# *Abstracts*

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HOWARD S. GROB, New York University.

*In vitro* cultivation of ovarian tissues.

Ovaries from rats and mice were dispersed by exposure to the proteolytic enzyme *Pronase*. The method utilized allows for the recovery of corpora lutea and follicles in all stages of maturation as well as suspensions containing all cell types present in the ovary. The tissue subunits and free cell suspensions have been cultivated in a number of tissue culture systems including hanging drops, roller tubes, flasks and petri dishes, all with a variety of commonly available culture media.

Reaggregation studies performed with both mixed tissue populations from whole ovary dispersals and cell suspensions from single follicle dispersals indicate that the surface components of the tissue are apparently normal.

Enzymatically dispersed ovarian tissues exhibit histotypical growth in all systems studied. The outgrowths from isolated follicles are epithelioid in nature. Isolated corpora lutea have been maintained for periods of 35 days and are capable of progesterone synthesis and release throughout this period. Modification of the culture system, and the addition of hormones to the medium allows organotypic growth of isolated follicles. These cultivated follicles appear normal histologically and may be endocrinologically functional. (Supported by grant HD-01541 from the USPHS.)

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JOSEPH T. VELARDO, BARBARA A. KASPROW and VINCENT R. KAVAL, Stritch School of Medicine, Loyola University, Chicago, Illinois, and Institute for the Study of Human Reproduction, Cleveland, Ohio.

Histochemical activity of the uterus of the rat exposed to chronic estrogenicity.

Experiments involving implantation of pellets containing 10.0 mgms. estradiol monobenzoate in rats 52-54 days of age, were performed so as to elucidate the effects of chronic estrogenicity on different histochemical reactions in the uterus. Concurrently, non-treated, intact control rats of similar ages were studied. Experimental and control data revealed: (a) final body weights of experimental animals given estrogen pellets for 161, 168 and 203 days were 241, 227 and 228 grams respectively; controls ranged 317-368 grams; (b) gravimetric data (mgms.%) of uteri of experimentals ranged 268-294; controls during the six stages of estrous cycle ranged 145-196; (c) morphologically, estrogenized animals had enlarged uteri, which contained several changes: squamous metaplasia of glandular and luminal epithelium, inflammatory states—endometritis, myometritis, pyometra and leukocytosis, and glandular cysts; non-treated controls were normal; (d) prolongedly estrogenized animals had histochemical activities resembling those of estrous and proestrous controls, especially noteworthy being localizations and activities of DNA, RNA, glycogen, mucopolysaccharides, collagen, nonspecific esterases, alkaline phosphatase, acid phosphatase, SDH, NAD diaphorase, and NADP diaphorase; (e) estrogenized-induced metaplastic conditions revealed less intense reactions than normal uterine columnar epithelium. Thus, whereas prolonged estrogenization induces atypical uterine changes, the eleven cellular and subcellular substances were maintained and extended for prolonged periods in excess of that seen during proestrus and estrus. (Supported by USPHS grant)

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WILLIAM SCHRADER and D. L. GREENMAN, Johns Hopkins University

Ribonuclease: estrogenic control in rat uterus.

Female albino rats were bilaterally ovariectomized thirty days before the start of experiments. Twice-daily subcutaneous injections of estradiol-17$\beta$ (1.6 $\mu$g per injection) were begun on day 30, and three animals were sacrificed per day. Uteri obtained from these animals were pulverized under liquid nitrogen and homogenized separately in 30 volumes of cold 0.25 M sucrose by ten strokes of a teflon-pestle tissue homogenizer. The homogenates were centrifuged at 34,000 rpm for 2 hours in a Spinco Model 40 rotor. The supernatant fluids, containing most of the ribonuclease activity, were saved for assay.

Ribonuclease activity was assayed by a modification of the method of Spahr and Hollingworth (*Jour. Biol. Chem. 236*:823, 1961). This procedure measures the release of acid-soluble material from RNA; unreacted RNA is precipitated with 0.75% uranyl acetate in 25% perchloric acid. The amount of acid soluble material in the supernatant fluid is measured by its absorbency at 260 m$\mu$.

Protein concentration in the uterine enzyme preparations was assayed by the biuret method.

Uterine extracts show two pH optima for ribonuclease activity; in 0.05 M Tris-Malonate buffers, the optima are at pH 5.7 and at pH 8.4. The pH curve peaks are sufficiently sharp to permit assay of either enzyme activity with only slight contamination by the other.

Uterine extracts from castrate rats receiving estradiol for up to two days show low alkaline ribonuclease specific activity (enzyme units per milligram of extracted protein). Ribonuclease specific activity rises on days 3 and 4, and shows an apparent plateau after the fifth day; the specific activity is about twice that seen in controls receiving no estradiol. (Supported by USPHS Predoctoral Training Grant 5 T01 GM 57-10, and USPHS Grant AM 09324).

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HELEN E. KELLEY and F. STORMSHAK, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

Effect of luteinizing hormone on luteal function in the rabbit.

Thirty mature Dutch Belted female rabbits were assigned randomly to six groups of five animals each in an experiment of 2 × 3 factorial design. All animals were made pseudopregnant by mating with vasectomized males (day of mating = day 0 of pseudopregnancy). Each of the animals

in three groups received a single IV injection of 50 μg NIH-LH-S-9 on day 9 of pseudopregnancy. The animals in the remaining three groups were similarly injected on day 9 with sterile saline. Corresponding treated and control groups were autopsied at time intervals of either ½, 24 or 48 hours post injection. Corpora lutea (CL) were dissected from the ovary, weighed and stored in 95% ethanol until analyzed for progesterone and $\Delta^4$-pregnene-20α-ol-3-one (20α-ol). Corpora lutea in treated animals regressed in size with time (LH × time interaction, $P<.01$). Progesterone content of CL was markedly decreased as a result of treatment with LH ($P<.05$). Luteal progesterone concentration was lower in treated than control animals, particularly at 48 hours, but the differences were not significant statistically. Administration of LH resulted in an initial increase in luteal content and concentration of 20α-ol, followed by a decrease over the ensuing 48-hour period (LH × time interaction, $P<.01$).

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RUSSEL J. REITER, University of Rochester.

Pineal function in androgen-sterilized female rats.

Postnatal treatment of female rats with testosterone propionate (TP) causes maldevelopment of the hypothalamo-pituitary-gonadal axis; when adults these animals exhibit persistent vaginal cornification and anovulatory ovarian cycles. The pineal gland, influenced by the absence of light, delays the onset of puberty in rats but blinded adult animals can reproduce. The combination of early androgen treatment and light deprivation (by blinding) causes striking changes in reproductive organs of adult rats. After receiving various combinations of treatment, ovaries of 7 groups of rats at 75 days of age, weighed 57.8, 38.3, 64.9, 31.0, 27.0, 14.8 and 27 mg for untreated, blinded, blinded pinealectomized, TP-treated, TP-treated pinealectomized, TP-treated blinded, and TP-treated blinded pinealectomized rats, respectively. TP was administered to 4- or 5-day-old animals while blinding and pinealectomy were done on 22-25-day-old rats. Uterine weights of the same groups of rats averaged 342, 171, 335, 306, 301, 125 and 291 mg, respectively. Vaginal smears of TP-treated blinded rats showed mostly leucocytes with occasional nucleated and cornified cells; TP treatment alone or TP treatment, blinding and pinealectomy resulted in vaginal smears that were primarily cornified (70% of the smears contained cornified cells). Histologically, ovaries and uteri of light-deprived androgen-treated rats exhibited changes associated with hypofunction. Speculatively androgen treatment in neonatal rats interferes with the normal differentiation of the anterior and/or preoptic hypothalamus. It is possible that pineal substances also act on the hypothalamo-pituitary axis. (Supported by grant HD-02937 USPHS.)

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LAURENCE HEDLUND and C. L. RALPH, University of Pittsburgh.

Daily variation of pineal serotonin in Japanese quail and Sprague-Dawley rats.

Ninety-day-old male rats and adult quail housed in environment rooms (Hotpack) with a daily light period from 0600-2000 were sacrificed by decapitation at 3-4-hour intervals. Pineals were immediately removed, homogenized in Mg-free Krebs saline and frozen in a dry ice-acetone bath. Bioassays utilized rat stomach muscle strips suspended from a lever and in a tissue bath of Mg-free Krebs saline. Muscle contractions were detected by a strain gauge transducer connected to the lever and recorded by a Grass polygraph. For each assay, single rat pineals were used; 1-3 quail pineals were pooled. At selected times of each cycle the fluorescence of ninhydrin-reacted butanol extracts were measured with a Turner fluorometer.

Serotonin in the quail pineal reaches a high level of 1.4 ng/pineal at 0700, declining to an afternoon low of 0.8 ng/pineal. This level is maintained after the onset of darkness and by 0500 shows slight increase to 1.0 ng/pineal. Fluorometric determination of afternoon serotonin levels agree closely with bioassay results.

Rat pineal serotonin increases from 66 ng/mg at 0800 to 71 ng/mg at 1030 and rises rapidly to a mid-day high of 87 ng/mg at 1300. There is an afternoon decline to 75 ng/mg at 1930 and a rapid fall after the onset of darkness to 39 ng/mg at 2300. Continuation of light to 2300 blocks this nocturnal decline. These results for rat pineal serotonin corroborate those presented by Quay (*Gen. Comp. Endocrinol.*, 3:473, 1963) and Snyder *et al.* (*Proc. Nat. Acac. Sci.*, 53:301, 1965). (Supported by grant GB-5606 from N.S.F.)

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LEONARD A. CIACCIO and R. D. LISK, Princeton University.

Facilitation and inhibition of estrous behavior in the spayed female golden hamster (*Mesocricetus auratus*). (Introduced by R. D. Lisk)

Estrogen treatment is ineffective for elicitation of the lordosis response in the spayed female golden hamster. Following spaying the females were given daily mating tests with a vigorous male. Nine days after spaying crystalline estradiol was placed subcutaneously and mating tests continued for a further 7 days. No lordosis was observed. When a 45 mg pellet of progesterone was added the lordosis response was displayed after two hours. One day later the lordosis response became more difficult to elicit. By day 4, following progesterone placement, no lordosis was observed; only fighting in the presence of the male. A similar response pattern was observed in another group of spayed females which were given a 200 μg pellet of progesterone, following estrogen priming. After inhibition of the behavior developed, removal of the progesterone pellets did not result in a reappearance of lordosis. Upon replacement of the original 45 mg pellets the lordosis response reappeared after two hours; inhibition developed after one day. The response was also reproducible using the 200 μg pellet. Progesterone within the lumen of a 27 gauge tube failed to facilitate estrous behavior. Progesterone treatment, in the absence of estrogen priming, did not produce behavioral estrous. In the spayed golden hamster (*Mesocricetus auratus*) estrogen and progesterone are necessary for behavioral estrous: progesterone first facilitating and then inhibiting the lordosis response. (Supported by grant GM-457 from the N.I.H. and grant DO-2651 from the N.I.H.)

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ROBERT K. CHIPMAN, The University of Vermont

Factors causing estrous inhibition in mice

When female mice are grouped six per cage the number of females showing estrous cycles as well as the frequency of the cycles decreases from that exhibited by single-caged females. The possibility that the inhibition was caused by an ovarian product was investigated by housing a female with five gonadectomized mice (both sexes were used, but not in combination). For these females, the percent of females cycling (65%) was greater than when the same females were grouped six per cage (35%) and less than when they were singly caged (87%). The frequency of cycling (as measured by the number of cycles per female per 12 days) showed the same relationship. Data for single females housed with five preputialectomized females were the same as for females housed with gonadectomized mice. Substituting preputialectomized-ovariectomized females did not appreciably increase the percent cycling or number of cycles per female.

It is postulated that the inhibition of estrus is the result of an ovarian secretion and a preputial substance. However, an additional factor such as density per se is probably operating as well. (Supported by grant HD-01141 from the USPHS.)

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SANDRA L. L. GAUNT, The University of Vermont

Classification and effect of the preputial pheromone in the mouse

The sex attraction of C57BL/6J female mice to the male preputial secretion has been demonstrated by Bronson (Amer. Zool., 6:535 (1966). Abstract). The purpose of the present investigation was to verify this function in ICR-Swiss mice and to isolate the attractant, or pheromone, from preputial gland secretion. Further, a possible function of this attractant in estrous acceleration (Whitten effect) was determined.

Female mice, grouped six per cage for two weeks prior to testing, were placed singly in a circular test cage, 43 cm in diameter, and observed five minutes a day for five days. The cage, divided into equal quadrants, was placed under even illumination and rotated 90 degrees after each test. The quadrants were supplied with various substances on gauze sponges within wire envelopes and relative attraction measured by the time in each quadrant. State of estrus was determined by vaginal smears.

Test I measured the attraction of extracted preputial secretion. Control cages contained only blank gauze. Experimental mice showed preference for the secretion, and estrous activity was significantly higher than controls. In test II, free fatty acids (FFA) and neutral lipids were separated from the preputial secretion using a silicic acid column according to McCarthy and Duthie (J. Lipid Res., 3:117 (1962)). These fractions were tested against each other. Control animals were presented various reagents used in separation. Test mice were attracted to the FFA quadrant and Whitten effect was again demonstrated. Controls showed no preference and no change in degree of estrous inhibition. Test III presented the FFA fraction only. Controls received the neutral lipid fraction. Again, mice were attracted to the FFA and showed a significantly higher estrous synchronization than controls. No attraction to neutral lipids was observed.

It is concluded that the free fatty acid component of the preputial secretion operates as an attractant and accelerates estrus in grouped females. (Supported by grant HD-01141 from the USPHS.)

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ROBERT ORTMAN, City College of New York

Three separate tests of Dawson's hypothesis concerning the thyrotropic cell of adult *Rana pipiens*.

About 90% of the Crossmon-purple cells in the pars distalis (Ortman, 1961) are localized rostral to the lobe's midpoint. These cells are also aldehyde fuchsin-positive (Ortman, et al., J. Histochem. Cytochem. 14:1966). Dawson (Anat. Rec., 1957) hypothesized on the basis of this latter fact that these cells produce thyrotropin.

Dawson's hypothesis has been subjected to three tests. Rostral and caudal half-lobes have been cut using cold acetone (exp. 1) or Ringer's fluid (exps. 2 & 3) as immersion media. The half-lobes were pooled and dehydrated in cold acetone. Suspensions (exp. 1) or extracts (exp. 2 & 3), derived by centrifuging at 0°C the suspension at 10,000 rpm for 10 min, were prepared in Ringer's fluid and assayed by the goldfish microhistometric method of Ortman and Billig (1966) using as a total dose per fish the active material contained in, or extractable from, 1.6 mg of dried powder.

Exp. 1: suspensions gave mean thyroid cell heights of 12.1 micra (rostral half, 5 fish), 13.1 micra, (caudal half, 7 fish); 2.8 micra (Ringer's-injected, 4 fish) and 3.1 micra (uninjected, 4 fish).

Exp. 2: extracts produced mean cell heights of 11.4 micra (rostral, 4 fish), 7.7 micra (caudal, 4 fish) and 2.6 micra (Ringer's-injected, 2 fish).

Exp. 3: thyroid cell heights of 11.3 micra (rostral, 4 fish), 12.2 micra (caudal, 5 fish) and 3.1 micra (Ringer's-injected, 4 fish) were found.

No consistent, and appropriately large, potency difference has been found. Thyrotropin, or thyrotropin-like activity is fairly evenly distributed through the pars distalis. (Supported by grant AM 07204-03 from USPHS.)

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LINDA C. SALAND, The City University of New York and Albert Einstein College of Medicine

Electron microscopic studies of frog pars intermedia as related to neural control of hormone release (Introduced by William Etkin)

Previous ultrastructural studies (Saland, '67, *Anat. Rec. 157*) indicate that dark adapted bullfrog (*Rana catesbiana*) pars intermedia is involved in active protein synthesis, whereas light adapted glands appear relatively inactive. Both gland types contain numerous stored cytoplasmic granules (2000-3000 Å. Nerve fibers and synaptic terminals containing dense core vesicles (600-1000 Å and small, clear vesicles (200-300 Å are observed among secretory cells in all bullfrog specimens. The pars intermedia of bullfrogs fixed while in

transition from dark to light backgrounds appears similar to dark adapted glands, with expansions and whorl formations of rough ER cisternae, and increased numbers of dense, membrane-bounded cytoplasmic granules in the Golgi region. These observations are interpreted as indicating that inhibitory hypothalamic control of the gland first occurs at the level of hormone release, and only as granules accumulate in the cytoplasm is synthesis inhibited. Glands from light adapted animals undergoing darkening show many characteristics of dark adaptation, which suggests that MSH release is promptly followed by initiation of synthesis of new hormone. There is no morphological evidence for dual control of the gland by both neurosecretory (peptidergic) and monoaminergic nerve fibers, respectively, as suggested by Knowles ('65) for elasmobranchs. Neural control of the pars intermedia in the frog is believed restricted to inhibition at the release level by aminergic fibers with intracellular feedback of stored hormone controlling synthesis. (Supported by NDEA predoctoral fellowship, NSF grant GB-5913, and NIH grants 5TO1-HDO116 and NB-05219.)

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E. ASHBY, PENELOPE COATES, L. KRULICH, A. DHARIWAL, and S. McCANN, University of Texas Southwestern Medical School, Dallas.

Early ultrastructural changes in somatotrophs under the influence of hypothalamic releasing factors.

An ultrastructural study was undertaken to determine the effects of crude fractions of sheep hypothalamic releasing factors on the anterior pituitary of the rat. Young adult male Sherman rats were divided into uninjected controls and injected experimentals. Groups were sacrificed at 5, 15, and 30 minute intervals. One-half of each pituitary was prepared for E-M investigation and the other for bioassay. Results of changes in the morphological profiles of somatotrophs (growth hormone producers) are reported in this study.

Control animals presented typical profiles of normal somatotrophs. Injected animals sacrificed at 5 minutes showed somatotrophs with increased granule content over the controls while the 15 minute group resembled the controls. By 30 minutes the somatotrophs showed evidence of granule depletion as indicated by decreased numbers of granules, and a redistribution of the remaining population to the periphery of the cell where the granules were frequently seen in rows subjacent to the cell membrane.

Results from the bioassay of the remaining half-pituitaries were consistent with the interpretation that data on granule frequency and distribution, apparent in the ultrastructure, may serve as an index to storage and release of hormone by these cells. In addition, the early increase in granule counts at five minutes supports other evidence that growth hormone releasing factor may function in both synthetic and release mechanisms.

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RICHARD S. NISHIOKA and HOWARD A. BERN, University of California, Berkeley

Ultrastructural study of the innervation of the pituitary of the teleost *Tilapia mossambica*.

Considerable interest has developed in the nature of the "neurosecretory innervation" of endocrine organs (cf. Knowles and Bern, 1966). The adenohypophysis of *Tilapia mossambica* is invaded by projections of neurohypophysial tissue which penetrate among groups of the various types of cells. Several types of inclusions are found in the axons making up the neurohypophysis (cf. Knowles and Vollrath, 1966). The contents of at least three recognizable types of neurohypophysial fibers are listed in order of frequency: (1) medium-sized granules about 600-1000 Å of variable density, (2) large dense granules (typical elementary neurosecretory granules) often in close association with capillaries, and (3) small electron-luscent vesicles.

In the rostral lobe of the pituitary the axons are separated from the adenohypophysial cells by a thick basement membrane within which are processes of dense cytoplasm presumably originating from specialized pituicytes. Most common are axons containing the smaller granules, although the other axon types are also present. In other parts of the pituitary some axons leave the neurohypophysial projections and are in direct contact with adenohypophysial cells. These fibers generally contain small granules and vesicles. Although some clusters of vesicles are seen at one side of some axons, no synaptoid thickening of the membranes occur.

The kinds of association between fibers and endocrine cells would seem to include both direct nonsynaptic contacts, terminations on basement membranes, and terminations on blood capillaries. Thus, control of the adenohypophysis by a type of neurosecretomotor innervation and by a neurosecretory neuropil are both possible in this species. (Aided by NSF grant GB-6424X).

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JAMES NORMAN DENT, University of Virginia

Survival and function in hypophyseal homografts in the spotted newt.

Earlier work has shown that the hypophysectomized newt (*Triturus viridescens*) at 24° ± 0.5°C ceases to eat and to shed its molts so that it accumulates numerous layers of stratum corneum. It usually dies within three or four weeks, but when implanted autoplastically and ectopically with its own pituitary gland it eats voraciously, molts regularly and lives indefinitely (Dent, 1966). Removal of the autograft is followed by death within about ten days.

Eighteen hypophysectomized newts were implanted ectopically with pituitary homografts. For a time these animals ate and molted regularly but eventually loss of appetite, thickening of the corneum and death occurred, apparently as a result of the immunologic rejection of the graft. The mean survival time of these animals was 171.1 (S.E. = 25.6) days. This is of interest since Cohen (1966) has shown the survival time of skin grafts in this species to be only about 22 days.

Prior to the rejection of the homografts the host animals were indistinguishable in vigor and activity from animals bearing autografted pituitary glands. A radioiodine turnover study, however, indicated the thyroid glands and presumably the thyrotropic cells of the grafts to be significantly less active than those of control animals. This latter finding was supported by histological observations. (Supported in part by U.S. A.E.C. Contract)

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RICHARD L. SWALLOW and W. R. FLEMING, University of Missouri

Effect of hypophysectomy on the metabolism of liver glycogen of *Tilapia mossambica*.

*T. mossambica* (2 to 4 grams) were adapted to a fish ringer (Handin *et al.*, 1964) for one week, hypophysectomized or sham-hypophysectomized, and returned to the saline. The animals were not fed after hypophysectomy. Seven days after surgery, 2 groups of six animals were killed and liver weight-body weight ratios, and liver glycogen levels, measured. Both the control and hypophysectomized animals showed a drop in liver weight from a mean of 2.13 to 1.44 percent of body weight, and no differences in liver glycogen levels were found.

A second set of animals were given a single intraperitoneal injection of 4 mg of glucose and killed 12 hours later. Both groups showed similar increases in liver weight and a net synthesis of liver glycogen.

A third set of animals were injected with a mixture of 15 uniformly labelled $C^{14}$ amino acids. Each animal received 0.33 $\mu$c of isotope and, in addition, 0.67 mg of glutamine. The animals were killed 12 hours later and the specific activity of the liver glycogen measured. Neither group showed a net synthesis of glycogen nor of liver tissue; however, the specific activity of the liver glycogen of the hypophysectomized animals was slightly less than 25% of that found in the control animals.

