the nucleus and neurofibrils and is prolonged as an axon leading toward the brain. The membranes of the endoplasmic reticulum of the middle segment and the bases of the microvilli are continuous at some points. Unlike the structure of the photoreceptor cells in *Dendrocoelum lacteum* as reported by Röhlich and Török (Quart. J. Micr. Sci., 104:543, 1962), there are no vast differences between the ultrastructure of dark-adapted and light-adapted photoreceptor cells in these three species. Each pigment cell contains

many membrane bound pigment granules, some vesicles forming pigment, mitochondria, a sparse endoplasmic reticulum and a nucleus. A sheath covers the outer surface of the pigment cells. It is formed by a layer of extremely attenuated cells. Despite the complex nature of the eyes, eyeless worms seem to orient away from the direction of sunlight as readily as worms with well developed eyes. An investigation of the possible usefulness of the eyes is now underway. (Supported by grants from the NSF and USPHS.)

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RONALD FAYER and DATUS M. HAMMOND, Utah State University.

In vitro cultivation of first-generation schizonts of *Eimeria bovis*. (By title only)

Monolayers of secondary cultures of embryonic bovine spleen and kidney cells were used to support development of certain asexual stages of *Eimeria bovis*.

Oocysts were sterilized in undiluted chlorox for 1 to several hours. They were rinsed twice, resuspended in phosphate-buffered saline and then ruptured in a motor-driven Teflon-coated tissue grinder. The liberated sporocysts were suspended in saline A containing trypsin and bovine bile, and incubated for 2 to 3 hours at 37°C. The free sporozoites were rinsed twice with phosphate-buffered saline, suspended in tissue culture medium (lactalbumin enzymatic hydrolysate, Earle's balanced salt solution, and lamb serum), and inoculated into Leighton tubes containing nearly complete cell monolayers. After incubation at 39°C for 3 hours, the cultures were rinsed with fresh tissue culture medium. Subsequent medium changes were made as necessary. Coverslips were fixed and stained at 24-hour intervals.

The best results were obtained in spleen or kidney cells that had been maintained and transferred over a period of several months. Trophozoites were seen in spleen and kidney cells as early as 4 hours after inoculation of sporozoites. No binucleate forms appeared until 8 days later. In spleen cells, nearly mature first-generation schizonts with some merozoites were seen after 18 days of incubation. Similar development, but without merozoites, occurred in kidney cells. The schizonts were smaller and required a longer time to reach maturity than those in natural infections. (Supported by NSF Grant GB-785 and by an NSF Summer Teaching Fellowship.)

ERRATA

Two abstracts of papers listed and presented at the meeting of the American Society of Zoologists with the American Institute of Biological Sciences at College Park, Maryland, were inadvertently omitted from proper publication in *American Zoologist*, Volume 6, Number 3, August, 1966. These abstracts are printed below and their numbers correspond to the printed numbers in issue Number 3. (Ed. and Prog. Off.)

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HOWARD L. GILLARY, The Johns Hopkins University.

Stimulation of the salt receptor of the blowfly. (Introduced by M. L. Wolbarsht)

Application of alkali halide solutions to the tip of a labellar sensillum of the blowfly, *Phormia*, elicited from the salt receptor repetitive action potentials, which were recorded using the technique of Evans and Mellon (J. Gen. Physiol. 45:651, 1962). Th response (defined as the number of these action potentials in the .5 sec interval beginning .1 sec after the onset of stimulation) exhibited reproducibility, permitting quantitative study. Above a threshold concentration, the response to NaCl solutions increased linearly with the logarithm of the molarity. The responses to LiCl and CsCl saturated to maxima at very high concentrations. The response to solutions of NaCl was independent of pH between 3 and 10. Beyond this range, it was inhibited or irregular. The response was markedly dependent on the temperature of the stimulus, the Q_{10} probably being greater than two. The alkali halides exhibited a regular pattern of relative stimulating effectiveness. The effectiveness of the anions increased monotonically with atomic number. The effectiveness of the cations was greatest for potassium and declined as the atomic number was increased or decreased. The response to a mixture of two salts was the average of those to the single salts at concentrations equal to the total concentration of the mixture. Cross-adaptation was observed between the alkali halides. Receptor sensitivity increased with fly age. One should be cautious in attributing all of these results to effects at the receptor membrane. (Supported by USPHS Grant 5-F1-GM-16,472-03.)

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JOHN L. FREHN, Illinois State University.

Oxidative metabolism of liver mitochondria from cold-exposed hamsters.

Studies were made of the effect of cold exposure (1-3 months at 5°C) on the respiratory activity of liver mitochondria in hamsters. All determinations were made polarographically at 25° and 35°C using the YSI oxygen monitor system and included measurements of the efficiency of oxidative phosphorylation as well as respiration rates in the presence of excess substrate (succinate and beta-hydroxybutyrate) and excess substrate plus ADP. The efficiency of oxidative phosphorylation appears to be unaffected in mitochondria from cold-exposed and hibernating hamsters at both temperatures using both substrates. Respiration, however, was consistently reduced in hibernating animals. Although the succinate-ADP respiration rate is significantly increased in cold-exposed active hamsters when measured at 35°C, the results indicate that at both 25° and 35°C, substrate respiration (with both substrates) and beta-hydroxybutyrate-ADP respiration is unaltered by cold exposure. These results agree with previous studies on chipmunk liver mitochondria (Frehn, *et al.*, Am. Zool. 1(3):355) in which respiration was unaffected at 25°C in cold active animals. (Supported in part by Research Grant GM 13358-01 from the USPHS.)