It is suggested that hypophysectomy of *T. mossambica* does not affect the incorporation of glucose into liver glycogen, nor does it affect the utilization of liver glycogen. Such treatment does, however, affect the formation of liver glycogen from amino acid precursors. (Supported by a NASA Traineeship to the senior author and by NSF GB 2264.)

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PETER K. T. PANG, Yale University

The effect of pinealectomy on adult male killifish, *Fundulus heteroclitus*. (Introduced by Grace E. Pickford)

Epiphysectomized, sham-operated and intact fish were studied in two experiments at 20°C on an 8 hour day in recirculating seawater. Successful removal of the epiphysis was shown histologically. The first experiment (December-March) was within the initial phase of growth and sexual maturation. The second experiment (February-June) included the height of growth and reproduction. Results showed a faster growth rate in pinealectomized fish and this difference was highly significant in the second experiment. Conversely, there was a delay in sexual maturation in pinealectomized animals as shown by the development of nuptial coloration. In the second experiment this delay was accompanied by a significantly lower gonadosomatic index in the controls which were sexually regressed at the time of autopsy. Control thyroid cell height was greater in the first experiment but no significant difference was seen in the second experiment. The hepatosomatic index was significantly lower in the controls in the first experiment but in the second experiment the difference was not significant. There was no difference in the nuclear diameter of the interrenal cells in either experiment but in the second the height of the interrenal zone was greater in the controls. No differences in melanin pigmentation and nocturnal pallor were observed. Hematological studies and serum osmolality showed no differences. (Supported by the Harrison Fellowship, Yale University and a grant from the National Science Foundation.)

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RICHARD A. FLETCHER, University of California, Berkeley.

Effect of vitamin A on reproduction in California Quail (*Lophortyx californicus*). (Introduced by S. Nandi)

Eighteen pair of quail were subjected to long days (15 hours light) from December 13, 1966, to February 13, 1967. The quail were separated into four groups receiving the following I.U. of vitamin A (Nopco Chemical Co.)/lb of diet: I (4 pair)—0; II (4 pair)—1000; III (4 pair)—2500; IV (6 pair)—6000.

No Group I females laid eggs. Three of four females in Groups II and II laid eggs. The eggs of two of the three laying females in each group underwent partial embryonic development but did not hatch. Four of six Group IV females laid eggs and two of these four females laid eggs which hatched.

Based on testicular volume, seminiferous tubule diameter, interstitial cell nuclear diameter and lipid deposits there was no difference in testicular development among these groups. However, no Group I testes contained sperm, whereas 3 Group II males, 4 Group III males and 4 Group IV males showed sperm.

All Group I males died 21 to 33 days after the start of the experiment. Two Group I females died within 45 days. Lack of germ-cell production in Group I without other gonadal changes appears symptomatic of the avitaminosis-A which results n early mortality. Although vitamin A levels in Groups II and III did not appear to interfere with gonadal recrudescence, they were insufficient to allow normal embryonic development. Interference with FSH production is suggested, and pituitary cytology is presently being studied. (Supported by NSF grant GB-2484, NIH grant CA-5045, and a grant from the Union Foundation Wildlife Fund)

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RICHARD E. JONES, University of California, Berkeley

Lack of effect of estrogen on the incubation patch of California Quail (*Lophortyx californicus*). (Introduced by Howard A. Bern)

Before or during egg-laying the ventral integument of most birds undergoes defeathering, vascularization, edema and hyperplasia producing an incubation patch. In passerine birds both estrogen and prolactin are necessary for patch development; estrogen alone is effective because it stimulates endogenous prolactin secretion.

The present study was an attempt to stimulate patch development in California Quail, a galliform, with estrogen alone (estradiol-17$\beta$ or diethylstilbestrol) or in combination with prolactin. Estrogen alone was ineffective, whereas estrogen with prolactin induced patch formation. This difference between passerines and quail can be interpreted in

one of several ways: (1) Estrogen produces a behavioral change ultimately resulting in prolactin secretion in passerines but not in quail; this is improbable, since no behavioral change associated with high prolactin levels (*e.g.*, incubation) was produced by estrogen treatment either in passerines or in quail. (2) Estrogen stimulates the hypothalamo-hypophysial complex to release prolactin in passerines but not in quail; since pituitary prolactin content in estrogen-treated quail was the same as in non-breeding control birds, estrogen did not stimulate prolactin synthesis. (3) Winter prolactin levels are low in quail but high in passerines. Assays of pituitary prolactin content in wintering quail were lower than in quail studied at any other season. However, prolactin content in passerines has been shown to be high in winter; thus estrogen may stimulate patch formation in passerines because prolactin levels are high at this time. (Supported by NSF grant GB-2484, NIH grant CA-5045, and a grant from the Union Foundation Wildlife Fund.)

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JACK E. YOUNG and WALTER CHAVIN, Wayne State University

Effect of various vertebrate insulins upon the serum glucose level of the goldfish, *Crassius auratus* L.

A total of 485 goldfish (1.5 to 5.0 g) maintained under constant conditions (25°C, 12-hour photoperiod and diet) was used. Serum glucose was determined with glucose oxidase on duplicate 10 $\mu$l samples of deproteinized plasma. Fish were injected intramuscularly with U.S.P. insulin vehicle (vehicle control), bovine albumin (protein control) or insulin (commercial bovine, Iletin, 500 U; crystalline bovine, 23.5 U/mg; bonito, 18.3 U/mg; tuna, 16.7 U/mg: *Hydrolagus collei,* 61 mU/mg; *Squalus acanthius,* 2.6 mU/mg).

Serum glucose levels of vehicle or protein controls were not signficantly different 30 min to 7 days post-injection. Iletin (5,000 U/kg) did not effect serum glucose levels 15 min to 4 hr post-injection compared to protein controls but produced significant hypoglycemia at intervals from 6 hr to 7 days. Bonito insulin (1,000 U/kg) produced moderate hyperglycemia 30 min to 4 hr post-injection. Return to control levels was achieved at 6 hr.

Insulin dose response was determined 6 hr post-injection. Compared to vehicle controls, 1.0 U/kg of bovine, bonito, tuna, or *Hydrolagus* insulin or 0.5 U/kg of *Squalus* insulin produced signficant hypoglycemia. Significant hyperglycemia followed 5,000 U/kg of bonito insulin or 1,000 and 5,000 U/kg of tuna insulin.

Glucagon contamination accompanying large doses of insulin may obviate hypoglycemia. Thus, in goldfish, insulin mediated hypoglycemia is measurable when both the glucagon contamination is minimal (low insulin dose) and at a time (6 hr) when the glucagon effect has subsided. Further, similar dose levels of a variety of vertebrate insulins effectively evoke hypoglycemia in goldfish indicating a lack of species specificity. (Supported by USPHS Grant Number AM-04876-06 MET.)

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WILLIAM H. VENSEL, University of Kansas

Reptilian insulin: its extraction and potency. (Introduced by Michael J. Maher)

The purpose of this experiment was to compare the activites of mammalian and reptilian insulins in the species from which the reptilian insulin was extracted. Pancreatic tissue, 1.6 g, from 360 *Anolis carolinensis* was homogenized at 2°C in 16 ml of 85% ethanol (pH 2). Particulate and insoluble material were removed by centrifugation, reextracted in 16 ml of 65% ethanol (pH 2) and centrifuged. Supernatants were combined, defatted with dichloromethane, dialyzed against 1 N acetic acid, flash evaporated, applied to a 2 × 100 cm column of Sephadex G-50 superfine and eluted with 1 N acetic acid. The peaks were isolated, flash evaporated, dialyzed against distilled water and freeze dried. Using a six point *in vivo* assay, the activity of the peak with an elution pattern similar to that of mammalian insulin was determined.

Because previous studies in this laboratory have shown that the temperature at which reptiles are maintained before and during treatment with insulin has a significant effect on the magnitude and duration of the hypoglycemia, the assay was carried out at 33°C, the preferred body temperature of *Anolis carolinensis*. Equal weight log doses of the mammalian standard (bovine insulin, Lilly Lot PJ-4609) and the reptilian extract were administered i.p. in 0.7% saline. Blood samples were taken 4 hours after injection and blood glucose was determined using Glucostat (Worthington Biochemical Corp.). One mg of the reptilian extract was found to be equivalent to 19.1 mg ± 2.1 mg of the mammalian standard. The precision (s/b) of the assay was 0.046.

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GREGORY J. PATENT, University of California, Berkeley, and University of Washington Marine Laboratories, Friday Harbor.

Effects of glucose, mammalian insulin or chondrichthyean insulins on blood glucose levels in an elasmobranch (*Squalus acanthias*) and a holocephalan (*Hydrolagus colliei*). (Introduced by Max Alfert)

One hundred and fourteen dogfish (*Squalus*) and ratfish (*Hydrolagus*) of both sexes were used. An indwelling cannula was implanted into the dorsal aorta *via* the celiac (dogfish) or posterior mesenteric (ratfish) artery to obtain serial blood samples with minimum disturbance. Glucose was determined enzymatically on deproteinized filtrates of whole blood. Glucose (0.5 gm/kg), mammalian insulin (5 IU/kg), and lyophilized acid-ethanol preparations of dogfish (0.3 IU/kg) and ratfish (2 IU/kg) insulins were administered intravascularly in elasmobranch saline. Controls were saline-injected.

Fasting blood glucose levels were approximately 50 mg% (dogfish) and 60 mg% (ratfish). A pronounced hyperglycemia resulted in dogfish (377 mg%) and ratfish (368 mg%) 5 minutes following glucose injection. Blood glucose levels fell rapidly during the next 1½ hours (to 200 mg%); however, normal values were not re-established for 48 to 72 hours.

Maximal hypoglycemia (14.6 mg%) was attained 24 hours following injection of mammalian insulin into dogfish and was maintained for 7 days. Insulin hypoglycemia (22.1 mg%) in ratfish was most pronounced 48 hours after injection.

Dogfish insulin produced maximal hypoglycemia

(12.3 mg%) 24 hours after injection into dogfish, followed by a return to normal levels 72 hours later. When injected into ratfish, maximal hypoglycemia (11.0 mg%) was attained after 48 hours.

Ratfish insulin produced severe hypoglycemia in dogfish (13.6 mg%) and ratfish (20.8 mg%) 24 hours following injection. Hypoglycemia was maintained for at least 3 days in dogfish, and there was no return to normal values. (Supported by NIH predoctoral fellowship, NIH grant AM-07896, and NSF Marine Sciences Training Grant GB-3386).

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HARRY A. KENT, JR., University of Georgia.

*In vitro* hormonal control of hexose active transport in liver cells.

Hamster liver cells were separated on a Tri-R homogenizer in .067 M phosphate buffer, then washed in ion-free water to effect disruption of of erythrocytes and finally suspended in the same phosphate buffer in a 37°C bath. The cell suspension was agitated automatically to maintain an aerobic condition. One ml samples were drawn at 110, 115 and 120 minutes after sacrifice. At 120 minutes exogenous glucose (0.3 mg/ml) was added and samples drawn at five second intervals for one minute. The samples were added to ethyl alcohol (final concentration at 80%), as they were drawn, to halt cellular activity. External carbohydrate, internal hexose and internal polysaccharide were determined.

Removal of both ovaries from the golden hamster, *Mesocricetus auratus* (Waterhouse), resulted in liver cells demonstrating the establishment of a definite ($c_i/c_o=8$) transient active transport of hexose to the cell interior. Treatment of the isolated liver cell suspension with estrogens resulted in a decreased active transport of hexose (a state found in the intact, unoperated animal). The conclusions to be drawn from these observation will be presented. (Supported by NIH Career Development Special Fellowship #1 F3 HD-31, 595-01).

36

MICHAEL J. MAHER, ROSS J. HALE and BARRY SHERR, University of Kansas.

The effect of corticosterone on liver glycogen in the eastern ring-necked snake, *Diadophis punctatus*.

In all experiments discussed below, ring-necked snakes, *Diadophis punctatus*, were injected daily with 0.1 mg corticosterone; controls received saline. A preliminary experiment was performed on a small number of animals maintained at 28°C to determine the number of days of injection with corticosterone necessary to elicit a rise in liver glycogen. No change in liver glycogen, relative to control levels, occurred until the sixth day of treatment. Experiments were then conducted on groups of snakes maintained with controls at 28° or 18°C, and injected with the hormone for six, seven or eight days. Results for snakes maintained at 28° were as follows: Corticosterone-treated animals—4.68, 3.92 and 4.02 gms of glycogen per 100 gms of liver for six, seven and eight days of treatment, respectively; controls—2.80, 3.06 and 2.27. Results from 18° experiment: Corticosterone-treated animals—2.92, 3.50 and 3.24; controls—2.55, 2.35 and 3.48. All the means for groups of hormone-treated animals maintained at 28° are statistically different from all means of controls maintained at that temperature as determined by analysis of variance; whereas, none of the means for hormone-treated snakes maintained at 18° is different from control means. The lack of significant response from snakes maintained at 18° indicates that, although this species can respond to corticosterone with a rise in liver glycogen, temperature may play an important role in the response. (Supported by grant AM-06839 from the USPHS)

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DANIEL H. GIST, University of Missouri.

Effects of fasting, feeding and mammalian ACTH administration on liver glycogen levels in the South American caiman (*Caiman* sp.) (Introduced by Roger deRoos)

Immature caimans of mixed sexes were maintained in a greenhouse under a natural photoperiod. Liver and muscle glycogen levels were measured by the anthrone method. Caimans fasted for 20 days were separated into two groups: one group continued the fast for an additional 20 days, and the second group was fed *ad libitum* for 20 days. Body weight was recorded at the start and end of the experimental period.

Caimans which were fed showed an 11% increase in body weight. Liver glycogen levels were 4.4 ± 1.00 mg % and muscle glycogen levels were 0.8 ± 0.06 mg %. Fasted animals showed a 9.9% decrease in body weight at the end of the 20 day experimental period. Liver and muscle glycogen levels were 3.1 ± 0.47 mg % and 0.7 ± 0.04 mg % respectively; the decreases in glycogen were not significant.

ACTH (ACTHar, Armour) in saline was administered intraperitoneally as daily injections for 15 days to fasted caimans at the level 1.0 unit per 100 gm body weight; control animals received an equal amount of saline. Liver glycogen levels in the control animals were 4.4 ± 1.16 mg % and muscle glycogen levels were 1.3 ± 0.21 mg %. The administration of ACTH resulted in a significant increase in the amount of liver glycogen (12.0 ± 0.54 mg %), but did not alter the amount of muscle glycogen (1.1 ± 0.12 mg %).

ACTH administration resulted in a significant increase in adrenal weight from 12.1 ± 0.7 to 19.5 ± 1.5 mg per 100 gm body weight. (Supported by U.S.P.H.S. Predoctoral Fellowship F1-GM-29, 457 (D.H.G.) and U.S.P.H.S. grant AM-06259 (R.dR.)

38

S. J. GLUCKMAN, Brown University, L. SMITH, University of Rhode Island, F. HUNTER, Brown University.

Sexual dimorphism of glucose-6-phosphate dehydrogenase in the chicken.

The rat exhibits both a sexual dimorphism and a number of variations in glucose-6-phosphate dehydrogenase (G6PD) concentrations, which may be correlated with sexual development. The reaction catalyzed by G6PD is the primary source of reduced NADP which is essential in a number of steriod metabolic reactions.

In an attempt to see if such correlations exist in other types of animals with regard to sexual

dimorphism and attainment of sexual maturity, G6PD concentrations were measured in liver, kidney, gonad, cerebral cortical and brain stem tissues of the White Leghorn fowl (*G. domesticus*). Normal males, normal females, capons (castrated males), and poulards (females sexually reversed by oophorectomy) were sacrificed at intervals from 10 to 180 days of age.

In the fowl, as in the rat, significant G6PD concentration changes do occur with the advent of sexual maturity—but not in the same tissues. In the rat the liver exhibits higher and more dynamic G6PD concentration changes than the kidney; however, in the fowl this relationship is reversed and the kidney appears to be the more active of the two with regard to G6PD levels.

In the fowl sexual dimorphism is observed in two organs. In the normal male, kidney and gonadal G6PD concentrations both increase with the onset of puberty and then plateau. In the normal female, kidney and gonadal enzyme concentrations do not show marked changes at the time of puberty. G6PD tissue concentrations in both the capon and the poulard approximate those of the normal female rather than the normal male. (Supported by PHS FR-07085 and NSF GY 2631-URP.)

39

JOSEPH L. SCOTT, JOHN J. TRIMBLE and KENNETH I. LICKER, University of Connecticut.

The Metabolism of DL-Epinephrine-7-$C^{14}$ by the Western Painted Turtle, *Chrysemys picta belii*.

Three fresh-water turtles, with average carapace lengths of five inches, received a single dose of 0.062 mg of DL-epinephrine-7-$C^{14}$ (Spec. Act. = 38.6 mc/mmole) as a deep, subcutaneous injection administered at the base of the right forelimb. Each animal was kept in a covered glass dish, 8" in diameter, partially immersed in tap water in which the excreta containing the $C^{14}$-metabolites were collected for 24-hour periods. The radioactivity of the tap water was determined to indicate the amounts of metabolites of epinephrine discharged. This excretion was a slow process in the turtle for only 65% of the activity injected was recovered in the 45 days that followed.

Collections of excreta with large quantities of activity were selected for a qualitative study of catabolites present. Aliquots of excreta were first subjected to acid (HCl) hydrolysis for 30 minutes at pH 1. The metabolites were separated from the excreta, at both pH 1 and pH 10, by extraction with ethyl acetate using a modified procedure of Armstrong *et al.* (*J. Biol. Chem. 218*:293). By these means fifty-five percent of the starting activity of the aliquot, at pH 1, was removed and only 15% of the activity of the aliquot at pH 10. All extracts were examined further by two phase, ascending, paper chromatography augmented by autoradiographic studies. Five prominent, separate and reproducible spots were obtained from the pH 1 extracts while only two from pH 10 extracts. The identification studies are being continued. (Supported by Research Grant H-3829 from U.S.P.H.S.)

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J. R. CORTELYOU, D. J. McWHINNIE and N. SORGENTE, De Paul University.

Some responses of iguanid lizards to mammalian parathyroid extract.

Parathyroid regulation of mineral metabolism in mammals has been extensively studied. Less extensive investigations have been made in this area on birds and amphibians, and relatively little has been done with reptiles. Reports available have dealt largely with location of their parathyroids, their histology, or effects of parathyroidectomy Umanski and Kudokotzev (1951, Kokl. Akad. Nauk SSSR, 77:533), however, found that the lizard, *Lacerta agilis*, treated with parathyroid extract (PTE) had an increased number of osteoclasts in bone, and Clark (1964, Gen. Comp. Endocrinol., 5:297) reported that the turtle, *Chrysemys picta*, was hyperphosphaturic, and showed small elevations in serum and urine calcium after PTE injection.

In light of the few studies on reptiles, this investigation was initiated to determine if PTE influences mineral distribution in lizards. The desert iguana, *Dipsosaurus dorsalis*, after 7 days of PTE treatment (15 units/day), became significantly hypercalcemic. In contrast, there was no change in plasma phosphorus. The calcium and phosphorus levels of solid urine pellets produced by these lizards were increased 8.4- and 19-fold, respectively, following PTE injection. The hypercalciuria and hyperphosphaturia were significant ($p = 0.01 - 0.0005$) whether based on mineral content/100 mg urine, or on total weekly excretion. A slight, but non-significant increase in bone citric acid occurred after PTE-treatment.

Similar changes in mineral excretion were noted in PTE-injected *Sceloporus grammicus*. Animals were kept as controls until a urine pellet was produced, and subsequently, each was treated with 10 units of PTE daily for 7 days. Again, a significant hypercalciuria and hyperphosphaturia occurred, whether mineral levels were expressed per 100 mg urine or per total weekly clearance. These data indicate that, as in mammals, PTE shifts the mineral distribution of iguanid lizards, with a consequent elevation of urinary calcium and phosphorus. Unlike mammals and amphibians, however, no hypophosphatemia occurs; lack of a plasma phosphorus decrease with PTE treatment has also been reported for turtles (Clark, N., 1964, Gen. Comp. Endocrinol., 5:297) and birds (Urist, M., *et al.*, 1960, Am. J. Physiol., 199:851). (Supported by Grants G-13403 and GB-1485 from the NSF).

41

WILLIAM J. DAVIS and RONALD R. NOVALES, Northwestern University.

Adenosine 3',5'-cyclic monophosphate and MSH darkening of the frog skin.

In view of prior, conflicting reports, the effect of cyclic 3',5'-AMP on frog skin melanophores has been examined. The ability of 3',5'-AMP to produce pigment dispersion was confirmed. Futher investigations have been directed to this problem in an attempt to shed additional light on the phenomenon and its possible relationship to hormonal control of melanophores.

*In vitro* darkening of skin from the frog *Rana pipiens* was measured by standard reflectance techniques and confirmed by microscopic observation. A dose-dependent response was obtained

with concentrations of 3',5'-AMP ranging from 1.0 to 10 mM. The action was specific to 3',5'-AMP, since 10 mM 5'-AMP and 3'-AMP were without effect. 3',5'-AMP was fully active in sodium-free Ringer, in contrast with the established sodium requirement for MSH action on the frog skin.

The relationship between 3',5'-AMP action and hormonal control of melanophores was investigated. The action of 3',5'-AMP and MSH in combination was examined. In addition, combinations of either 3',5'-AMP or MSH and other agents, known to influence cellular levels of 3',5'-AMP, were tested. The metabolic requirements for the action of MSH and 3',5'-AMP were compared. The results, to be presented, form a body of circumstantial evidence which supports a model for the action of MSH on *Rana* melanophordes through the intermediacy of cyclic 3',5'-AMP. (Supported by Grant GB-4956X from the N.S.F.)

42

ALBERT DERBY, The City University of New York and Albert Einstein College of Medicine.

An *in vitro* quantitative analysis of the response of tadpole tissue to thyroxine. (Introduced by William Etkin)

Dorsal fins of tadpoles in various stages of metamorphosis were cut into discs and cultured in Hanks balanced salt solution with penicillin-streptomycin. Discs generally heal-over completely within two days. Discs were then placed singly in petri dishes (60×15) containing 10 ml of medium. Experimental dishes contained concentrations of thyroxine. (3, 9, 27, 81, 250 or 750 parts per billion) or KI, DL-thyronine or DL-diiodothyronine in concentrations of equivalent molality to 27 and 250 ppb of thyroxine. The shrinkage of discs was determined quantitatively by measurement of area.

In salt solutions disc from early prometamorphic donors first show shrinkage after two weeks in culture whereas discs from late prometamorphic and climax animals begin shrinking from day one. The rate of such shrinkage increases markedly between E+0 and E+1.

Thyroxine accelerates shrinkage and this response varied proportionately to the concentration used; 27 ppb was the lowest dosage which gave the maximum response. No acceleration of response over controls was obtained from KI or DL-thyronine and only a minimal response was found to high concentrations of DL-DIT. Late prometamorphic donors show an increase in response to thyroxine over early prometamorphic ones.

It is concluded that the response of tail fins to thyroxine is stoichiometric, not threshold dependent. The increase in sensitivity of tail fins to thyroxine during metamorphosis is interpreted as the result of a low but increasing level of thyroxine during prometamorphosis. This level appears to jump in the donor tissues at the beginning of climax. (Supported by NSF grant GB-5913, and the National Institute of Child Health and Human Development, NIH Training Grant ITI-HD-116-02.)

43

WILLIAM ETKIN, ALBERT DERBY and AMOS G. GONA, Albert Einstein College of Medicine.

Inhibition of metamorphosis by pituitary grafts in tadpoles.

Previous experiments had shown that prolactin (ovine, NIH) at high dosage inhibits metamorphosis acting, at least in part, as a goitrogen (J. E.Z., Endocrinology, 1967). Since pituitary grafts in tadpoles, as in mammals, appear to secrete prolactin at a high rate, experiments were run to determine if they would retard metamorphosis. Twenty bullfrog (*R. catesbeiana*) tadpoles in late prometamorphosis were each implanted with two pituitaries taken from early prometamorphic donors. Twenty-eight comparable animals, ten implanted with other tissues served as controls. The resorption of the tail and other metamorphic events were found to be retarded beyond the range of variation of controls in most experimentals. However, all eventually completed metamorphosis. Histological studies indicate that many grafts were still viable at the completion of the experiment. The results are interpreted as indicating that the action of prolactin previously reported is not merely pharmacological but that the animal's own gland can exert metamorphosis inhibiting effects. Hypothalamic control of tadpole growth and metamorphosis operates by reciprocal regulation of the antagonistic effects of prolactin and TSH. (Supported by NSF Grant No. 5913).

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JOHN J. JUST, University of Iowa.

The plasma protein-bound iodine (PBI) concentration of *Rana pipiens* larvae. (Introduced by J. J. Kollros).

It is widely held that an increased thyroid hormone supply becomes available to anuran tissues at metamorphic climax. As a test of this view, an ultramicro PBI method (Malkin, *J. Clin. Endocrin.*, *25*:28 (1965)) was modified for PBI determination in plasma of individual *R. pipiens* tadpoles.

The PBI means, in μg iodine/100ml. of normal plasma, increase slightly or remain constant between stages XIII and XVII, as indicated by the following representative values: 2.66 μg at stage XIII, 3.85 μg at stage XV, 3.47 μg at stage XVII. Beginning with stage XVIII when 4.99 μg are noted, a sharp rise is observed with a peak of 10.52 μg at stage XIX. Following this peak the values decline until at stage XXII the PBI measures 4.42 μg. Levels immediately after metamorphosis have fallen to 3.05 μg.

The plasma protein concentration of normal *R. pipiens* larvae increases steadily from stage XIII to XXIII. Mean values in gm. protein/100 ml. plasma are: stage XIV—0.85 gm., stage XVII—0.99 gm., stage XX—1.38 gm. The protein concentration peaks at 2.13 gm. in stage XXIII, then declines to 1.53 gm. in froglets.

The present investigation supports the conclusion that the increased thyroid gland activity demonstrated at metamorphic climax results in elevated hormonal levels in the blood, and therefore may cause an increase in amount of hormone available to tissues. (Supported by U.S.P.H.S. Training Grant HD-00152 and U.S.P.H.S. Grant AM02202)

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JACK HARCLERODE, NED A. UNDERWOOD, Bucknell University, and HENRY

VALLOWE, Ohio University.

Effect of temperature on the thyroid physiology of the watersnake, *Natrix rhombifera*.

A total of 26 watersnakes, *Natrix rhombifera*, were exposed to either 50°F, 70°F, or 90°F for seven days. At the end of this period they were injected (i.p.) with 5 microcuries of $I^{131}$ and re-exposed to the same temperatures for another seven-day period. At the end of this period various tests were made to determine the functional state of the thyroid gland.

Generally, exposure to warmer temperatures (90°F) caused an increase in both absolute and relative thyroid weight, absolute $I^{131}$ uptake, percent $I^{131}$ uptake, DIT/MIT ratio, and amount of $I^{131}$ accumulated per mg thyroid tissue. Exposure to warm temperatures caused a decrease in protein bound $I^{131}$ and conversion ratio.

46

MAX GOLDMAN, Long Island University.

The effect of a single neonatal dose of thyroxin on the iodide concentrating mechanism of the thyroid gland in adult female rats.

A decreased capacity to concentrate radioiodide by the thyroid iodide trapping mechanism in adult female Long-Evans rats, 9 months of age, resulted from the injection of a single dose of thyroxin (1 mg.) in neonate 5 day old rats. The thyroid: serum radioiodide concentration ratio (T/S) was 40.2 $\pm$ 1.5 for the thyroxin-injected group and 22.2 $\pm$ 2.2 for the control group.

Other indices of thyroid function such as 24 hour $I^{131}$ uptake and distribution of $I^{131}$ in the components of pronase hydrolyzed thyroidal iodoprotein were not significantly different in the thyroxin-injected and control groups; however, the mean serum $PBI^{131}$ was much lower in the thyroxin-injected-group.

Average body, thyroid, adrenal, ovarian and uterine weights of treated adult rats were similar to those of the control group. Vaginal smears in the two groups of rats indicated no alteration in estrous cycles.

Results suggest that neonatal administration of a single dose of thyroxin altered thyroid function in adult female rats as evidenced by diminished T/S values and serum $PBI^{131}$. The impairment in thyroid function, however, was not marked since body weights, organ weights and estrous cycles were not affected. (Supported by a grant from The Committee on Research of L.I.U.)

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BRAHIM LAHLOU and WILBUR H. SAWYER, Columbia University, College of Physicians and Surgeons.

Sodium and chloride balance in hypophysectomized goldfish, *Carassius auratus* L.

Goldfish survive several weeks after hypophysectomy if placed in 0.4% NaCl and 0.1% $CaCl_2$ for the first three postoperative days and then adapted gradually to fresh water. Three weeks after operation hypophysectomized fish have very low plasma levels of Na (86.7 $\pm$ 4.3 mEq/l) and Cl (45.3 $\pm$ 3.5). Intact and sham-operated control fish have plasma Na levels of 130.5 $\pm$ 2.0 and 134.6 $\pm$ 4.0, respectively, and Cl levels of 100.3 $\pm$ 1.9 and 98.7 $\pm$ 7.2.

Extrarenal fluxes were measured three weeks after operation using $^{22}Na$ in fish in which urine was collected through bladder catheters. Net extrarenal Na flux is strongly negative in hypophysectomized fish (mean is $-28$ $\mu$Eq/100g per hour in contrast to $-10$ in intact and $-16$ in sham-operated control fish). The negative net flux results largely from increased outflux since influx is nearly normal (about 13 $\mu$Eq/100 g per hour in hypophysectomized fish, 16 in intact, and 17 in sham-operated controls.

Urinary Na concentration is slightly increased in hypophysectomized fish but urine flow rate is reduced. Total urine Na excretion is not significantly different from that of control fish. The Na deficit in hypophysectomized fish thus seems related to abnormal extrarenal exchange, probably across the gills. In this respect goldfish resemble the euryhaline killifish *Fundulus heteroclitus* (Maetz *et al.* 1967). (Supported by grant AM-01940 from the U.S.P.H.S.)

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DANIEL E. OVERACK and ROGER deROOS, University of Missouri

Comparative effects of neurohypophysial hormones on body weight in the newt, *Notophthalmus viridescens*.

Body weight changes of the newt, *Notophthalmus (Diemictylus) viridescens*, were measured in response to the administration of the following neurophyophysial hormones in an amphibian Ringer solution: synthetic arginine vasotocin, arginine vasopressin, oxytocin and acetic acid extracts of chicken posterior lobe powder (PPE). Body weights were determined immediately prior to the injection, immediately following the intraperitoneal injection, and then at intervals for the subsequent 24 hours.

Uninjected and Ringer-injected control animals showed a small continuous decline in body weight throughout the experimental period. The administration of 5 mU of vasotocin resulted in an 11% increase in body weight; 10 mU resulted in an increase of 23%. The maximum increase in weight occurred within 3 to 4 hours; thereafter the weight declined to that of the preinjection weight, or slightly lower, within 24 hours. The administration of 20 mU of vasopressin resulted in a 9% increase in body weight. Oxytocin failed to cause an increase in body weight even at a dose of 40 mU. PPE at doses of 2.5, 5.0 and 10.0 $\mu$g resulted in weight increases of 17, 65 and 91%, respectively, of the response attained with 10 mU of vasotocin.

In preliminary experiments, the response of the aquatic larvae to vasopressin and PPE was approximately 50% of the response of the aquatic adult at equivalent dose levels: the increase in body weight of the terrestrial eft maintained on damp sphagnum was approximately equal to that of the aquatic adult. (Supported by Grant AM-06259 from the USPHS)

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V. G. KRISHNAMURTHY, University of California, Berkeley.

Cytophysiology of Stannius corpuscles in some tele-

ost fishes. (Introduced by Jean Nandi)

The function of Stannius corpuscles remains uncertain. The several possible functions (cf. Fontaine, 1967; Nandi, 1967; Chester Jones *et al.*, 1966), the variable origin of the corpuscles (DeSmet, 1962; Krishnamurthy, 1967, and the presence of at least two kinds of cells (cf. Nadkarni and Gorbman, 1966; Oguri, 1966) suggest a multiple role for these organs. Various histological and cytochemical methods are being used to analyze the types of cells present in several teleost species, at different ages and subjected to various manipulations.

Sections of Stannius corpuscles of a variety of freshwater and marine teleosts have been stained with two staining sequences. It is becoming obvious that no decision about the presence or absence of several cell types can be based upon a single staining method. For example, the azocarmine-orange G-aniline blue sequence showed the presence of some cells with aniline blue-positive granules and other cells devoid of granules in the Stannius corpuscles of *Gymnothorax flavimarginatus* and *Salmo gairdnerii*. In both these species, the aldehyde fuchsin-ponceau de xylidine-light green sequence also revealed two cell types. However, in *Conger bowersi, Gillichthys mirabilis, Hypsurus caryi* and *Tilapia mossambica* only granule-containing cells were observed with these staining sequences. It is hoped that studies of these kinds will lead to information relating the presence of specific cell types to specific functions. (Aided by NIH grant AM-07896)

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CLAUDE DESJARDINS, The Jackson Laboratory and BASIL E. ELEFTHERIOU, Kansas State University.

Diurnal variations in plasma and brain biogenic amines in the rabbit.

Blood plasma levels of serotonin, epinephrine, norepinephrine, glucose, and liver glycogen were measured in adult rabbits in order to determine whether or not these plasma constituents varied diurnally in this species. The norepinephrine content of the hypothalamus and amygdala also were determined in these same animals. The animals were maintained individually at 70 $\pm$ 4°F on 14 hrs. light/day (6 AM-8 PM) and fed 15 g/kg of body weight of a standard rabbit chow at 9 AM and 8 PM, respectively. The rabbits were killed by cervical dislocation and tissues were removed immediately from each of 5 animals at 12 M, 6 AM, 10 AM, 2 PM, and 7 PM. The mean plasma glucose levels were significantly higher ($P<0.05$) at 12 M (43.01 mg%) than at 2 PM (34.70 mg%) and liver glycogen values were inversely related to those for plasma glucose. Mean plasma norepinephrine values were significantly lower ($P<0.05$) at 2 PM (1.07 $\mu$g%) than at 12 M (1.57 $\mu$g%). Average titers of plasma serotonin were significantly higher ($P<0.05$) at 12 M than at 2 PM (792.82 and 501.03 $\mu$g/ml, respectively). The hypothalamic levels of norepinephrine also were significantly higher ($P<0.01$) at 12 M (13.47 $\mu$g/g) than at 2 PM (6.63 $\mu$g/g) while levels of norepinephrine in the amygdala followed parallel variations with mean values of 0.789 and 0.606 $\mu$g/g at 12 M and 2 PM, respectively. These data suggest that the rabbit is a species that exhibits maximum physiologic activity during nocturnal hours. (Supported by PHS grants FR-05545 and AM-11195)

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JOHN A. LAMPING and ROBERT C. THOMMES, De Paul University.

Endocrine control of electrolyte distribution in the chick embryo. II. Allantoic fluid sodium and potassium.

The effect of hypophysectomy upon allantoic fluid levels of sodium and potassium has been studied in the developing chick embryo.

Animals were hypophysectomized at 36 to 40 hours of incubation by the "partial decapitation" method of Fugo (1940). Allantoic fluid samples were collected at 24-hour intervals from day 10.5 through 17.5. Sodium and potassium levels in allantoic fluid of normal and hypophysectomized White Leghorn chick embryos were determined by means of a Beckman DU spectrophotometer with flame attachment.

The results of this investigation have demonstrated a statistically significant difference in allantoic fluid sodium concentrations between intact and hypophysectomized embryos from 12.5 to 16.5 days of incubation. Normal allantoic fluid sodium levels range from 96.7 mEq/l on day 12.5 to 53.4 mEq/l on day 16.5, whereas experimental levels range from 115.9 mEq/l on day 12.5 to 86.2 mEq/l on day 16.5. However, potassium levels in the allantoic fluid of control and operated animals were of the same order of magnitude.

Transplantation of pituitaries from day 10.5 donors to the CAM of hypophysectomized, day 9.5 host embryos results in a reversal of hypophysectomy-induced hypernaturia as observed on days 13.5, 14.5 and 15.5 of incubation. (Supported by grant HD 01475 from the U.S.P.H.S.)

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BERTA SCHARRER, Albert Einstein College of Medicine.

Ultrastructural specializations of neurosecretory terminals in the corpus cardiacum of cockroaches.

In the corpus cardiacum of insects, products of intrinsic and extrinsic neurosecretory neurons, in order to reach the hemolymph, must pass through an extracellular stroma consisting of the organ's sheath and interconnecting trabeculae. Therefore, in an analysis of the release mechanism of neurohormones, structural specializations at the axon-stroma junction, or at more distant sites of termination of neurosecretory neurons, are of particular interest. Neurosecretory terminals, and localized preterminal areas facing the stroma, frequently show features comparable to synaptic specializations: electron opaque thickenings of the plasmalemma, preterminal densities, and, subjacent to these, clusters of small, clear vesicles. Furthermore, such vesicles may be intermingled with neurosecretory granules in other parts of the axon. Electron lucent vesicles at non-synaptic sites may be interpreted as either "ghosts" of neurosecretory vesicles or containers of neurohumors such as acetylcholine. In the latter case, the neurohumor must be assumed to perform a special, i.e., non-transmitter function, one presumably related to the release of neurohormone. As in various known cases of cellular contiguity,

including the synapse, membrane thickenings at non-synaptic contact areas of neurosecretory neurons might serve as adhesion devices. Or, perhaps, part of the terminal densities consist of neurosecretory material that has been released from vesicles but has not yet left the neuron. Occurrence of "synaptoid" specializations at neuronal junctions not engaged in regular synaptic transmission exemplifies the fact that different, though somewhat comparable, functional requirements may express themselves in similar structural details. (Supported by grants RO1-AM-3984 and NB-00840 from the U.S.P.H.S.)

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STANLEY B. KATER, University of Virginia.

Release of a cardioaccelerator substance by stimulation of nerves to the corpora cardiaca in *Periplaneta americana*. (Introduced by Dietrich Bodenstein)

Electrical stimulation of one of the ten nerves associated with the corpus allatum-cardiacum complex in *Periplaneta americana* evokes the release of a factor (s) with cardioaccelerating and neural activating activity. Threshold levels of *in vitro* stimulation indicate that nervus corporis cardiaci I is the specific efferent tract for these functions. Removal of the corpora allata has little effect on the system and therefore it appears that release of this factor (s) is from the corpora cardiaca.

Using a mechanical transducer on the exposed heart of an isolated abdomen, measurements were obtained which were extremely reproducible and easily quantified. Dosage response curves are linear over a range which is often as broad as 0 to 300% of the normal rate. The effect of the cardioaccelerator is expressed within 1 second after the application. Trypan blue and carmine applied to the heart for up to 4 hours prior to testing have no effect on the response to the cardioaccelerator. This makes it highly unlikely that the cardioaccelerator is acting via the pericardial cells as previously supposed.

The half life of the cardioaccelerator is only ten hours. The substance is heat stable, but sensitive to pronase and chymotrypsin. On gel filtration with Bio-Gel P-6 the cardioaccelerator is in the included fraction with a mobility suggesting a molecular weight of approximately 1300. Heating, followed by gel filtration and isolation of the active fractions fails to retard the loss of biological activity. It is unlikely that the decreased activity is associated with enzymatic degradation. (Supported by Grant GB-4847 from NSF and an NDEA Title IV Fellowship)

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NED A. SMITH and CHARLES L. RALPH, University of Pittsburgh.

A comparison of properties of some heart-accelerating substances from tissues of the American cockroach.

Studies were carried out to determine and compare some of the physical and chemical properties of heart-accelerating substances in tissues associated with the heart of the cockroach, *Periplaneta americana* (L.). The semi-isolated heart assay was used to measure the concentration of accelerator materials extracted from three tissue sources: the corpora cardiaca, the ventral nerve cord, and the heat complex. The latter includes the heart muscle itself, the pericardial cells, the nervi aortici, connective tissue, and trachea. The techniques employed to study the properties of accelerator substances included sucrose and saline extraction, heat treatments, dialysis, centrifugation, sucrose gradient ultracentrifugation, and enzyme treatments. Results indicate that the principal kinds of accelerator substances from these three tissue sources are different from each other. The heart-accelerating activity of extracts of the corpora cardiaca is associated with a heat-stable, proteinaceous material which dialyzes, and has a sedimentation coefficient, $s_{20,w} - 1.51$, indicative of a material with a molecular weight of about 11,000. In extracts of the ventral nerve cord, the heart-accelerating activity is associated with very light materials which are only partially proteinaceous. These substances are heat-stable, and will dialyze. Activity in extracts of the heart complex is about 60% heat-stable. It is associated with a broad spectrum of materials with an average sedimentation coefficient, $s_{20,w} = 10.32$. This material does not dialyze, and its activity decreases by about 50% after treatment with proteolytic enzymes. (Supported by USPHS fellowship 1-F1-GM 33, 738-01)

55

AVERETT S. TOMBES, Clemson University and DIETRICH BODENSTEIN, University of Virginia.

The neuroendocrine system of the adult alfalfa weevil, *Hypera postica*.

The histology of the neuroendocrine system (neurosecretory cells, corpora cardiaca and corpora allata) has been studied using principally Ewen's alhehyde fuchsin technique in laboratory reared colonies of diapausing and reproducing *Hypera postica* females. Examinations were made of both populations at 0, 14 and 28 days following adult emergence.

A and B cells were present in the protocerebrum with approximately 58 staining in the reproducing and 28 in the diapausing female. The degree of staining varied considerably with the greatest affinity appearing in the reproducing adult.

The volume change in both glands was very significant during the first 14 days with approximately a twofold increase in the cardiaca of both populations and the allata of the diapausing colony. A threefold increase was noted in the allata of the reproducing female.

Preliminary experimental procedures attempting to modify the physiology of the female destined to diapause have been performed through environmental modifications, gland implants and synthetic hormone applications. (Supported by NIH Postdoctoral Fellowship 7-F3-GM-28, 152-01A1 and NSF grant GB-5445)

56

KENNETH N. KATO and FRED I. KAMEMOTO, University of Hawaii.

Implications of neuroendocrine regulation of the osmotic concentration in the grapsid crab, *Metopograpsus messor*.

The grapsid crab, *Metopograpsus messor* (Forskal) is an excellent osmoregulator. Within a range

of 25% to 125% sea water, the blood osmotic concentration varies only 14% from one extreme to the other. In 25% sea water the blood osmotic concentration decreases by 6% of the normal value; it increases by 8% in 125% sea water.

Bilateral eyestalk ablations or ligations result in a slight blood osmotic concentration increase in normal sea water after 24 hours. Subsequent exposure to 25% sea water for 6 hours results in a decrease in the blood osmotic concentration of approximately 9% as contrasted to a 4% decrease in normal animals. After 12 hrs., the ablated or ligated animals realize an 11% decrease as contrasted to a 5% decrease in normal animals. There is little change in the blood osmotic concentration of both ablated and normal animals between 12 hrs. and 24 hrs. of exposure to 25% sea water. Injection of eyestalk extracts results in partial replacement of the blood osmotic concentration to the normal value.

Bilateral eyestalk ablations or ligations appear to render the animal more permeable to water influx. This is also reflected in the weight changes of normal and ablated animals in a dilute medium. The ablated animals realize a greater weight increase over their normal controls after the nephropores have been plugged. (Supported by grant GB-673 from the N.S.F.)

57

DENIS G. BASKIN and DAVID W. GOLDING, University of California, Berkeley.

The infracerebral gland: a possible neuroendocrine complex in *Nereis*. (Introduced by Ralph I. Smith)

The infracerebral gland of *Nereis* is an epithelial structure underlying the brain. Two cell types have been recognized with the light and electron microscopes. The more common cell is a modified pericapsular epithelial element in direct contact with the fibrous brain capsule and containing abundant mitochondria and golgi centers. Less numerous are cells with considerable amounts of endoplasmic reticulum and large numbers of granules resembling those encountered in many neurosecretory neurons. Swollen cell processes of unknown origin, packed with mitochondria, terminate at the base of the brain. Several nerve tracts run from the neuropil to the base of the brain, and some of these axons also form enlarged terminals on the fibrous capsule separating the brain from the gland. These latter endings contain elementary granules. Occasional axons appear to pass into the gland through perforations in the capsule. These axons appear to divide and ramify within the gland forming a possible "neurosecretory neuropil". The size of the gland varies among different species. It is considerably larger in *Nereis limnicola* than in any other species yet examined. The structure of the infracerebral gland and its relationship to the brain suggest that it may be an endocrine organ which, together with neurosecretory neurons from the brain, may constitute a neuroendocrine complex. The functional significance of this complex and its possible relationship to the hormone production presently ascribed to the brain are being investigated. (Supported by a PHS Predoctoral Fellowship and NSF grant GB-6424X)

58

FRED I. KAMEMOTO and JOYCE K. ONO, University of Hawaii.

The effects of eyestalk ligation on salt and water balance in the crayfish, *Procambarus clarkii*.

It has been suggested that a factor, presumably secreted in the brain and released in the eyestalk, maintains the normal permeability of the body surfaces to water in the freshwater crayfish, *Procambarus clarkii* (Kamemoto, Kato and Tucker, Amer. Zool. 6:213, 1966). It was demonstrated that there is a greater increase in the weight of animals with bilaterally ligated eyestalks and plugged nephropores as compared to animals with normal eyestalks and plugged nephropores. The injection of brain homogenates into these eyestalk-ligated animals prevents to some extent the great increase in their weights. The injection of brain homogenates or eyestalk extracts also prevents the decrease in the chloride concentrations of the blood normally resulting from the removal of the eyestalks. Brain homogenates seem to be more effective than the eyestalk extracts.

These studies have suggested that the removal of the eyestalks in crayfish resulted in the increased influx of water accompanied by the increased production and release of urine by the antennal glands. Polyethylene catheters were attached over the nephropores for the continuous collection of crayfish urine. Results indicate that crayfish with ligated eyestalks release nearly twice the volume of urine released by normal crayfish over a 24 hour period. (Supported by grant GB-673 from the NSF)

59

JERREL L. WILKENS, University of California, Los Angeles.

The control of egg maturation in *Sarcophaga bullata* (Diptera). (Introduced by Franz Engelmann)

In *Sarcophaga* vitellogenesis appears to be controlled by two endocrine organs, the corpus allatum and the neurosecretory cells of the pars intercerebralis. Yolk was not deposited after the neurosecretory cells were removed ($NSC^-$). Reimplantation of neurosecretory cells into $NSC^-$ females stimulated vitellogenesis, whereas implanting corpora allata did not. Adult females allatectomized ($CA^-$) at pupation matured eggs fully if fed liver, but only partially when fed defined medium. Seventy percent of females $CA^-$ at emergence deposited yolk whether fed liver or defined medium.

Blood protein (B.P.) concentrations were low in newly emerged and sugar-fed females. Egg maturing females fed liver had an increased blood protein level. Allatectomized females which did not deposit yolk had low B.P. levels, whereas those maturing eggs (on the same diet) possessed moderate to high levels. $NSC^-$ females had high B.P. concentrations, but deposited no yolk.

Immuno-diffusion electrophoresis of hemolymph indicated that egg maturing females possessed a protein fraction not found in sugar-fed females or meat-fed males. This "female" protein was extractable from oocytes containing yolk, but not from oocytes without yolk. The "female" protein was also present in $NSC^-$ females and in those

CA⁻ females which deposited yolk.

It is concluded that egg maturation is a two-step process. The synthesis of yolk proteins appears to be controlled by a corpus allatum hormone, or by an analogue supplied in the diet or produced elsewhere; a hormone produced by the median neurosecretory cells of the brain governs the incorporation of these poteins into the oocytes. (Supported by a Ford Foundation fellowship and grant 4-F1-GM-31,026-02 from NIH.)

60

TERRANCE S. ADAMS, Metabolism and Radiation Research Laboratory, USDA, ARS, ERD, Fargo, North Dakota.

The relationship of ovaries and the corpus allatum to mating in the house fly, *Musca domestica*.

A correlation between ovarian development and mating exists in the house fly. No females from the population with stage 2 ovaries mated; stage 3 females rarely mated; most mating occurred in stage 4 through 10 females. In this study, males and females were of the same age and held together continuously. When one 3-day-old male was confined with five females of different ages and replicated 200 times, no females with stage 2 through 5 ovaries mated. All mating was with stage 6 through 10 females. When given a choice, males would mate only with stage 6-10 females, but when no choice was involved, males mated with stage 4 through 10 females. Allatectomy of females at an age of 12 hours inhibited ovarian development at the initial stages of vitellogenesis (stage 4), and only 4% of these females mated. Check operated females had mature ovaries (stage 10) and demonstrated 84% mating. Ovariectomized females mated normally, whereas ovariectomized + allatectomized and allatectomized females demonstrated reduced mating. Thus the relationship between ovarian development and mating is due to a response of both these parameters to the titre of juvenile hormone in the insect.

61

PAUL C. SCHROEDER, University of California, Berkeley.

Eleocyte nucleolus formation in relation to development of female nereid polychaetes.

The eleocytes, the most characteristic cell type in the coelomic fluid of nereid polychaetes, may be recognized by their content of fusiform muscle fragments (sarcolytes) and lipid droplets, and in females by their adherence to developing oocytes. Female heteronereid eleocyts contain a prominent nucleolus and granular endoplasmic reticulum not present in equivalent cells of immature specimens (A. Dhainaut, C. R. Acad. Sci. 262.2740, 1966).

Paraffin sections from the metamorphosing region of a series of 33 female *Nereis grubei* (oocyte diameters: 35-200 $\mu$) indicate that the prominent nucleolus appears in the eleocytes when the oocytes are about 140 $\mu$ in diameter. This is during stage II of the development of the heteronereid setae (P. Schroeder, *Biol. Bull. 133*, 1967), a part of the somatic metamorphosis which begins when the oocytes are 90-100 $\mu$ in diameter. The nucleolus was found to occur sporadically in females with oocytes greater than 175 $\mu$ in diameter.

The appearance of the nucleolus in these cells may be accelerated by decapitation (removal of brain hormone) as can both somatic metamorphosis and gametogenesis. Sections from 18 females (oocyte diameter: 35-165 $\mu$) sacrificed 18 or more days after decapitation indicated nucleoli in the oocytes of animals with oocystes up to 97 $\mu$ (9 animals; in the older animals the nucleolus had presumably appeared and later disappeared. This conjecture is supported by the occurrence of eleocyte nucleoli in animals with oocytes over 100 $\mu$ in diameter sacrificed at intermediate times after decapitation. The hormonal inhibition of eleocyte nucleolus formation is being investigated *in vitro*. (Supported by USPHS Fellowship GM 17,007, NSF Fellowship 46015, and NSF grant 6424X.)

62

GEORGE A. CLAY and F. A. BELAMARICH, Boston University.

Histamine content of hearts and pericardial organs of the crabs *Cancer borealis* and *Carcinus maenas*.

The histamine content of the hearts and pericardial organs of *Cancer borealis* and *Carcinus maenas* was found to be significantly lower than had been reported for *Carcinus maenas*. Previous investigators had used chromatographic and colorimetric techniques that utilized a non-specific diazo color reaction for the identification and quantitation of histamine. These methods were found to be useful only if histamine was purified prior to coupling with diazotized sulfanilic acid.

Extracts of hearts and pericardial organs were examined by the following specific methods: (1) an orthophthalaldehyde derivative of extracted tissue histamine was assayed by spectrofluorophotometry, (2) histamine was labelled with a $C^{14}$ methyl group transferred by the enzyme methylhistamine transferase from S-adenosylmethioninemethyl $C^{14}$. Trace amounts of $H^3$-histamine were added and the ratio of $C^{14}$ to $H^3$ of extracted 1,4-methylhistamine was measured on a scintillation counter, (3) bioassay of histamine extracts on the guinea pig ileum. Histamine was present in all tissues examined but in amounts less than 1.5 $\mu$g histamine/g tissue. These results are similar to the tissue histamine levels reported for many vertebrate and invertebrate tissues.

Bioassay of histamine and certain derivatives on the isolated lobster heart indicates that none of these compounds has a direct role in cardioregulation. (Supported by grant HE 08172 from the U.S.P.H.S.)

63

JAMES HANEGAN, L. ANOLI, and C. W. MAJOR, University of Maine.

Organic binding of iodide in Nemerteans *in vitro*.

A nemertean, *Lineus ruber*, concentrates iodide$^{131}$ from a sea water solution and the iodide appears in organically bound form. The organification is confined to the mucous coating which is able, *in vitro*, to produce the iodide binding. The compounds produced have been resolved by butanol-acetic acid-water (78-10-12) and butanol-ethanol-2H $NH_4OH$ (100-20-40) ascending paper chromatography and have mobilities similar to iodotyrosines and iodothyronines. Another nemertean, *Amphi-*

*porus angulatus,* shows negative results in these tests despite taxonomic similarity and has only 1% as much tyrosine/ml of mucous (Folin-Ciocalteau) as *Lineus ruber.* (Supported by the Coe Fund.)

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NARAYAN G. PATEL and KORNATH MADHAVAN, Western Reserve University.

Synthetic capacities of imaginal wing discs of the Ricini silkworm.

The imaginal wing discs of Lepidoptera are relatively homogeneous tissues, can be isolated with ease and are suitable for biochemical studies of development. Wing discs at specific times during larval and larval-pupal development in *Samia cynthia ricini* were incubated *in vitro* in insect Ringer with $C^{14}$ amino acids and $H^3$ uridine and the incorporation of the labelled precursors was measured by conventional methods. Amino acid incorporation into protein was highest during ecdysis, decreased immediately thereafter to a low level and started to increase again about two-thirds of the way through the instar. RNA synthesis followed a different pattern and began to increase about midway in the instar and decreased to a low level at ecdysis.

How are the normal patterns of synthesis affected by injection of ecdysone or juvenile hormone? To answer this question, at specific times during the six-day-long fourth (penultimate) instar, larvae were injected with ecdysone or dodecyl methyl ether (DME), an analogue of juvenile hormone. The synthetic capacities of wing discs were determined following the treatments. Injection of ecdysone into 3- and 5-day-old larvae increased RNA synthesis by 20- and 2-fold, respectively, whereas protein synthesis was stimulated only slightly. Injection of DME under similar conditions stimulated RNA synthesis 15-fold in younger larvae but much less in older ones. The effect of DME on protein synthesis was only slight.

The results indicate that in both normal and injected insects ecdysone stimulates both RNA and protein synthesis in fourth instar wing discs. The action of juvenile hormone and DME are less clear.

65

MARTIN P. SCHREIBMAN and KLAUS D. KALLMAN, Brooklyn College and Osborn Laboratory of Marine Sciences, New York Zoological Society.

Sterility in hybrid fishes of *Xiphophorus.*

$F_1$ hybrids of *Xiphophorus maculatus* (strain Jp 163A or Jp 163B) × *X. couchianus,* their $F_2$, and backcross generations are fertile. However, when the *maculatus* parent belongs to the strain Hp-2 ($X_4Y_{Sd}$ ♂ ♂) both + and Sd offspring are sterile. At the age of three months the anal fin of the Sd fish fully differentiates into a gonopodium, a secondary sex character under androgenic control. The Sd fish possess no spermatogenic tissue. Their vestigial gonads consist of large branching ducts filled with a PAS-positive secretion. These ducts are lined by large cuboidal cells that are faintly acidophilic and PAS-negative and contain a centrally-placed, spherical nucleus with few clumps of chromatin and a small nucleolus. Among other intertubular elements there are clusters of large ovoid cells with a coarse, acidophilic (PAS-negative) cytoplasm that surrounds a dark-staining, eccentrically-placed nucleus. To determine if this genetically controlled sterility is the result of a pituitary abnormality, the immature gonads from male *X. couchianus* (Xc-G) were implanted into the caudal musculature of nine-month old hybrids. Not one graft developed and histologically they resembled the host's undeveloped gonad. In 14- to 15-month old sterile hybrids the meso-adenohypophysis is markedly enlarged and has local areas of hypertrophy. The abnormal shape of these glands can be readily recognized on gross examination.

The wild type (+) hybrids assume a deep-bodied shape, a characteristic of castrated platyfish, and their anal fin remains unmodified. Lack of estrogenic hormone is indicated by the persistence of the first three hemal spines which disappear in maturing females under the influence of these hormones.

The presence of secondary sex characters under androgenic control in Sd fish but the lack of spermatogenic activity in host and grafted testes may provide a system for studying the pituitary control of gonadal function in fish. (Supported by grant CA-06665 from the U.S.P.H.S. and by grant GB-6614X from the N.S.F.)

66

ROBERT C. THOMMES and GEORGE MATTHEW, De Paul University.

Endocrine control of yolk sac membrane glycogen levels in the developing chick embryo: IV. Insulin effects.

The study of the effects of exogenous insulin on glycogen concentrations in the yolk sac membrane of the developing chick embryo has been extended to include earlier stages of development.

Four series of embryos were prepared: series I received 0.02 unit of protamine zinc insulin placed on the blastoderm at day 3 of incubation; series II and III were comprised of vehicle-treated and normal embryos respectively while series IV was hypophysectomized by the "partial decapitation method" of Fugo (1940) and insulin-treated.

From day 4 through day 10 a marked increase in yolk sac membrane glycogen was observed in insulin-treated embryos. Recovery occurred on about day 12. Following recovery there was a compensatory undershoot on days 14 and 15, glycogen levels again returning to normal on day 16. In hypophysectomized-insulin-treated embryos, glycogen concentrations were determined only on days 14, 15, and 16. No statistically significant difference between these and intact-insulin-treated embryos was observed on days 14 and 15. However on day 16, hypophysectomized-insulin-treated embryos did not show the recovery seen in intact-insulin-treated embryos: in fact yolk sac membrane glycogen concentrations actually declined.

The present investigation indicates that the yolk sac membrane is capable of responding to insulin during a period which corresponds in time to the initial appearance of beta granulations in the pancreatic anlage. Reestablishment of normal glycogen levels is either directly or indirectly pituitary-dependent. (Supported by Grant HD 01475 from USPHS.)

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JAMES E. WOODS and RICHARD WEEKS, De Paul University.

Ontogenesis of histochemically-demonstrable $\Delta^5$-3$\beta$-HSD activity in the gonads of the chick embryo. I. Pituitary influence.

The time during incubation of the white Leghorn chick embryo when sex hormones are first produced by the gonads is not known. Also, the period when the pituitary-gonadal axis in this embryo is initially functional has not been established.

Frozen sections of gonads from chick embryos of 3.5 to 20.5 days of incubation were examined histochemically for the presence of $\Delta^5$-3$\beta$-hydroxysteroid dehydrogenase ($\Delta^5$-3$\beta$-HSD), a key enzyme in steroid hormone biosynthesis. Sexually undifferentiated, as well as differentiated gonads (testes and ovaries), of intact, hypophysectomized, and hypophysectomized embryos with pituitary transplants to the chorioallantoic membrane were examined for the presence of this enzyme.

It was observed that $\Delta^5$-3$\beta$-HSD is present in the genital ridge of the 3.5-day-old chick embryo, and that in the gonads enzyme activity, as indicated by the amount of formazan deposition, increases with developmental time.

Gonads of embryos hypophysectomized by the partial decapitation method of Fugo (1940) at 33- to 38-hours of incubation exhibit a diminution in $\Delta^5$-3$\beta$-HSD activity, when compared to gonads of intact embryos, which begins at day 13.5 and continues to day 20.5 of incubation. Testes of such pituoprivic embryos exhibit a remarkable decrease in enzyme activity throughout this time period. Ovaries of hypophysectomized embryos also show a similar reduction in $\Delta^5$-3$\beta$-HSD activity; however, this decrease is markedly less than that demonstrated by the testes of operated embryos and is restricted to the cortex.

When pituitaries of 9.5-day-old chick embryos are transplanted to the chorioallontoic membrane of hypophysectomized embryos of the same age, $\Delta^5$-3$\beta$-HSD activity in the gonads of the latter is restored to levels characteristic of gonads of intact individuals.

These findings indicate that synthesis of $\Delta^5$-3$\beta$-HSD in the chick embryo occurs prior to sex differentiation of the gonads (ca. 3rd day of incubation) and that enzyme activity in the gonads increases during the embryonic period. The rate of $\Delta^5$-3$\beta$-HSD synthesis in the testis does not appear to be pituitary-dependent until the middle of the incubation period. The latter observation supports the hypothesis that in the chick embryo the pituitary-gonadal axis first becomes functional after 13 days of incubation.

69

BARBARA R. BEITCH, University of Virginia.

The roles of adenosine triphosphate and divalent ions, ionic strength, and pH in the contraction of hydra cell models. (Introduced by S. P. Maroney, Jr.)

The chemical mechanism of contraction in gastrodermal and epidermal myo-epithelial cells of hydra was investigated, using cell models. These models were prepared by extraction for 8 minutes in buffered 0.1% saponin solution (pH 6.8) at low ionic strength (0.02 to 0.05 $\mu$), followed by thirty minutes of washing in 1.5 M urea (same pH, ionic strength).

Contraction is stimulated by ATP in the presence of either Ca or Mg. Evidence is presented for competitory activation of contraction by either $CaATP^{2-}$ or $MgATP^{2-}$, and for inhibition by free ATP ions and possibly by free Mg ions. The pH optima for contraction are 6.8 and 7.6 for the epidermal and gastrodermal myo-epithelial cells, respectively. The optimum ionic strength for preparation and activation of models is 0.02 $\mu$ for *Hydra viridis* and 0.05 $\mu$ for *Hydra littoralis*.

It was concluded that while contraction of hydra resembles that of higher muscle in its response to ATP and its pH range, it differs in that it does not require Mg and it is activated by a much lower ionic strength. (Supported by training grant 5-T1HD2902 from the N.I.H.)

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GILBERT A. SHIBLEY and MARILYN MANCHESTER, Lawrence University.

Nutritional condition and contraction activity in *Hydra pseudoligactis*.

Contraction of the longitudinal muscles in the body column of hydra follows spontaneous electrical pulses which may or may not be largely patterned into bursts. Species vary in their degree of burst type activity (Rushforth, Am. Zool. 6:524, 1966). *H. pseudoligactis* shows less bursting than most other species and also shows considerable variability in contraction pulse patterns. We have recorded electrical signals from animals in a graded series of nutritional conditions and have found regular differences in total contraction pulse activity, in burst/pulse ratios, and in certain other aspects of the patterning. Animals fed daily contract most, and most often in bursts. Animals fed every second, third, fourth, or fifth days show progressively less contraction activity and smaller burst/pulse ratios. Activity in the rhythmic potential system and the tentacle-contraction potential system (Passano & McCullough, J. Exp. Biol. 42: 205-231, 1965) is also dependent on feeding schedule. Rhythmic potential activity is lower, but tentacle activity is greater in the less well fed groups.

These findings, taken with our observation that daily feeding of this species does not insure equal intake from day to day by individuals, help explain the variability in contraction pulse patterning characteristic of this species. The correlation of total contraction pulses with burst/pulse ratio suggests that bursting (i.e., patterning) is a function of whatever drives the system to spontaneous activity and probably not something imposed secondarily upon it by another system. (Aided by a grant from Research Corporation)

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JOHN BUCK, FRANK E. HANSON, JR., and GEORGE T. REYNOLDS, National Institutes of Health and Princeton University.

Colony coordination of luminescence in *Renilla*. (Motion Picture)

Parker (1920), Buck (1953) and Nicol (1955) have reported that the waves of luminescence that sweep over the rachis of the colonial alcyonarian *Renilla* following electrical or mechanical stimulation are

all-or-none, polydirectional, uniform in velocity, non-decremental, facilitatible, and involve several ranks of both autozooids and siphonozooids, with the leading edge brighter than the trailing. To date it has not been known how properties of the individual zooids, such as glow duration and intensity changes with time, compare with those of the whole colony, whether facilitation involves recruitment, whether particular zooids can fire repetitively and whether the excitation of zooids occurs via fixed spatial pathways.

Several of the above questions have now been answered by cinematography of luminous *Renilla* colonies using an image intensifier tube. Records will be presented of zooid participation and sequential action during normal rachidial waves, facilitation, fatigue, "scintillation" (asynchronous repetitive unit firing), "flare" (explosive high frequency discharge ending in a series of waves of progressively decreasing brightness and frequency) and "frenzy" (long-continuing autoexcitatory rachidial waves from multiple and shifting foci) and of their relation to an apparent reticulate pattern of zooid excitation. (Some of this work was supported by AEC Contract AT (30-1)-3406.)

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MARI-LUZ HERNANDEZ-NICAISE and L. M. PASSANO, Friday Harbor Laboratory, University of Lyon, France, and The University of Wisconsin.

A physiological analysis of the feeding behavior of *Sarsia tubulosa* (M. Sars), a hydrozoan jellyfish. (Motion picture)

*Sarsia* is an indiscriminant predator on copepods, chaetognaths and various meroplankters of suitable size. Initial capture is by nematocyst discharge from one or more of the four tentacles following chance contact with any portion of the moving prey. The catcher tentacle then contracts proportionally to the activity of the prey, initiating a margin pulse burst which triggers a bout of swimming contractions, and also an epithelial pulse which is conducted to the pendulous manubrium, causing the latter to contract. These responses may be repeated several times, and the epithelial-induced manubrial contractions may cause further manubrial shortening due to additional epithelial pulses emanating from the mouth region. The mouth bends at a right angle and shows seeking movements.

Further prey struggles cause the catcher tentacle to contract further and be pulled against the manubrium by a volley of contractions of that quadrant's subumbrellar radial musculature, with repeated manubrial contractions. Eventually, even slight contact between prey and manubrial lips cause an abrupt shift in *Sarsia* behavior: lips open and commence engulfment of prey; manubrium and catch tentacle commence relaxation; swimming bouts are immediately inhibited. Behavioral control via suppression of margin pulse and swim pacemakers, and epithelial pulses, is effected by distinctive small quick "lip pulses" emanating from the mouth region and conducted via manubrium and radial nerves to the marginal tentacle bulbs, the ganglionic control centers. With the completion of swallowing intermittent swimming resumes. (Supported by grant NB 06009 from the N.I.H.)

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G. O. MACKIE and L. M. PASSANO, University of Alberta and University of Wisconsin.

Non-nervous conduction in the epithelia and myoepithelia of hydromedusae.

New data are presented for the non-nervous conducting tissues of jellyfish of the genera *Sarsia, Euphysa* and *Phialidium*. Pulse forms are recorded from simple epithelia with suction electrodes as 1-2 mV biphasic events, 5-15 msec in duration, conducted at velocities between 15 and 35 cm/sec at 18-20°C. Potentials recorded from the subumbrellar myoepithelium (swimming muscle) are of complex form, long duration (>300 msec) and are slowly propagated (5.0-8.5 cm/sec). Intracellular recordings (obtained in collaboration with Dr. Mahlon Kriebel) from *Sarsia* myoepithelium show a resting potential of 65 mV negative, so it is reasonable to assume that the propagated events are depolarizations.

The conducting tissues, like those previously studied in siphonophores, show no capacity for spontaneous activity but are excited by direct electrical or tactile stimulation (sometimes firing repetitively), or indirectly through the nervous system. There is increasing evidence that nervous activity can, in turn, be caused by excitation in the epithelia. Non-nervous conduction is typically associated with the *en bloc* activation of widespread effector units, as in swimming and in certain protective reactions, rather than in local, graded activities. (Supported by major equipment and operating grants of the National Research Council of Canada.)

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BRUCE OAKLEY and JOHN M. PALKA, The University of Michigan and Rice University.

Prey capture by dragonfly larvae.

The labium of the aquatic larvae of dragonflies is a hinged, protrusible, grasping organ that strikes rapidly at suitable moving preg (e.g., *Daphnia*). *Anax* stalks the prey and also tracks its movements with the head, sometimes for many seconds, and then strikes by quickly extending the labium in line with the head axis. *Leucorrhinia* rarely stalks or makes tracking movements. Instead it strikes abruptly from rest, reaching targets over 90° off the body axis and 45° off the head axis. Slow motion cinematography shows that *Leucorrhinia* reaches its target by simultaneously flicking its head to one side, extending the labium, and twisting it outwards at the mental ("elbow") joint. (The head flick has a duration of about 200 msec and, as in *Anax*, the labial strike lasts about 100 msec.) Thus, in *Leucorrhinia* achievement of an accurate strike is complicated by simultaneous lateral movements of the head and labium.

The labial strike in *Anax* was shown to be dominated by visual control. Food intake in complete darkness or under a moonless sky was markedly reduced and the normal full strike rarely occurred. With sight in one eye the strike was inaccurately aimed. In contrast, feeding in *Leucorrhinia* was virtually unaffected by blinding either one or both eyes, owing to its sensitivity to mechanical stimulation by the prey. (Supported in part by a Univ. of Michigan Rackham Faculty

Research Grant, USPHS Grant NB07072-01, and NIH Training Grant 2T1GM989.)

75

JOHN PALKA, Rice University.

Head movement inhibits locust visual unit response to target movement.

The largest axon in each connective of the thoracic ventral nerve cord of locusts is a dimming detector receiving input from the contralateral compound eye. It responds vigorously to movement of objects in an approximately hemispheric receptive field (J. Palka, *J. Insect Physiol.* 13, 235, 1967). I now find that when the head itself is moved—freely by the animal or forcibly by the experimenter—the unit is silent even though the eyes seem the same target. A powerful inhibition accompanies and outlasts the head movement.

Adult *Schistocerca lata* were prepared for recording as before. The head was waxed to the shaft of a precision potentiometer which monitored head rotation. A black card mounted on the pen carrier of a potentiometric recorder was the moving target.

The fiber's normal response to a 20° movement of the card is a burst of 5 to 10 spikes at 50 to 100 per second. In marked contrast, head rotation of the same magnitude and with the eye viewing the same card, elicits no response.

Inhibition must be present because normally effective card movement becomes ineffective when presented concurrently with or following head movement. In a sample experiment, card movement elicited no response even 250 msec after head rotation, and at 420 msec the response was still reduced.

Input from the other eye cannot be the source of the inhibition since painting it (and the ocelli) has no effect. Visual input from the excitatory eye and mechanosensory feedback are two possible explanations.

76

WILLIAM E. BRADSHAW, The University of Michigan.

The role of morphological type and food in the termination of larval diapause in *Chaoborus* (Diptera, Culicidae). (Introduced by D. G. Shappirio)

On long day (17:7) without food, or short day (8:16) with food, *Chaoborus* larvae at 25°C exhibit up to 50% pupation, which occurs at scattered times from the 5th to 20th day of experimental treatment. However, on long day with food, larvae exhibit at least 90% pupation, which peaks abruptly at 6-7 days but gradually declines thereafter. Wild populations produce morphologically indistinguishable adults but reveal two larval varieties: large yellow and small pale individuals. Pupation patterns for populations of large yellow larvae resemble those of natural populations in showing the typical 6-7 day peak but with a sharper decline. Small clear individuals under the same conditions showed only 5% pupation, and no recognizable pattern. Percent and relative distribution of surviving adults indicate that this difference derives from a physiological or developmental, rather than a sexual dimorphism. No significant difference appears in either wild or large yellow populations between (1) animals exposed to long day and food for one day and then short day without food for two weeks, and (2) animals exposed to short day with food for one, two, three, or four days and then short day without food for the balance of 15 days. Distribution of pupation tended to be peaked for the former and scattered for the latter. Thus food appears to be acting as a physiological cue apart from its nutritional benefits. (Aided by PHS Grant GM-06101)

77

WILLIAM F. ZIMMERMAN, Amherst College.

An analysis of temperature effects on the pupal emergence rhythm in *Drosophila pseudoobscura*.

The circadian oscillation which gates pupal emergence in *Drosophila pseudoobscura* exhibits a remarkable conjunction of temperature properties: although the oscillation's free-running period, $\tau$ ($\tau$, measured as the interval between successive emergence peaks in constant conditions) is little affected by different *constant* temperatures ($Q_{10} = 1.02$), the oscillation is phase-shifted by and entrained to temperature *changes*.

Concerning the phase-shifting effects of temperature changes, temperature steps up cause only phase advances, temperature steps down cause only phase delays, and temperature pulses cause both phase advances and delays. The magnitude—and for pulses, the direction—of phase-shift depends on the point in the oscillation's cycle exposed to the signal.

An empirical relationship is found between the effects of temperature steps and pulses: the phase-shifting effect of a pulse may be obtained by adding the phase-shifting effects of the two steps comprising the pulse.

From the phase-shifting effect of a single pulse—plotted as a "response curve"—one can, in turn, correctly predict the phase angle difference, $\Psi$, between the emergence rhythm (driven oscillation) and pulses repeated at 24 hour intervals (driver oscillation). $\Psi$ is found experimentally to vary with the duration of the repeated pulse; that is, $\Psi$ is different for different 24 hour thermoperiods. The experimental pattern of $\Psi$ dependence on 24 hour thermoperiod is close to that predicted from the phase-shifting effects of single pulses which, in turn, were derived from the phase-shifting effect of steps. (Supported by research grants from the Office of Naval Research, Nonr 1958 (28) and the National Science Foundation, GB 5819.)

78

J. ROSS STEVENSON and T. Y. ADOMAKO

Diphenol oxidase in relation to sclerotization of the cuticle in the crayfish.

Frozen sections of cuticle of the crayfish *Orconectes obscurus* were incubated in phenolic substrates, and formation of colored product was used to detect sites of phenol oxidase activity. Enzyme activity was found in the newly formed epicuticle and outer pre-exuvial endocuticle shortly before the molt in stages $D_2$, $D_3$, and $D_4$ and after the molt in stages $A_1$ through $C_2$. Apparently the enzyme is incorporated in these layers when they are first secreted. Activity decreased rapidly during stage B, when sclerotization (tanning) is thought to occur, leaving a small residual activity in stages $C_1$ and $C_2$ and no activity after stage $C_2$. The loss

of activity during stage B is in itself evidence that sclerotization occurs during this stage. Although the formation of colored product was inhibited by 0.005 M KCN during stages $D_2$ through B, color developed in the outer pre-exuvial endocuticle even in the presence of 0.005 M KCN in all stages later than stage B. This cyanide-insensitive reaction may be produced by the natural quinone-protein products of sclerotization.

The enzyme was found to be active without the activator substance sodium oleate, and appeared to catalyze oxidation of the diphenols dopa (3,4-dihydroxyphenylalanine) and dopamine and not the monophenols tyrosine and tyramine. (Supported by USPHS research grant GM 08363)

79

DOROTHY M. SKINNER, Biology Division, Oak Ridge National Laboratory.

Isolation and characterization of the satellite DNAs of the land crab, *Gecarcinus lateralis*.

The DNA satellite "d(A-T)" of the marine crabs *Cancer* sp. (Smith, BBRC, 1963, *10*, 67) and of the land crab *Gecarcinus lateralis*, (Skinner, PNAS, 1967, *58*, 103) is a DNA composed of more than 90% alternating deoxyadenylate and deoxythymidylate residues. In several tissues of *Gecarcinus*, the "d(A-T)" satellite (density = 1.677 g/cm$^3$) makes up 18% of the total DNA and another satellite, rich in deoxyguanylate and deoxycitidylate residues (density = 1.721 g/cm$^3$) makes up 4%. The main band DNA has a density of 1.701 g/cm$^3$. The proportion of each of these three species of DNA is the same in the tissues studied and at various stages of the molt cycle. Studies on the biological role of the "d(A-T)" satellite are in progress. (Research jointly supported by USPHS grant AM-06268 and the Atomic Energy Commission under contract with the Union Carbide Corporation)

80

ALEASE L. SULLY and WILLIAM M. BANKS, Howard University.

Muscle metabolism in the cockroach, *Blaberus giganteus* L.

Red flexor femoris and white flexor and extensor tibialis muscles of meso- and metathoracic legs of adult roaches were dissected, kept separate male from female, and chilled in Pringle's saline solution. Muscle fibers were teased apart with glass probes, weighed on a torsion balance and transferred, in 50 mg portions to 6 ml Warburg flasks. Oxygen consumption was determined in $Ca^{++}$ and glucose free Pringle-phosphate buffer, pH 6.8, in 20% $O_2$, at 26°C for 1 hr. Dry weight determinations were made and results expressed as $\mu l$ $O_2$/mg dry tissue/hr. The effects of added substrates, 0.01 M glucose, 0.01 M pyruvate, 0.01 M succinate, 0.03 M malonate and 0.03 M fumarate on oxygen consumption of male and female muscle were compared. Glycogen content was measured by the method of Neptune and Foreman (1959), glucose by that of Somogyi and Nelson (1944). Mitochondria were isolated by a modification of Cooperstein's method (1950) and counted with the aid of a hemocytometer.

Oxygen consumption was significantly higher in red than in white muscles. Mean per cent increment of red over white in buffered Pringle's was 266. Mean per cent increments with added substrates, pyruvate, succinate and glucose were 175, 76, 139 respectively. Neither the degree of respiratory inhibition by malonate nor its reversal by fumarate differed significantly in the muscles of either sex. These results appear to be correlated with a higher concentration of mitochondria, enzymes and substrate in red rather than in white muscles. In contrast to observations reported on muscle metabolism in *Periplaneta americana* (Barron and Tahmisian, 1948), oxygen consumption of skeletal muscles does not differ significantly between the sexes of *B. giganteus*.

81

JAMES L. BAIRD, JR. and ROBERT P. NUTTALL, Lafayette College.

An analysis of the carbohydrate content of the flight muscle of the large milkweed bug, *Oncopeltus fasciatus*, Dallas, as a function of age.

As a part of a larger study on the flight performance of the Heteroptera the carbohydrate content of the flight muscle of the large milkweed bug, *Oncopeltus fasciatus*, Dallas was determined as a function of age. Dingle (J. Exp. Biol., 44:335-343, 1966) has reported that *Oncopeltus* shows peak flight activity 8-10 days after the final molt. The literature concerning the fuel for flight in insects with fibrillar muscle strongly suggests that carbohydrates, and more specifically glycogen, are the principal sources of metabolic energy for flight. It was hypothesized that *Oncopeltus* uses glycogen as an energy source for flight and, furthermore, the amount of muscle glycogen increases as a function of age toward the peak flying period. Flight muscle from adults of age 1-2 days and 7-8 days was dissected out and inactivated in boiling water. A modification of the anthrone technique of Kubista and Bartos (Physiol. Bohemoslov., 9:228, 1960) was employed to determine total carbohydrate, non-glycogen carbohydrate, free glycogen, and fixed glycogen. Results indicate that the total carbohydrate content of the flight muscle of *Oncopeltus* is considerably lower than might be expected. Further the total carbohydrate decreases as a function of age, being 25% lower in the 7-8 day adults as compared to the 1-2 day adults. Work is underway which seeks to clarify these apparent discrepancies and determine the probable flight fuel in *Oncopeltus*.

82

ROGER H. LUMB, Western Carolina University, LARRY T. WIMER, and B. THEODORE COLE, University of South Carolina.

Fatty acid analysis of different lipid classes during the life cycle of *Sarcophaga bullata*.

One adult, 5 pupal and 8 larval stages were selected from the life cycle of *Sarcophaga bullata*, and lipid was extracted from these whole animal samples using 2:1 chloroform:methanol. The lipid extract was washed using a modified Folch procedure and fractionated by thin-layer chromatography into triglyceride, free fatty acid, partial glyceride and phospholipid. The fatty acid patterns of these four fractions were ascertained using gas-liquid chromatography.

Palmitic, palmitoleic, stearic and oleic were found to be the major fatty acids accounting for 90% of the total in most stages. The minor fatty acids detected were lauric, tridecanoic, myristic, myristoleic, pentadecanoic, heptadecanoic and linoleic acid.

In most stages palmitoleic acid predominated in the free fatty acid, partial glyceride and phospholipid fractions, but not in the triglyceride. Palmitoleic and oleic acids of the free fatty acid fraction tended to increase in preparation for pupation and eclosion. Oleic acid of phospholipid increased during larval development, while palmitoleic acid decreased.

In general the fatty acid distribution was consistent with most other analyses on Diptera, in that there were high amounts of fatty acids with less than 18 carbons, and large percentages of palmitoleic acid. (Supported in part by NSF Grant GB-926)

83

WALTER R. TSCHINKEL, University of California, Berkeley.

Defensive secretions of the beetle *Zophobas rugipes* F. (Introduced by Rudolph Pipa)

The Central American tenebrionid bettle, *Zophobas rugipes,* has a pair of prothoracic defense glands secreting a mixture of phenols and a pair of abdominal defense glands secreting a mixture of quinones. The free phenols were separated and the peaks collected by GLC on a 15% FFAP column or by column chromatography on alumina. Cresol is the major component as judged by UV and NMR of the n-phenyl urethane. Ethylphenol and phenol were also identified as minor components by UV and by comparison of retention times on three columns (FFAP, SE-30, NPGS). Two quinones were separated and collected from a 5% SE-30 column. They appeared to be toluquinone and ethylquinone (UV, NMR and m.p.). Some minor, as yet unidentified, peaks were also present. The phenol-secreting prothoracic glands consist of a pair of reservoirs opening on the membrane between the head and prothorax. The reservoir is covered with glandular-appearing tissue. The orifice is controlled by a muscle, but the milky secretion is presumably expelled by hemolymph pressure. The abdominal quinone-secreting glands are a pair of eversible sacs located between the fifth and sixth sternites. Their gross anatomy is similar to the homologous glands in *Tenebrio molitor.* When disturbed, larvae of *Z. rugipes* and several other tenebrionids squirt hemolymph from predetermined breaks in the lateral body wall.

The composition of the quinone secretion of *Z. rugipes* has been compared by GLC with the homologous secretion of other tenebrionids. Taxonomic relationships appear to be reflected in the composition of these secretions. (Aided by NIH predoctoral fellowship and NSF grant GB-6424X)

84

DANIEL E. MARVIN and ALAN G. HEATH, Virginia Polytechnic Institute.

Cardiac and respiratory responses to gradually induced hypoxia in three species of freshwater fish.

Rainbow trout, *Salmo gairdnerii,* bluegill sunfish, *Lepomis macrochirus,* and brown bullhead catfish, *Ictalurus nebulosus,* were subjected to a gradual reduction in ambient oxygen over an eight hour period. The fish were inrestrained in experimental chambers just large enough to permit free fin movement. Heart rate, ventilation rate, and oxygen consumption were measured at one-half hour intervals. Ten individuals of each species were used and the data computer analyzed.

Catfish showed the lowest lethal level of oxygen of the three species examined (0.5-1.0 mg $O_2$ per liter). Under hypoxia ventilation volume was increased in trout by increased ventilation depth, while bluegills showed an increase in ventilation rate. Ventilation was regular in all species although catfish did show long periods of apnea under severe hypoxia. Bradycardia was common to all species and occurred immediately at the beginning of stress in catfish, but was delayed in trout and bluegills.

Data on oxygen consumption indicate that trout and bluegill are oxygen regulating species, while catfish appear to be an oxygen conforming species. As ambient oxygen decreased catfish showed a gradual reduction in heart rate and ventilation rate coupled with a reduction in oxygen consumption.

Heart rate and ventilation rate were found to place a similar metabolic demand on the animal in trout and catfish. In bluegills, however, the heart places a significantly higher metabolic demand on the animal than does the ventilation mechanism.

85

ALAN G. HEATH and DANIEL E. MARVIN, Virginia Polytechnic Institute.

Cardiac and respiratory changes in three species of freshwater fish during and following severe hypoxic stress.

Rainbow trout, *Salmo gairdnerii,* bluegill sunfish, *Lepomis macrochirus,* and brown bullhead catfish, *Ictalurus nebulosus,* were exposed to a rapid reduction in ambient oxygen followed by a period of recovery in well oxygenated water. The fish were confined to experimental chambers large enough to allow fin movement but only minor body movement. During and following the period of hypoxia, frequent (every 15 minutes) measurements of heart rate, ventilation rate and oxygen uptake were made. Ten individuals of each species were used.

Physiological responses during the stress were similar to those seen in gradual stress (Marvin and Heath, Amer. Zool. 7, 750, 1967) except catfish tended to show a 1:1 relationship between heart rate and ventilation rate at the peak of stress.

Trout showed a 100%, bluegills 22%, and catfish 0% increase in oxygen uptake following the stress. Recovery generally required at least 5 hours in trout and bluegills. Ventilation rate returned to the control level quickly with no overshoot during recovery in bluegills and trout. A slightly elevated breathing occurred in catfish following the stress.

A significant bradycardia was evident in trout for one hour following the stress, while catfish showed a significant tachycardia after hypoxia. Heart rate in bluegills following the stress did not differ from the control rate. The slow return of

cardiac function in trout may help explain the delayed rise in blood lactate following hypoxia as seen in earlier studies.

86

BRUCE L. UMMINGER, Yale University.

Sub-zero temperatures and supercooling in *Fundulus heteroclitus*. (Introduced by Grace E. Pickford)

Serum from *Fundulus heteroclitus* acclimated to 20° freezes at —0.62°C. Acclimation to —1.6°C only lowers serum freezing point to —0.86°C. Clearly, *Fundulus* survives sub-zero temperatures in a supercooled state and does not form an "antifreeze."

Four groups of 25 adult male *Fundulus* were acclimated to 20°C, 10°C, 2°C and —1°C. Serum was analyzed to determine the substance permitting permanent supercooling. Serum chemistry was not significantly different in fish acclimated to 20°C and 10°C. Acclimation to 2°C, however, induced significant increases in some serum components (sodium, 7.4%; calcium, 43.8%; magnesium, 38.5%; chloride, 15.5%; non-protein nitrogen, 41.3%; total cholesterol, 73.1%; glucose, 119.1%; osmolality, 30.9%) and no change in others (potassium, bicarbonate, inorganic phosphate, total protein). These levels were maintained at —1°C with the exception of an increase in bicarbonate (51.1%) and a further increase in glucose (440.4%: 84.7 mg% at 20°C and 10°C, 185.6 mg% at 2°C, 457.6 mg% at —1°C). Identical levels of most serum constituents at 2°C and —1°C indicate general metabolic adaptations to low temperature whereas the dramatic increase in glucose at —1°C represents a specialized physiological adaptation to survival in a supercooled state. (Supported by a N.A.S.A. Traineeship and National Science Foundation grants to Grace E. Pickford.)

87

WALTER G. WHITFORD and THOMAS O. BOSWELL, New Mexico State University.

The effects of water hardness on the upper lethal temperature of the Green Sunfish *(Lepomis cyanellus)*.

The effects of water hardness on the upper lethal temperature of the green sunfish *(Lepomis cyanellus)* were examined. Fish were acclimated and tested at hardness levels of 30, 180, and 400 p.p.m. Both 48-hour $LD_{50}$ upper lethal temperatures and critical thermal maxima were recorded for acclimation temperatures of 20°C and 30°C. It is suggested that the critical thermal maximum determination is a useful tool in comparative lethal temperature studies with fish which involve only one variable.

Acclimation and 48-hour $LD_{50}$ tests were conducted in ten-gallon aquaria designed to eliminate behavioral interaction. No significant effects of water hardness on upper lethal temperatures were noted in either the 48-hour $LD_{50}$ determinations or in the critical thermal maxima determinations. Winter collected fish showed lower levels of thermal resistance than summer collected fish.

Behavioral evidence is presented supporting the hypothesis that the primary lethal mechanism in heat death in green sunfish is failure of the nervous system.

It is suggested that, due to differences in metabolic and activity patterns between cold water stenothermic fishes and eurythermic fishes, variations in hardness level may be significant only in upper lethal temperature relations of cold water stenothermic fishes. (Supported by WRRI grant NM3109-23)

88

JOHN L. ROBERTS, University of Massachusetts, and GEORGE M. HUGHES, University of Bristol.

Gill ventilation and stress in trout.

Hydrostatic pressure changes in the mouth and opercular cavities of rainbow trout have been measured both directly and differentially during normal ventilation cycles at 15°C and during warming (1°C/3 min) from 15°C. Procedures used generally have followed those employed previously by one of the authors (G. M. Hughes & G. Shelton, 1958, J. Exp. Biol. *35*) with lightly anesthetized fish (MS 222. 0.04 g/l). Pressures were recorded continuously with Sanborn gauges (268B) via needle catheters inserted into the buccal and opercular chambers as the water bath temperature was raised from 15°C and until respiratory failure became obvious. This usually occurred between 28° and 29°C or at a brain temperature equivalent to 25°-26°C (implanted bead thermister).

The observed variations in amplitude and phasing of pressure waves generated during ventilation cycles in trout will be illustrated and discussed relative to the degree of accommodation (safety factor) for changes in stream flow to the mouth and for thermal stress. (Supported by a Public Health Service Special Fellowship to J. L. Roberts, 1-F3-GM-2531-01, NIGMS.)

89

ROBERT C. LASIEWSKI, MARVIN H. BERNSTEIN and WESLEY W. WEATHERS, University of California, Los Angeles.

Oxygen consumption, evaporative water loss and body temperatures of the Giant Hummingbird, *Patagona gigas*.

*Patagona gigas*, the largest living hummingbird, may weigh as much as 22 g and measure 23 cm in length. Three Giant Hummingbirds were purchased from a commercial source, maintained in the laboratory for over three months, and their physiological responses studied. Standard metabolic rate of *P. gigas* (mean weight, 19.1 g) was 2.7 cc $O_2$/g/hr (5.9 kcal/day), and thermal conductance was 0.17 cc $O_2$/g/hr/°C. Torpid metabolism increased exponentially with ambient temperature, ranging from 0.25 to 0.87 cc $O_2$/g/hr at 16.4 and 25.6°C, respectively ($Q_{10} = 3.7$). Rates of entry into and arousal from torpor were inversely related to body weight in birds studied. Evaporative water loss was inversely related to ambient water vapor pressure at ambient temperature of 25°C. Homeothermic heart rates ranged from 300-1020/min, and minimum recorded breathing rate was 106/min. Wing beat frequency during hovering flight was 15/sec.

Thermal conductance in birds is inversely related to body weight, and can be described by the equation: $\log C = \log 0.848 - 0.508 \log W$, where C is thermal conductance in cc $O_2$/g/hr/°C, and W is body weight in g. Thermal conductances, calcu-

lated from records of body temperatures of hummingbirds entering torpor, were similar to minimum themal conductances obtained from ambient temperature-metabolism curves. (Supported by NSF Grants GB 3017 and GB 5347.)

90

C. M. WINGET, C. A. WARREN and C. W. DeROSHIA, Ames Research Center, NASA, Moffet Field, California.

Interrelationships of the pineal gland, the diencephalon and the pituitary *(Gallus domesticus)*. (Introduced by J. Oyama)

The interrelationships between certain daily rhythms were studied in male adult chickens (n=34) maintained on a 12 L:12 D photoperiod. After decapitation the pineal gland (excluding meningeal tissues), diencephalon and pituitary were dissected out and immediately frozen. These tissues were later weighed and then assayed for monoamine oxidase (MAO), cholinesterase (CHE), acid and alkaline phosphatases (AP) and hydroxyindole-O-methyltransferase (HIOMT-pineal gland only). The data were reduced in several steps to evaluate phase relationships between the various organs in terms of maximum and minimum fluctuations in enzyme activities. Pineal MAO and HIOMT exhibited a daily activity cycle with maxima during the lights-on phase. The AP maxima occurred just before lights-off. CHE activity was not detected in the pineal gland. Diencephalon had higher average enzyme activities (counts/mg/min) than either pineal gland or pituitary but the amplitudes of the diencephalon cycles were lower. The greatest deviation from the means of the pituitary enzymes activity occurred at the time of lights-off. Pituitary CHE activity which is localized in specific areas varied least from its mean. This may result from an enzyme dilution effect. The results indicate that the pineal gland maintains maximal enzyme activity during the light phase while the pituitary is most active during the dark phase.

91

R. GLENN NORTHCUTT, JAMES EDWARD HEATH, and ROBERT P. BARBER, University of Illinois.

Rotational optokinesis of reptiles.

Some reptiles show a marked ability to rotate the optic bulb in response to the pitch of the head. The eye rotates through 50° in crocodiles, 20-30° in snakes, and 60° in rhyncocephalians. The response is less developed in lizards, and absent in turtles. The eye fully compensates for changes in pitch through about ½ the total range of the response. Ablation or procaine treatment of the semicircular canals abolishes it.

92

ALLAN BERLIND, Harvard University.

Release of a neurosecretory hormone in an invertebrate: effects of calcium and 5-HT. (Introduced by Ian Cooke)

The isolated pericardial organ (PO) of the spider crab *Libinia emarginata* was used to study the effects of calcium on electrically elicited release of a neurosecretory hormone, and on the electrical activity of the cells involved. The PO was stimulated electrically in normal crab ringer, then in ringer with calcium omitted, and again in normal ringer. The fluid bathing the PO was assayed for hormone on the isolated, perfused heart of the same animal, which exhibits an increase in frequency and/or amplitude in the presence of the hormone. In 15 experiments the amount of hormone released by stimulation in normal ringer was significantly greater than that released in the absence of calcium. The amount of hormone released by stimulation in the absence of calcium did not differ significantly from that released spontaneously. The amplitude of the compound action potential recorded extracellularly was not altered in the calcium-free medium in most experiments but was increased in several. The conduction velocity was increased by an average of 15% in the absence of calcium.

5-Hydroxytryptamine is present in higher concentration in the PO than in other crab nervous tissue (Maynard and Welsh, J. Physiol. *149*, 215. 1959). The effect of 5-HT on release was tested by bathing the PO in a 1.0 $\mu$M solution of that substance in crab ringer. The bathing solution was assayed on a heart made tachyphylactic to 5-HT by continuous perfusion with 0.1 $\mu$M 5-HT. Release in 5-HT did not differ significantly from spontaneous release. (Supported by an NSF graduate fellowship and NSF grant GB-4315)

93

I. M. COOKE, Harvard University.

Potentials recorded intracellularly from neurosecretory terminals.

Neurosecretory terminals in the sinus gland of the land crab, *Cardisoma guanhumi*, reach a size permitting penetration under visual direction with a microelectrode. Electrodes were filled with 1.5 M $K_3Fe(CN)_6$ permitting verification of recording sites by histological examination. The majority of penetrations were signalled by a potential shift of —30 to +70 mV which then declined to —15 mV or less within a few seconds to a minute, presumably as a result of damage from the electrode. About half of these units showed rapid spontaneous depolarizations. These were of constant or discretely varying form in a particular unit: the amplitude appeared to be a function of resting potential. In a number of units the potentials were overshooting. Sometimes positive-going potentials persisted for some seconds after the resting potential had disappeared. A small number of penetrations gave maintained resting potentials of —50 to —80 mV. Some of these were quiescent and did not respond to stimulation of the X-organ nerve nor to depolarizing current through the electrode of up to $5 \times 10^{-9}$A. Others responded to nerve stimulation with overshooting potentials or with smaller, complex depolarizations exhibiting amplitude variations in discrete steps. None of the units would follow stimulation rates greater than 4/sec. In a few cases responses to nerve stimulation could be compared to spontaneously occurring 'spikes' and to 'spikes' produced by depolarizing current. An invariable feature of spontaneous and stimulated activity is its long time-course, half-amplitude durations being always in excess of 10 msec. The limited data suggest the possibility that regenerative sodium conductance changes can occur in neurosceretory

terminals. (Histology by Martha W. Goldstone. Supported by grant GB-4315 from N.S.F.)

94

DANIEL HARTLINE, Harvard University.

Integration in the lobster cardiac ganglion (*Homarus americanus*).

This ganglion offers the attractive possibility of determining the role each neuron plays in generating a complex pattern of activity, the periodic "burst." With multiple-electrode recording almost every burst impulse can be identified. Cell 7 usually initiates the burst. The usual initial large-cell order is 5, 4 and $3_C$, 1 and 2. Cells 8 and 9 trigger distally at first, then proximally later in the burst. Small cells fire a uniform impulse train with frequency slowly declining from a moderate peak near the beginning. Cells 6 and 7 fire most rapidly, cell 8 less so, and cell 9 least rapidly. Large-cell frequency drops precipitously from a high initial transient to a low plateau. Cells 5 and 4 tend to fire fastest, cell 3 less rapidly, and cells 1 and 2 least rapidly. Cell 1 and 2 impulses cause firing in the $3_A$ and $3_B$ axons, respectively, and in corresponding truncated cell 4 and 5 axons. Impulses in certain large cells can cause early firing of the burst.

An antidromic impulse "resets" a train of large-cell escape impulses, with the following interval greater than normal. Additional impulses further increase this interval. In contrast, insertion of an antidromic impulse into certain large-cell burst patterns simply eliminates the firing of the next expected orthodromic impulse, usually without changing the remainder of the pattern. Additional antidromic impulses eliminate more impulses from the burst pattern, the remainder of which either is unchanged or assumes only certain discrete alternative configurations.

Possible explanations for these observations will be discussed. (Supported by grants 5-F1-GM-22,300, 5-TO1-GM-0036, and NB-00623 from U.S.P.H.S. and grant GB-4315 from NSF)

95

BRIAN MULLONEY, University of California, Berkeley.

Patterns of activity in motor neurons of the dorso-longitudinal flight muscles of the bumble bee, *Bombus californicus*. (Introduced by D. M. Wilson)

The dorso-longitudinal muscles of bees are fibrillar muscles, and are part of a mechanical resonator—the thoracic flight apparatus. Their contractions are phasically independent of the action potentials in the neurons which innervate them. The frequency of their contractions is determined mainly by the resonant frequency of the thorax. The action potentials of the motor neurons can be monitored by electrodes in the muscles, since the muscle action potentials follow one to one each nerve action potential (Ikeda and Boettiger 1965 J. Insect Physiol. *11*:779-789).

Counts of the number of distinguishable classes of muscle action potentials recorded from a single dorso-longitudinal muscle indicate that there are five motor units in each of these muscles. No single experiment has yielded data from every unit that was good enough to permit analysis of the temporal relationships of all five units recorded simultaneously. However, if one assumes that there is an equal chance of recording from each of the five in any one experiment, the results of different experiments in which two or three units were recorded and analysed can be combined.

Each unit tends to fire regularly, without much short term irregularity. Interspike interval histograms are broad, skewed, and unimodal. Different units in the same muscle not only have similar average firing frequencies, but also follow short term variations together.

Some units within the same muscle are loosely phase-coupled, tending to fire either at the same time, or with one unit leading the other slightly. The latency of any unit of the phase coupled pairs with respect to its partner is not correlated with the interspike interval. Other pairs of units are temporally independent except for the common frequency trends.

The relevance of this study to other work on motor control of insect flight behavior will be discussed, and comparisons will be made with similar work on flies, beetles, and bugs. (Supported by a grant of computer time from the UC Computer Center, Berkeley, and by grant NB 03927)

96

NANCY S. MILBURN and ROGER D. FARLEY, Tufts University and the University of California at Riverside.

Physiology and morphology of the cercal and giant nerve fibers in *Periplaneta americana*.

Mapping of the giant nerve fiber system in the American cockroach has been initiated with studies of normal animals and those undergoing retrograde and Wallerian degeneration. The nerve cord or cercal nerves were sectioned or hemisectioned and three to thirty-seven days were allowed to elapse. Following this some of the nerve tissue was prepared for light or electron microscopy. In other cases electrophysiological studies were performed before fixation.

There are sixteen giant axons which travel the length of the abdominal nerve cord, (Hess, 1958, J. Morph. *103*) five located dorsally and three ventrally in each connective. A smaller diameter fiber is grouped with the ventral giants. Giant fibers are syncytial. They originate at least in part from a group of large ganglion cells located primarily in the contralateral ventral caudal area of the last abdominal ganglion. Cell bodies in no other ganglia are associated with the giant axons. All giant axons travel up the abdominal cord to the metathoracic and mesothoracic ganglia where the dorsal ones seem to synapse and end. The ventral giant fibers extend into the subesophageal ganglion and may continue to the other ganglia of the head.

The primary input to these axons is the cercal nerve sensory fibers. These apparently cholinergic synapses are characterized by accretions of 500 A diameter membrane-bound vesicles over thickened synaptic membranes. A subsynaptic web and presynaptic laminar apparatus are also seen.

All giant axons normally conduct in the ascending direction and may be electrically stimulated to conduct antidromically. Exposure of the animal to excess $CO_2$ can also trigger descending giant

fiber action potentials. (Supported by NIH postdoctoral grant, USPHS grant TIA132 and NSF grant GB2971)

97

DAVID F. WILSON, University of Delaware.

Electrical and mechanical responses of the pallial muscle in the mantle of a clam. (Introduced by R. A. Nystrom)

The mantle edge of *Spisula solidissima* is richly endowed with loosely packed muscle fibers. Application of a single electrical or mechanical stimulus to the mantle induces a large ($35\pm5$ g) double contraction followed by a prolonged relaxation time (several minutes). The contraction consists of two components, called $\alpha$ and $\beta$, which respond differently to drugs and threshold stimulation. The prolonged relaxation phase is due to activity of nerve elements: MS222, a nerve blocking agent, hastens relaxation; release experiments show prolonged activity in the contractile elements; electrical activity can be recorded during relaxation; and ganglion cells can be seen in histological preparations of the mantle. The mantle edge is innervated by the anterior and posterior pallial nerves and both are capable of initiating and propagating only low-tension contractions throughout the mantle. The anterior pallial nerve can also inhibit by inducing a quick relaxation in a large contraction that was locally initiated. 5-HT mimics the inhibitory effect. (Supported by NASA Predoctoral Fellowship.)

98

BERT SHAPIRO, Harvard University.

Mechanism of action of anemone toxin on crustacean nerves.

The tentacles of *Condylactis gigantea* contain a protein toxin which paralyzes crustacea. The initial rigid paralysis is due to spontaneous repetitive firing in the nerves in high-frequency bursts. The toxin has no effect directly on muscle cell membranes or on neuromuscular junctions. Direct application of toxin to crayfish and lobster axons transforms the normal action potentials into long plateau-potentials which can last several seconds. The rising phases of the action potentials are only slightly altered. The resting potential and resting resistance of the cell are unaltered by the toxin. The only cell characteristic apparently changed is the falling phase of the action potential.

The cell resistance during the plateaus is greatly reduced. As the plateau continues, the cell resistance increases but is still below resting level at the termination of the potential. The resistance rapidly returns to its resting level after cell repolarization. During the plateau the membrane potential is very sensitive to external sodium. Increasing sodium increases plateau height and duration. Decreasing sodium decreases these parameters, and the plateau may revert to an action potential.

This indicates that the cell is permeable to sodium during the plateau. All evidence agrees with the model that anemone toxin increases the time constant of sodium inactivation.

The plateaus formed in this manner can induce repetitive firing farther down the axon by generating DC currents. Low-frequency trains can be generated by a slight increase in spike duration.

99

KENNETH J. FRIEDMAN, State University of New York, Stony Brook.

Peripheral and central effects of curare in the cockroach. (Introduced by E. Eisenstein)

Evidence indicates that curare blocks peripherally as well as in the central nervous system of the cockroach, *P. americana*. Elimination of the leg reflex for one metathoracic leg may be achieved by injection of 25 $\mu$l of a dTC solution (25 mg crystalline d-tubocurarine chloride/ml Pringle's insect saline) into the coxa of that leg as per the technique of Kerkut ('65). The reflex is maintained in the contralateral leg. Perfusion of the metathoracic ganglion with 25 $\mu$l of the dTC solution blocks reflexes to both legs. Both peripheral and central blockage may be reversed by perfusing the affected area with saline.

The mode of curare entry into the roach determines the drug's effectiveness. Whereas an intra-abdominal injection of 1.1 $\mu$gm/mg body weight will curarize the intact animal as shown by Larsen, *et al* ('66), we have found that oral administration of 3.0 $\mu$gm/mg body weight is without effect. (Supported by USPHS NB-05827-03; SUNY Research Foundation 31-186A; NDEA Title IV Graduate Fellowship Program.)

100

JOHN M. LUCH and ELDEN W. MARTIN, Bowling Green University.

Evidence for osmoregulatory activity by scyphistoma of *Aurelia aurita*.

A study was done to determine if scyphistoma of *Aurelia aurita* carried on osmoregulatory activity in varying salinities. Scyphistoma were conditioned in salinities of 15, 20, 25, 30, 35, 40 and 45 parts per thousand for a two-week period. Internal fluids of the animals were extracted and the osmotic concentration of the fluids determined. The respiration rate of the animals was determined in order to investigate possible active transport mechanisms in osmoregulation.

Extraction of internal fluids showed that animals from a salinity of 25 parts per thousand had internal fluids whose concentration almost equaled that of the external environment. The internal fluid concentration of animals from salinities lower than 25 parts per thousand was higher than the external medium while the concentrations of the fluids from animals above 25 parts per thousand salinity were lower than the external medium. This would indicate the ability of*Aurelia aurita* to partially regulate their internal concentration against a concentration gradient.

The oxygen consumption rate of animals from a salinity of 25 parts per thousand was significantly lower than those from 15, 20 and 30 parts per thousand salinity. This would indicate that a salinity of approximately 25 parts per thousand is the optimum salinity in which the least osmoregulatory energy is expended. Osmoregulation does occur at salinities either higher or lower than 25 parts per thousand, probably by active transport mechanisms.

101

THOMAS H. DIETZ, Oregon State University.

Ionic regulation in *Lumbricus terrestris*. (Introduced by R. H. Alvarado)

The earthworm, *L. terrestris*, normally occupies a moist, semi-terrestrial environment. They also survive for several months in an artificial pond-water (PW) (0.50 mM NaCl, 0.05 mM KCl, 0.40 mM $CaCl_2$, 0.20 mM $NaHCO_3$).

Transfer of worms from soil to PW results in a 15% increase in weight within 2 days due to the uptake of water. There is a concomitant dilution of the coelomic fluid. Within 7 days the Na and Cl concentrations in the coelomic fluid return to a level similar to worms in soil, reflecting a net uptake of these ions.

Sodium fluxes have been determined on worms acclimated to various concentrations of PW. Individual animals were placed in a small volume of PW labeled with $^{22}Na$. Bath samples were removed at intervals and assayed for radioactivity. The bath sodium concentration was determined by flame photometry. From these measurements the influx and net flux were estimated. The efflux was calculated by difference between the influx and the net flux. The influx of sodium is a function of the external concentration. Over the concentration range of PW to 8 times PW, the influx varied directly from 0.03 to 1.30 $\mu$eq Na/10 g-hr. Preliminary chloride flux data indicate similar kinetics. (Supported by USPHS Predoctoral Fellowship 1-F1-GM-35,422-01 and NIH grant AM 10508-03)

102

RAGHUNATH A. VIRKAR, Wisconsin State University, Superior, and KENNETH L. WEBB, Virginia Institute of Marine Science.

Salinity-induced variations in the free amino acid composition of the soft-shelled clam *Mya arenaria*.

Soft-shelled clams, collected from an estuarine habitat, were maintained in the laboratory at a salinity of 20‰, and then transferred to various salinities ranging from 2‰ to 30‰. Pieces of adductor muscles were extracted in 80% ethanol and the concentration of ninhydrin-positive substances (NPS) in the extract measured colorimetrically. A linear relationship between salinity and NPS concentration was observed over the entire salinity range, the concentrations ranging from 50 to 390 millimoles per kg tissue water.

Ion-exchange chromatography showed that individual constituents of the free amino acid pool behaved differently in response to salinity change. At the highest salinity, alanine accounted for about 50% of the total free amino acids, the other major constituents being glycine, valine, taurine, arginine, and glutamic acid. At the lowest salinity, alanine formed only 10% of the total pool, while the contribution of glutamic acid was about 50%. Glutamic acid, ornithine and histidine showed an absolute as well as a relative increase at the lowest salinity. After correcting for increased tissue water at the lowest salinity, alanine, glycine, valine, and arginine showed a net decrease of about 38%, taurine decreased by about 28%, while glutamic acid increased by about 125%.

The response of the pool to a change in salinity is quite rapid. A measurable drop in the concentration of NPS occurs within two hours following transfer from a salinity of 20‰ to 5‰. The concentration continues to drop linearly with time, and the process is apparently completed in 48 hours.

103

C. S. HAMMEN, University of Rhode Island.

Aminotransferase activities and amino acid excretion of bivalve mollusks. (Introduced by Robert Barnes)

Tissue homogenates of six species of lamellibranchs were assayed for aspartate and alanine aminotransferase activities by measuring the amounts of oxalacetate and pyruvate formed. Aspartate enzyme activity ranged from 0.70 micromoles per gram-minute in *Mercenaria mercenaria* to 6.80 in *Donax variabilis*, the alanine enzyme from 0.20 in *Crassostrea virginica* to 13.55 in *Solemya velum*. In five species the conversion of alanine to pyruvate was catalyzed from 0.5 to 2.0 times as rapidly as the conversion of aspartate to oxaloacetate, but in the oyster the alanine reaction was only one-tenth as rapid. Whole animals were held in dishes of filtered sea water for 6 to 24 hours, and the water was analyzed for ammonia, amino acids, uric acid, and urea. Ammonia made up 50 to 72 per cent, and amino acids 23 to 31 per cent of the identified products. Small quantities of uric acid were given off by five species, and small amounts of urea by the oyster. Rates of total amino acid excretion were directly proportional to total aminotransferase activities, and inversely proportional to weights of the animals.

104

ROBERT H. PARSONS, Oregon State University.

Sodium metabolism in a fresh-water pulmonate snail. (Introduced by R. H. Alvarado)

Snails acclimated for 10 or more days at 20°C in an artificial pond water (0.13 mM NaCl, 0.012 mM KCl, 0.1 mM $CaCl_2$, 0.05 mM $NaHCO_3$) had an internal concentration of 41 mM of sodium and 39 mM of chloride and a total solute concentration of 105 millosmols/l.

Sodium influx was determined by placing individual animals in a bath containing $^{22}Na$ and following the rate of loss of isotope from the bath. Changes in sodium concentration of the bath were monitored by flame photometry which provided an estimate of the net flux. The efflux was determined as the difference between the influx and net flux. Animals in a steady state exchange sodium at a rate of 1.9 $\mu$eq/hr-10 g (weight includes shell).

Animals, salt depleted by placing them in running distilled water for five days, suffered a 20% decrease in their sodium and chloride concentrations. When they were placed in a limited volume (50 ml) of pond water the animals experienced a net uptake of sodium reducing the bath sodium to a steady state value of 0.03 mM. The positive net flux of sodium observed in a salt-depleted animal transferred to pond water can be attributed to a two-fold increase in the influx.

These preliminary studies suggest that these snails are well adapted to a low external concentration of sodium by possessing a very effective sodium uptake

mechanism coupled with an iono-regulatory system, which is probably sensitive to the internal salt concentration. The possibility that this is an endocrine controlled system is now under investigation. (Supported in part by grant Am 10508-03 from N.I.H.)

105

LAWRENCE C. THOMPSON, University of California, Berkeley.

Osmotic regulation in the Mexican freshwater crab, *Pseudothelphusa jouyi* Rathbun. (Introduced by R. I. Smith)

Crabs were collected from the freshwater (FW) Lake Chapala, near Guadalajara, Mexico, and transported to Berkeley, California. Animals were suitably acclimated to dechlorinated tap water and dilutions of seawater (SW). Total osmotic pressures of body fluids were measured by vapor pressure osmometer; ions were measured by chloridometer, flame photometer and atomic absorption spectrophotometer. Permeability to tritiated water was determined by liquid scintillation spectrometry, and was compared to several euryhaline and stenohaline marine crustaceans. $^{22}Na$ and $^{36}Cl$ fluxes in FW were determined similarly with scintillation techniques. Inulin volumes, clearances and urinary outputs were estimated using $^{14}C$-inulin. The effects of eyestalk ablation upon these several regulatory parameters are currently being investigated.

Results indicate that *Pseudothelphusa* regulates at tonicity levels comparable to the African FW crab *Potamon*. Of the several species tested, *Pseudothelphusa* shows least permeability to water, being at least twice as impermeable as the FW crayfish. Reduced permeability to salts is indicated by the relatively longer times required to achieve new steady state conditions in dilutions of SW. The FW crab and the FW crayfish (unpublished research on *Procambarus clarki*) appear unable to regulate hypo-osmotically. Ionic flux data show uphill solute transport with saturation kinetics. Urine of *Pseudothelphusa* is blood isosmotic and urinary output low, but higher than that estimated for *Potamon*. However, inulin clearance appreciably exceeds urinary output, presenting problems of interpretation. Eyestalkless animals show increased permeability to water.

The significance of these findings is discussed.

106

JAMES ROBERT NOLFI, II, University of California, Bodega Marine Laboratory.

Uric acid transport and ionic regulation in the renal vesicle of the tunicate, *Molgula manhattensis*. (Introduced by Ralph I. Smith)

The ascidian, *Molgula manhattensis*, is known to inhabit water of varying salinity. In San Francisco Bay, where this species has been introduced, *Molgula* thrives and reproduces in water with a salinity as low as 50% sea water. The possible osmoregulatory role for the renal vesicle in this species suggested by Das was examined further. When groups of animals were acclimated to water of various salinities, the chloride, sodium and potassium levels of the blood were essentially equal to those of the medium. Freezing point determinations on blood, renal vesicle fluid and medium at various salinities indicated that these three fluids were isosmotic. The potassium level of the renal vesicle fluid was essentially isotonic with the blood and medium, but sodium and chloride levels were always about half those of the blood or medium. Soluble urate in the renal vesicle fluid, determined enzymatically on centrifuged renal vesicle fluid from individual animals appears to make up the ionic deficit. The role of the ionic regulation of sodium, chloride, and perhaps other ions, is probably related to transport of urate from the blood into the vesicle where concretions are formed. The fine structure of cells which form the wall of the renal vesicle are compared with other known uric acid transporting and ionic regulating structures, and the role of ionic regulation as related to uric acid transport is discussed. (USPHS Predoctoral Fellowship 5-F1-GM-30,333-02.)

107

VAUGHAN H. SHOEMAKER, University of California, Riverside.

Renal function in the mourning dove.

Renal function of mourning doves *(Zenaidura macroura)* was studied in terms of glomerular filtration and net transtubular movements of water and solutes. Birds were restrained in the perching position and given water or a salt solution via stomach tube. Indwelling cannulae were used for venous infusion of inulin and p-amino hippurate (PAH) and for sampling arterial blood. Urine was collected by a cup-shaped cloacal cannula positioned beneath the ureteral openings.

The rate of fluid administration was varied to obtain a range of urine flow rates from 4 to 0.1 ml/hr. Glomerular filtration rate (GFR), measured as inulin clearance, was constant at about 16 ml/hr when rates of urine production exceeded 1 ml/hr. At lower rates of urine flow, urine production was proportional to GFR, while the fraction of the filtered water resorbed remained at about 95-97%. Renal plasma flow (RPF), measured as PAH clearance, varied with urine production in a manner similar to GFR, and the filtration fraction (GFR/RPF) was constant at about 13%. Uric acid clearance approximated the RPF.

When given tapwater, doves excreted less than 1% of the filtered Na and Cl and about 5% of the filtered K. When 150 ml mM NaCl was given, urinary Na and Cl levels gradually increased to ca. 150 meq/l as the result of diminished tubular resorption of these ions. KCl administration rapidly enhanced K excretion, and net tubular secretion of this ion was evident. There was no significant enhancement of Na excretion by K administration and vice versa. (Supported by NSF Grant GB 5804.)

108

DONALD M. MILLER, BERNARD C. ABBOTT, and JOHN D. ANDERSON, Southern Illinois University and University of Illinois, Urbana.

Potentials recorded from a slime mold plasmodium and an evaluation of the Kamiya Chamber Technique.

Potentials were externally recorded from oriented plasmodia of the myxomycete, *Physarum polycephalum*. Fast potentials from one to ten mv negative originate at the advancing front of a plasmodium or at a place of injury. Slow potentials of one

to five mv are recorded from any site on the plasmodium. Slow potentials are greater in amplitude when recorded from areas nearer the advancing front. Slow potentials from different areas of the same plasmodium are not in phase with each other. It is concluded that the bioelectrical potential measurement of a Kamiya chamber represents the differential recording of the potentials from two oppositely advancing plasmodia. Possible explanations for the origin of the externally recorded fast and slow potentials and their relation to the phenomenon of motility are discussed with reference to the results presented. (Supported by fellowship NIH-F1-GM23,041-01 from U.S.P.H.S.)

109

R. G. STROSS, Department of Biology, State University of New York, Albany.

Photoperiodism in arctic *Daphnia*.

The environments in which embryonic diapause is induced in *Daphnia middendorffiana* Fischer were studied in controlled experiments and in shallow tundra pools near Point Barrow, Alaska (71°N). Young from overwintering (ephippial) embryos, and from non-diapausing embryos, were cultured at fixed densities and photoperiods, and maintained for a period of 30 days at 12° or 20°C.

The shift to embryonic diapause is facultative and may be prevented at long daylengths (including constant light) and low density. The critical photoperiod at normal (1350 lux) and ¼-normal intensity was approximately L:D 22:2 at the lowest density (1/20 ml). In adults from non-diapausing embryos, non-permissively long daylengths were partially overridden at densities of 3 and 5/20 ml, and completely, at a density of 10/20 ml. The density effect was reversible at long daylengths following dilution. First generation adults produced one and usually two broods of non-diapausing embryos before responding to induction.

Photoperiod control is overridden in tundra pools near Barrow. The first one or two broods were non-diapausing. They were followed in mid-July by a 95%, or more, shift to the production of diapausing embryos, and at a time when the sun was continuously above the horizon. The shift is reversible at long daylengths in the laboratory.

The long critical photoperiod (L:D 22:2) contrasts with that of 13:11 measured in a population from 45°N (Stross and Hill, 1965) Science 150:1462-1464). Presumably, it may function in arctic lakes where density does not override photoperiod. The presence of photoperiod control in populations undergoing summer diapause suggests that all populations of *Daphnia* entering embryonic diapause may be controllable. (Project conducted at ARL Barrow with support from the Arctic Institute under ONR subcontr. 399.)

110

ROBERT E. HILLMAN and PAUL F. NACE Battelle Memorial Institute.

Histochemical studies of barnacle cyprid adhesive secretion.

Laboratory-reared cyprids of *Balanus eburneus* were induced to settle on and attach to balsa wood strips. The attached cyprids were fixed in Bouin's solution for simultaneous decalcification and subsequently imbedded in paraffin for sectioning and staining. Sections cut at 5$\mu$ were stained with hematoxylin and eosin, Mallory's triple stain, periodic acid/Schiffalcian blue sequence, aldehyde fuchsin-alcian blue, Millon reaction, pedioric acid/Schiff with diastase controls, Gomori's aldehehyde fuchsin, Sudan black B, azo blue-Congo red, the Sakaguchi reaction and toluidine blue 0. The results of these reactions indicate that the initial adhesive is secreted by nerve cells found in the cyprid's antennae. This material, however, may be basically different from most neurosecretory material in that it does not react with the usual reagents used in neurosecretory histochemistry. The difference may lie in the fact that the cyprid's nervous tissue undergoes further differentiation following metamorphosis of the settled cyprid to the young adult stage.

111

CLAUDIA F. BAILEY, Oberlin College.

Phospholipids of the hepatopancreas and brain of the crayfish. (Introduced by Jane Oppenheimer).

Phospholipid components of crayfish hepatopancreas and brain were identified chromatographically and localized histochemically. Tissue sections subjected to various lipid stains, e. g. Sudan Black B, Baker's Acid Hematin, Sudan II, *et al.*, were examined and compared to localize specific lipid components. Phospholipids could be demonstrated in the hepatopancreas, especially in the basement membrane, brush border and areas of mitochondrial and/or ribosomal concentration. The major phospholipids, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol and sphingomyelin were extracted and identified by thin-layer chromatography. The source of phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol is probably the membrane systems, especially mitochondria. Previous studies indicate large numbers of mitochondria in the cells of the hepatopancreas where phospholipids are found in structural association with protein. The phospholipids undoubtedly affect permeability and may participate in active transport. The presence of relatively high concentrations of phosphatidyl choline may be indicative of the role of the hepatopancreas in fat metabolism.

Phospholipids were also indentified in the brain. Sudan II located traces of myelin in fibers of diffuse neuropile. Results from Baker's Acid Hematin Test and the Smith-Dietrich Procedure suggest that phosphatidyl choline, sphingomyelin and possibly cerebroside were present in these same fibers. Phosphatidyl choline could also be demonstrated in stratified neuropile although no evidence of myelin was found. The presence of these phospholipids, indicated by histochemical reactions, was confirmed by thin-layer chromatography. Phosphatidyl choline and sphingomyelin, both components of myelin, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol were identified.

112

TOM ROOP and MICHAEL J. GREENBERG, Florida State University.

Acetylcholinesterase activity in *Crassostrea virginica* and *Mercenaria mercenaria*.

Isolated venerid hearts are about 10,000 times more sensitive to acetylcholine (ACh) depression than are ostreid hearts. However, eserine augmentation of the ACh effect in venerids is about 30 fold smaller than the potentiation in ostreids.

If transverse or longitudinal gill strips from *Crassostrea virginica* or *Mercenaria mercenaria* are suspended in a perfusion bath, the isotonic contractions of the branchial musculature can be recorded. ACh increases the rhythmical activity of oyster gills. Threshold is about $10^{-6}$M, but it drops to about $10^{-8}$ M in the presence of eserine ($10^{-6}$ g/ml). Eserine, alone, augments rhythmical activity. In contrast, the action of ACh on isolated *M. mercenaria* gills is scarcely affected by eserine.

Gills, hearts, and ganglia of *C. virginica* and *M. mercenaria* were stained for cholinesterase by a modification of Koelle's method. While oyster gill and heart stained strongly, little enzyme activity could be demonstrated in the homologous organs of the clam. But the ganglia of the two species reacted equally, and strongly.

The cholinesterase activity of these tissues was measured by spectrophotometry of SH groups released by acetylthiocholine hydrolysis. The soluble enzyme activity recovered from oyster heart and gill was 6-10 $\mu$M hydrolyzed/min/gm dry weight—at least 100 times greater than that of *Mercenaria* organs assayed with the same substrate concentration and method of enzyme preparation. Again, the ganglia of the two species had a similar high activity.

Differences in the cholinergic pharmacology of oysters and venerids are attributable to the comparatively high cholinestrase activity in oyster tissues. (Supported by the U.S.A.E.C. and NIH grant HE - 09283.)

113

ROBERT E. LOVELAND and DAVID S. K. CHU, Rutgers University.

Relating oxygen consumption to pumping rates in *Mercenaria mercenaria*. (Introduced by Robert E. Loveland)

Oxygen consumption measurements of the bivalve *Mercenaria mercenaria* were made at S‰=22‰ and T°=25°C. Oxygen uptake was measured with a volumetric differential respirometer, modified to internally circulate gas and monitor oxygen tension simultaneously. $Q_{O_2}$ values related to total wet weight according to the equation:

$$q = 0.207\ X^{-0.526} \quad (1)$$

where $q = Q_{O_2}$ in mls $O_2$/gm total wt/hr; X = gm total wt.

It was shown that $Q_{O_2}$ related to pumping rates determined by Coughlan and Ansell (J. du Conseil *29*:205) according to the equation:

$$\log Y = -21.359\ (q) + 1.145 \quad (2)$$

where Y = pumping rate in L/hr; $q = Q_{O_2}$.

Assuming $Q_{O_2}$ and pumping rate to be related, the following equation predicts the relationship between rate of oxygen consumption ($\Delta$p, in mls $O_2$), oxygen tension (p/cc), time (t in hours) and total wet weight in grams X):

$$Y = \frac{\Delta p}{(p/cc)\,(t)} \cdot X^n \quad (3)$$

For constant salinity and temperature, equation (3) converts to:

$$Y = k_c \cdot Q_{O_2} \cdot X^{n+1} = (0.01414)X^{n+0.474} \quad (4)$$

The relationship of oxygen demand to pumping rate is discussed in light of these equations.

114

SONIA RUDE, Harvard University.

Monoamines in Leech Ganglia (Introduced by John H. Welsh)

Using the formaldehyde condensation technique for the histochemical localization of monoamines, seven monoamine-containing neuron perikarya are found in a typical ganglion of *Hirudo medicinalis*. These cell bodies have an intense fluorescence which varies in color from bright yellow to orange-yellow or greenish-yellow.

The processes of the monoamine-containing cell bodies are normally only weakly fluorescent. However, various techniques can be employed to increase their monoamine content and render them more strongly fluorescent. Using these techniques the pathway through the ganglion of each of the seven monoamine-containing neurons has been traced.

The processes of the seven fluorescent neurons in each ganglion are large, smooth and yellowish. In addition to them, a ganglion receives two large, smooth, bright green fluorescent nerve processes from the periphery. The large, smooth, green and yellowish processes make up only a small part of an intensely fluorescent neuropile. Most of the fluorescence in the neuropile can be attributed to a dense feltwork of very fine green and yellow fluorescent fibers.

It is known that the ventral nerve cord of the leech contains large quantities of serotonin (Welsh and Moorhead, J. Neurochem., 6: 146-169). The finding of bright green as well as yellow fluorescence in leech ganglia suggests that not only serotonin, but also catecholamines are present. (Supported by grant 1-F2-NB-34, 824-01 from the U.S.P.H.S.).

115

MORRIS H. BASLOW, GEORGE W. READ and KENNETH DORMER, University of Hawaii

Hypotensive and paralytic agents from the sponge *Toxadocia violacea*.

Aqueous extracts of the littoral sponge *Toxadocia violacea* made from fresh frozen material or after heat drying at 100°C for 15 hours have been found to have hypotensive activity in the albino rat and paralytic activity in the carp *Cyprinus carpio*. Pentobarbital-anesthetized rats were monitored for changes in EKG, EEG, heart rate, arterial blood pressure, respiration and smooth muscle activity with a Grass model 7 polygraph. Intravenous administration of water-soluble material at a dose of 4-8 mg/kg causes an 80% reduction in systolic and diastolic blood pressures after 2-3 minutes. A single dose lowers blood pressure for 30-60 minutes and except for a slight lowering of heart rate and a brief attenuation of the EKG Q wave, all functions appear normal. Activity of the sponge extract in spinal-sectioned rats suggests that the mode of action is peripheral rather than central. Intraperitoneal injection causes a 5-10 minute cessation of gut activity and no hypotension. Twenty gram carp injected intraperitoneally with 2-4 mg of sponge extract

exhibit a syndrome of tonic rigidity and anesthesia. After 2-3 minutes there is difficulty with coordinated pectoral fin movements and at 5-6 minutes the pectoral fins stand at 90° to the body and the jaws are maintained in an open position. At this time, the fish sinks to the bottom, does not respond to strong tactile stimulation and exhibits a feeble opercular beat. At about 15 minutes jaw function returns followed shortly by pectoral fin movements. Within 30 minutes there is response to visual and tactile stimulation and a resumption of schooling behavior. The active agents from *T. violacea* are heat stable and non-dialyzable. Gel filtration using Sephadex G-50 indicates a molecular weight of around 9000 for the hypotensive component and it appears that these agents are unstable in aqueous solutions. The chemical nature and function of these substances in the sponge are unknown.

116

VICTOR A. CVANCARA, Unviersity of South Dakota.

Liver uricase in some Missouri River fresh water fishes. (Introduced by Fred Duerr)

Liver acetone powders prepared from 14 species of Missouri River fishes were assayed for uricase activity by a method similar to that of Brown and coworkers (Science, 153, 1659, 1966). Acetone powders were rehomogenized in 0.2M trishydroxyaminomethane (Tris) at pH 9.0, and centrifuged at 13,000 × g for 15 minutes at 2°C. Aliquots of the supernatant solution were incubated in rectangular, quartz cuvets at 22-38°C, with 0.018 to 0.18 μmoles of re-crystallized uric acid in the presence of 0.2M Tris buffer, pH 9.0-10.8. The final volume was 2.8-3.0 ml. The decrease in absorbance at 293 mu as a function of time was measured in a Beckman DK-2 recording spectrophotometer. Optimum substrate and homogenate concentrations were determined under the optimum pH assay conditions for each species. Under the conditions used, the pH optimum was found to be 9.5 to 10.4 at 38°C. depending on the species. The rate of the reaction for uricase using liver homogenates was linear in nearly all cases for up to 9 minutes. Specific activity based on bovine crystalline albumin was calculated for all species. The optimum temperature, Michaelis constant and effects of some inhibitors were determined in selected species.

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

Oxidoreductases in trypanosomes.

Respiratory rates and specific activities, intracellular localization and isozymes of selected oxidoreductases were compared for *Trypanosoma conorhini* and the kinetoplastic and dyskinetoplastic forms of *T. equiperdum*. 1) The respiratory rate of *T. equiperdum* was 6 times higher than that of *T. conorhini*. There appeared to be a slightly higher respiration rate in the kinetoplastic as compared to the dyskinetoplastic form of *T. equiperdum*. The respiration of *T. equiperdum* was unaffected when measured in the presence of cyanide. 2) Succinate:ferricyanide reductase and succinate oxidase were not detected in *T. equiperdum*. The succinate oxidase of *T. conorhini* was sensitive to cyanide. The succinate: ferricyanide reductase of *T. conorhini* exhibited 40% the activity of mouse liver. Differential centrifugation of the homogenates showed the activity to be sedimentable in mitochondrial fractions. 3) The glycerophosphate oxidase activity of *T. equiperdum* was 6 times greater than that found in *T. conorhini*. The rate of the oxidase in *T. equiperdum* was not inhibited by cyanide. 4) Malate dehydrogenase activity in *T. conorhini* was 25 times greater than the activity in *T. equiperdum*. The enzyme in *T. conorhini* was composed of 2 isozymes of which one was found to be associated with the mitochondrial fraction. The malate dehydrogenase of *T. equiperdum* was composed of 4 isozymes. 5) The lactate dehydrogenase activity in *T. conorhini* was found to increase with the age of the culture. The enzyme was composed of 5 isozymes that differed from mouse heart and muscle electrophoretic migration patterns. The lactate dehydrogenase of *T. equiperdum* was composed of 2 isozymes and exhibited 25% of the activity found in *T. conorhini* from 5 day old cultures.

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ESTHER M. LEISE, IRVING GRAY and MARTHA K. WARD, Georgetown University and U. S. Army Medical Unit.

Lactic acid dehydrogenase activity in rabbits following pneumococcal infection. (Introduced by George B. Chapman)

Lactic acid dehydrogenase (LDH) activity has been studied in rabbits, each infected with 800 *Diplococcus pneumoniae* cells. Prior to infection control values were obtained. Following the administration of the organisms, samples were obtained at four, twenty-four and forty-eight hours. LDH activity was estimated quantitatively and the isozymes characterized by acrylamide gel electrophoresis. Leucocytes were separated after suspension of the blood in 3% Dextran in normal saline containing 0.06% EDTA. LDH activity of leucocyte homogenates was followed. An increase in leucocyte LDH was observed as early as four hours after infection in some rabbits. Others showed an increase twenty-four hours after infection. The increase persisted until death of the animal. From the zymogram, it appeared that no one particular molecular species of LDH was responsible for the increased activity. The distribution of the different isozymes remained about the same, with some slight tendency toward an increase in cathodal enzymes following infection. Enzyme changes in serum and erythrocytes were compared under the same conditions and the changes in their activity summarized and compared. (Supported by Research Contract No. DA-49-193-MD-2598, U.S.A., Medical Service Research and Development Command.)

119

FRANK P. CONTE, Department of Zoology, Oregon State University.

Characterization of ribosomes isolated from chinook salmon, (*Oncorhynchus tshawytscha*) gill filaments.

Polyribosomes are conventionally prepared from the cytoplasmic supernatant remaining after the sedimentation of nuclei and mitochondria. Recent investigations (Loeb, J. N., *et al.*, J. Biol. Chem. 242 (a) 2069-2074, 1967) have shown that substan-

tial numbers of ribosomes sediment in weak centrifugal fields with nuclei and mitochondrial particles. These ribosomes are bound to large pieces of endoplasmic reticulum and following deoxycholate (DOC) treatment are released as polysomal units providing that degradation by ribonucleases is inhibited. In the present study, characterization of ribosomal aggregates by ultracentrifugation in sucrose density gradients (8.5 to 34%) from different cell fractions of gill filaments was performed. Gills, dissected from juvenile salmon adapted either to fresh water or sea water, were ground to fine powder in a mortar and pestle under liquid nitrogen. A medium which contained bentonite (2 mg/ml), a nuclease inhibitor; 0.01 M Tricine buffer, pH 8.0; 0.05 M KCl; and varying concentrations of Mg $(Ac)_2$ (0.0001 M to 0.01 M) was used to extract (w/v, 1:1) the cytoplasmic constituents. The nuclear fraction (I) and the post-mitochondrial supernatant fraction (III) gave sedimentation patterns similar to each other in the absence of 1.3% DOC treatment. However, no free ribosomes were found in the mitochondrial fraction (II). Following DOC treatment, all fractions gave similar sedimentation patterns. Each fraction yielded four distinct peaks, which had $S_{20;w}$ values of 80S, 120S, 156S and 176S respectively. (Supported by Grant AT (45-1)-2013 from the AEC.)

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H. SCHUEL, N. J. UNAKAR and R. SCHUEL, Oakland University, Rochester, Michigan.

Purification of lysosomes by combined rate—and isopycnic—zonal centrifugation.

Rat liver lysosomes can be obtained free of cross contamination during a single sedimentation velocity run in the A-XII zonal centrifuge (Schuel and Schuel, J. Gen. Physiol. *50*:1085, 1967; Unakar, et al., J. Cell Biol., *in press*). However the lysosomal fractions were still significantly contaminated by microsomal vesicles and glycogen particles. The lysosomal fractions initially obtained by rate-zonal centrifugation were further purified by subsequent isopycnic banding in cesium choride density gradients (Anderson, et al., Natl. Cancer Inst. Monogr. *21*:253, 1966). Acid nitrophenyl phosphatase was employed as the biochemical marker for the lysosomes. The acid phosphatase activity was banded sharply in the upper half of the cesium chloride density gradient, and was displaced slightly below the peaks in ultraviolet absorbance at 260 and 280 m$\mu$. Electron microscope observations showed that the distribution of lysosomes coincided with the distribution of acid phosphatase activity. This region of the gradient showed a significant reduction in contamination by microsomes and glycogen particles. These contaminants were found primarily in the heavy end of the cesium chloride density gradient. (Supported in part by N.S.F. grants #GB-3388 and GB-6307 and Oakland University faculty research grants.)

121

PAUL T. MEDICI, SAMUEL D. HUANG, St. John's University.

Uptake of tritiated uridine in tissues of kinetin treated mice.

The effect of a plant hormone, kinetin, was investigated in CF#1 strain mice grafted with C3H/BA mammary tumors. Kinetics of $C^{14}$-labelled kinetin incorporation into trichloroacetic acid insoluble fractions of whole cells from various organs was studied. The most rapid incorporation of $C^{14}$ kinetin (rate: 0.5 m$\mu$mole kinetin/mg of protein) was observed in liver, brain, and tumor tissues and reached a maximum level by twelve hours post-injection. Kidney and spleen showed a slower and more gradual uptake of kinetin (rate: 0.2 m$\mu$mole kinetin/mg of protein), the rise continuing for over 36 hours after the initial injection. Over 95% of the labelled kinetin appeared in the 105,000 $\times$ *g* supernatant fraction for each cell type studied.

The effect of a single intraperitoneal dose of kinetin (0.2 mole/animal) on the incorporation of $H^3$-uridine into trichloroacetic acid insoluble fractions was studied after a 15-minute pulse label. Marked inhibition of liver and spleen uptake of $H^3$-uridine (approximately 70%) was observed 24 hours after kinetin treatment. Similar inhibitory effects on $H^3$-uridine incorporation were noted after prolonged treatment with kinetin (10 daily injections: 0.1 m$\mu$mole/animal). Liver and spleen uptake of the tritium label corresponded to the value observed after a single kinetin injection while tumor and brain tissue exhibited little or no inhibition.

Experiments are now in progress to determine the mechanism of kinetin inhibition of uridine incorporation into trichloroacetic acid insoluble fractions of animal cells and its possible influence on nucleic acid metabolism. (Supported by Queens Hospital Center, Department of Radiation Medicine and Grant GZ 552: NFS Graduate Traineeship.)

122

TOD S. JOHNSON, Department of General Science, Oregon State University.

The effect of temperature and X-irradiation on the uptake and loss of $^3$H-thymidine in the intestine of the juvenile silver salmon *(Oncorhynchus kisutch)*.

The intestinal epithelial cell population of animals undergoes continuous renewal. This cell system is useful for the interpretation of environmental changes, which may significantly influence cellular dynamics. Data on cellular dynamics and DNA-synthesis in poikilotherms are very limited. The present study on juvenile silver salmon *(Oncorhynchus kisutch)* is concerned with the effects of temperature and X-irradiation on DNA synthesis in the intestinal tissue.

The intestinal epithelial cells of the silver salmon proliferate in basal regions of the intestinal folds as demonstrated by colchicine arrested mitotic figures and initial incorporation of DNA precursor ($^3$H-TdR) using radioautography. Cell migration occurs by transposition of the cells up the folds, with ultimate sloughing into the lumen.

Intestinal epithelial cell renewal times as evident from radioautography and $^3$H-TdR turnover studies is temperature dependent, with estimates of 13-15 days, 23-25 days, and 33-36 days for temperatures of 18°, 10° and 5°C, respectively. The incorporation of $^3$H-TdR into the intestine following X-irradiation showed a biphasic response which may be due to "competitive inhibition" from the endogenous thymidine pool. (Supported by an AEC

Richland Graduate Fellowship under Contract No. AT 45-1-1779.)

123

STANLEY N. OYAMA and FRED I. KAMEMOTO, University of Hawaii.

A study of ovarian development of a portunid crab, *Thalamita crenata* Latreille in organ culture.

By using the floating lens paper technique and a chemically defined medium, a method of organ culture for the study of ovarian development in a portunid crab, *Thalamita crenata* Latreille, was developed. The culture medium consisted of a crab saline formulated by Pantin for *Carcinus* and Medium 199 (Baltimore Biological Laboratory), a commercial nutrient without serum.

Ovarian tissues cultured in this medium were viable for at least seven days. The cultures were incubated in a constant temperature chamber equipped with a day-night light regulator and were maintained at 25°-26°C. A supply of oxygen-carbon dioxide mixture was found to be unnecessary for these cultures. The medium was changed on the third and fifth days of the incubation period and kept at a pH of 7.4 to 7.6.

Histological sections of the ovarian cultures demonstrated maintenance of the ovarian tissue and proliferation of oogonia in the germinative zone as indicated by the presence of meiotic figures. Maintenance of the developing oocytes was observed and, in some instances, there were indications that these oocytes moved to the periphery of the ovary into spaces created by ovulated ova from the previous reproductive cycle. Further *in vitro* studies to determine the role of neurosecretions in the development of the ovary are now in progress. (Supported by grant GB-673 from the N.S.F.)

124

LESTER Y. ICHINOSE, University of Hawaii.

Directional sensitivity of the posterior tentacle of the giant African land snail, *Achatina fulica* Bowdich. (Introduced by Dr. P. van Weel)

In addition to the tentacular nerve-tentacular ganglion sensory channel, the posterior tentacle of *Achatina fulica* Bowdich has a set of 4-6 nerves originating from the ventro-lateral epithelium of the tip. They extend centrally between the tentacle retractor muscle and the dermo--muscular wall of the tentacle to form a complex nerve net at the base of the tentacle. The net then concentrates into two nerve bundles, the left and right peritentacular nerves, that terminate in the posterior portion of the ipsilateral procerebrum.

The tip of the amputated tentacle was isolated in an olfactory chamber provided with inflow and outflow ports. The tip was stimulated with olfactory (vegetable juice odor) and tactile (deodorized air flow) stimuli directed at right angles to the long axis of the tentacle. Electrophysiological recordings of the activity of these nerves were obtained with the use of two external stainless steel electrodes 50 microns in diameter, and simultaneous recordings from two nerves were amplified with two single-ended Tektronix 122 preamplifiers.

When deodorized air impinged on the left side of the tentacle tip, the nerve serving that portion of the epithelium showed greater activity than the nerve serving the epithelium on the right side. Deodorized air impinging on the right side of the tip produced greater activity in the right nerve and less activity in the left. A greater difference in the activities of the left and right lateral nerves was exhibited when an olfactory stimulus impinged on the left side of the tentacle tip.

125

RICHARD F. OLIVO, Harvard University.

Mechanoreceptors of a bivalve mollusc. (Introduced by J. H. Welsh) (Motion picture)

Mechanoreceptors in the foot of the razor clam, *Ensis directus,* trigger the foot-withdrawal reflex and may also contribute to the digging reflex. It is possible to study single receptors by applying controlled mechanical stimuli to the foot through fine probes and recording from pedal nerves cut just before they reach the pedal ganglion.

The receptors are highly phasic and show no spontaneous activity. Their response to a long mechanical pulse is one (or rarely two) spikes at onset, and sometimes a spike at release. Using a stimulator which applies force at various controlled rates until a selected "plateau" force is reached, one can demonstrate a decrease in the force needed for a threshold response as the rise rate is made faster. Slow rise rates will not produce a spike even at very large plateau forces. Fast rates (rise times on the order of a few milliseconds) require forces equivalent to less than 0.5 gm.

The receptors will not follow a short-pulse mechanical stimulus at repetition rates greater than 20 or 30 pulses/second. Using a stimulator with a sensing probe (which monitors the force actually applied to the foot), one finds an increase in the force needed at threshold if the repetition rate exceeds several pulses/second, with a very great increase near the maximum rate.

Single receptive fields are typically a few millimeters in diameter. They overlap extensively, so that at any point on the surface of the foot several receptors can be stimulated. (Supported by NIH and NSF predoctoral fellowships.)

126

PHILLIP G. SOKOLOVE, Harvard University.

Some aspects of adaptations in crustacean stretch receptor. (Introduced by Ian Cooke)

Experiments were performed in the isolated slowly adapting stretch receptor of the crayfish *Orconectes virilis.* With the cell firing spontaneously at some convenient frequency (usually 8-10 sec) the cell was stimulated antidromically *via* a suction electrode and intracellular voltage recorded with a microelectrode penetrating the soma. De- and hyperpolarizing current could also be passed through the electrode by means of a Wheatstone bridge.

1) Trains of antidromic impulses at 100/sec containing various numbers of spikes caused a post-train frequency decrease approximately proportional to train duration. This was followed by a slow, exponential return to the spontaneous rate.

2) Single antidromic (A) impulses delivered at various times after an orthodromic (O) impulse resulted in a slight increase in the interval to the next O spike. This increase was 13% greater than the spontaneous interval when the O-A time was about 20 msec and decreased linearly to 0% as the

O-A time approached the spontaneous interval. Two A spikes separated by about 20 msec caused the $A_2$-O interval to increase by a discrete amount more, the maximum increase being about 27% over the spontaneous interval when the O-$A_1$ time was about 20 msec.

3) Short (10-20 msec) hyperpolarizing pulses of current delivered at various times after a spontaneous O impulse resulted in an increase in the O-O interval containing the pulse. The amount of increase depended linearly upon when in the interval the pulse was given. A pulse given late in the interval had a much larger effect than one given early in the interval. (Limited data indicate the reverse is true for short depolarizing pulses: the interval is decreased.)

A relatively simple "self inhibition" model is proposed to account for the main observations. (Supported by an NSF Graduate Fellowship.)

127

JEFFREY M. CAMHI, Harvard University.

Locust aerodynamic setae: sensory and interneuron responses. (Introduced by I. M. Cooke)

Desert locusts (*Schistocerca gregaria* Forsk.) possess facial aerodynamic sensory setae important in flight regulatory behavior. Fine suction electrodes can be used to record single unit activity of sensory and interneuron axons from teased out bundles.

The single sensory cell innervating a seta responds to wind with a tonic train of impulses. Different cells respond maximally to wind flowing in different directions, between 40° left and 40° right of the head-on direction. Three measurable factors produce this direction discrimination: variation with wind angle of the seta shaft's aerodynamic drag, force asymmetries of the socket cuticle, and the eccentric attachment of sensory dendrite to shaft base. The optimal direction has been determined for each seta on the face.

Electrical recordings made just posterior to the sensory axon terminations reveal that interneurons integrate the sensory information in at least four distinct ways. Two groups of interneurons give tonic responses and two are phasic. One tonic interneuron category responds to wind from only a very narrow range of directions, the other fom a very wide range. One phasic interneuron category responds selectively to changes of wind angle toward the head-on position, the final category responding only to wind accelerations.

The yaw stabilization reflex described by Weis-Fogh (Nature *163*, 873, 1949) probably results from activity of the first group of tonic interneurons, which discriminate the direction of the relative wind. The flight maintenance reflex (Weis-Fogh, 1949) appears to result from activity of the second tonic group, largely insensitive to wind direction. A newly discovered lift control reflex probably also involves this latter, non-directional, group of interneurons. (Supported by a predoctoral fellowship from NIH.)

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ROBERT C. TAYLOR, University of Georgia.

Interneuron responses to near field sound pulses in the crayfish *Procambarus spiculifer*.

Chronically implanted, teflon-coated, platinum iridium electrodes were used to detect the nervous activity in the circumesophageal connectives of unrestrained *Procambarus spiculifer*. With the use of a latency triangulation method and selective amputations the statocysts, antennules, and antennae were shown to be sensitive to near field sound pulses (water born vibrations) down to a calculated water displacement of 0.1 to 3 microns. Removal of the whole animal from the solid substrate by a number of means did not produce any detectable change in the sensitivity of the interneurons under study. The crayfish did not respond to the propagated pressure wave; tested according to the method of Enger (Enger, P. C. Comp. Physiol. Biochem. 18:859, 1966). A plot of the displacement transducer's displacement amplitude against its distance from the animal, for a constant response, gave a straight line curve on a log-log plot with a mean slope of 3.01 for seven trials, indicating near field reception (Harris, G. G. and W. A. van Bergeijk, J. Acoust. Soc. Amer. 34:1831, 1962).

The interneurons studied appear to be similar to C84 and C110; however, their sensitivity is also affected by optic stimulation. The probability of the interneurons responding to any one sound pulse is a function of stimulus strength (displacement) and frequency; 100% response, for the statocyst interneuron, occurring up to 8/second and 3 to 5 microns of water displacement, and 0% response somewhat below 0.1 micron and above 35/second. At a point where the cell responds to only 20 or 30% of the sound pulses and with only one spike per pulse, there is a grouping of the spikes in twos or threes with large intervening gaps. The production of doublets or triplets to any one sound pulse occurs when the displacement is larger than that needed to produce 100% response. (Supported by Grant GB-6234 from the N.S.F.)

129

GORDON A. WYSE, The University of Michigan and University of Massachusetts.

Properties of mechanoreceptors in the claw pads of *Limulus*.

The opposed grasping surfaces of the claws of *Limulus* walking legs are in the form of ridge-like pads of pliable cuticle. These pads contain both mechanoreceptors and chemoreceptors. The mechanoreceptors are either rapidly adapting (Type 1) or slowly adapting (Type 2); few intermediate units are found. Both Type 1 and Type 2 receptors have thresholds of 3 - 40 gm and 1.2 - 2.5 mm receptive fields along the pad. The response frequency of Type 1 units increases with increasing rate of change of stimulus force, while the number of spikes increases with increasing amplitude of force. Both frequency and duration of Type 2 unit responses increase with increasing amplitude of force, but with some movement sensitivity. Evidence is presented that the receptors involved are large multipolar cells lying under the pad and that they have large, rapidly conducting axons representing the fast sensory component of the large leg nerve. (Supported by a Graduate Fellowship from the NSF and by NIH Training Grant 5TIGM989).

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WARREN L. CLARK and LOUIS P. GRANATH, Worcester Polytechnic Institute.

A measure of the threshold sensitivity of *Gymnotus carapo* to electric fields.

The fish were trained to search for food following an electric stimulation. Pulses of one millisecond duration and 40 Hz repetition rate, which approximate the fish's natural output, were used for the stimulus. Once the fish were trained, the strength of the uniform field was progressively reduced to the minimum stimulus which would elicit the typical searching behavior prior to feeding. Valid responses were obtained which indicate a minimum threshold sensitivity of 0.04 microvolt/cm for two specimens and less than 4.0 microvolt/cm for for several specimens. The variation of sensitivity with pulse duration was also observed.

Another conditioned response experiment was devised as a check on the fish's sensitivity. The fish were trained to stop their electrical output when an electrical stimulus was applied. Preliminary results indicate a sensitivity comparable to that obtained in the preceding method. (Supported by USPHS Grant 1 RO1-NB06659-02.)

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LAWRENCE A. MINKOFF, WARREN L CLARK, and HOWARD G. SACHS, Worcester Polytechnic Institute and Clark University.

Interspike interval analysis of the discharge of a weakly electric Mormyrid fish. (Introduced by Louis P. Granath)

A method has been developed to allow the pulse-by-pulse analysis of the pulsing output of the weakly electric Mormyrid fish, *Gnathonemus petersi*. The output of the fish is picked up by stainless steel plate electrodes, amplified and recorded on a Sanborn F. M. data tape recorder at 60 inches per second. The tape is played back through the analyzer at 3¾ inches per second. Two pulse generators and an electronic counter form the basis of the interspike interval analyzer from which both a digital and an analog record are obtained.

Preliminary results with this method of analysis have shown that there appear to be two peaks in the interspike interval histogram under normal conditions. The short-period firing is almost exclusively made up of burst patterns, while the long-period firing is of nominally random variation. When the fish is placed in the stress situation of having to swim against a stream of water, the pulse distribution shifts to shorter interspike intervals as the flow rate increases. (Supported by USPHS Grant 1 RO1-NB06659-02)

132

IRVING GRAY and LINDA WOLFSON, Georgetown University.

Effect of hyperthermia on protein synthesis.

Chronic and/or acute febrile periods have long been known to cause increased nitrogen excretion including negative nitrogen balance. We have recently reported that induced hyperthermia in rabbits caused decreased protein synthesis when studied by *in vivo* or *in vitro* techniques. We have now studied the effect of increased temperature on cell-free protein synthesizing systems prepared from control and hyperthermia rabbits. Established methods for the preparation of the pH 5 enzymes, sRNA and ribosomes, taken from the literature, were used. The incorporation of 1-leucine-U-$C^{14}$ was followed at temperatures of 20° - 50° C. No differences were found in the activity curves of the two preparations. However, the decrease after the optimum of 35°C was extremely rapid. At 40°C the activity had decreased approximately 40%. It appears that the decreased synthesis found *in vivo* or in cells *in vitro* may be due to the decreased efficiency of the synthesizing system. (Supported by research contract DA-49-193-MD-2598, USA Medical Service Research and Development Command.)

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ROY F. BURLINGTON, U. S. Army Research Institute of Environmental Medicine, Natick, Massachusetts.

Distribution and activity of lactic dehydrogenase isozymes in tissues from active and hibernating ground squirrels.

The five electrophoretically distinct forms of lactic dehydrogenase (LDH) are the result of tetrameric combinations of two gene-regulated subunits, heart (H) type and muscle (M) type. The latter subunit has been associated with anaerobic function, whereas H subunits predominate in tissues which function aerobically. LDH isozyme synthesis has been shown to be influenced by changes in ambient oxygen tension (Dawson, *et al*, Science 143: 929-933, 1964) and Blatt, *et al* (Am. J. Physiol. 209: 785-789, 1965) found an increased proportion of H type isozyme in cardiac tissue form cold acclimatized rats. The ability of hibernating mammals to maintain physiological integrity, both in deep hypothermia and during exposure to severe hypoxia, suggests that these animals can adapt to environmental extremes at the cellular level. Accordingly, the electrophoretic distribution of LDH isozymes and the total LDH enzyme activity were determined in heart, brain, liver and skeletal muscle from normothermic and hibernating ground squirrels (*Citellus tridecemlineatus*).

During hibernation, the proportion of M type subunit increased significantly in cardiac tissue but the distribution of isozymes in skeletal muscle, brain and liver was similar to that in normothermic squirrels. No seasonal change in isozyme distribution was noted in heart tissue taken from normothermic squirrels during October, January and March. When measured at 32°C, total LDH enzyme activity was increased significantly in liver, brain and heart tissue from hibernating animals. These differences were not apparent in heart and brain at 15°C. LDH isozyme distribution and enzyme activity appear to be associated with adaptive metabolic changes during hibernation.

134

RICHARD S. CALDWELL, Duke University.

Effects of temperature acclimation on respiratory enzyme activity in goldfish.

The effect of temperature acclimation on the activity of electron transport enzymes in the goldfish (*Carassius auratus* L.) was studied. Cytochrome oxidase activity in brain, gill and muscle homogenates from 10°C acclimated fish was 50-100% higher than in preparations from 30°C acclimated fish when determined over the temperature range of 10-40°C. A similar compensation of this enzyme was observed in liver homogenates when measured

at 30°C and 40°C but was lacking at the lower assay temperatures.

Gill mitochondria from acclimated fish were isolated by differential centrifugation in 0.25 M sucrose containing 10 mM tris-HCl and 0.1 mM EDTA. In these preparations, the specific activity of cytochrome oxidase was 24-60% higher in the 10°C than in the 30°C mitochondria when measured at subsequent periods throughout the year. It appears, therefore, that the increase in activity of cytochrome oxidase in gill homogenates can be partially attributed to an increase in the specific activity of this enzyme in mithochondria. Similarly, the specific activity of succinate cytochrome *c* reductase was 23-39% higher and the specific activity of NADH-cytochrome *c* reductase was 63-82% higher in the 10°C than in the 30°C gill mitochondria. These data support the contention that cold acclimation of goldfish results in an overall increase in the capacity for electron transport in certain tissues. However, the concentrations of cytochromes $aa_3$, $b$, $c$, and $c_1$ in gill mitochondria did not increase at the lower acclimation temperature, sugesting that factors other than enzyme concentration are involved in the compensation of enzyme activity. (Supported by an NSF Predoctoral Traineeship in Biological Oceanography.)

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JOSEPH A. THOMAS and JOHN L. FREHN, Illinois State University.

Effects of cold-exposure and hibernation on selected tissues from golden hamsters.

Adult male hamsters, (*Mesocricetus auratus*, Waterhouse), were exposed to an environment of 5°C (two months and three to four months) in order to investigate the effects of cold-exposure and 2-5 days of hibernation on the following parameters: 1) body weights, 2) organ weights (testis, kidney), 3) histology and histochemistry (testis, kidney, epididymis and brown fat) and 4) mitochondrial succinate oxidation (liver). Controls were maintained at room temperature. Only the hibernating animals lost a significant amount of weight during exposure. Although the testis and epididymis regressed in both cold-exposed and hibernating animals, the testes of hibernators showed some signs of recrudescence relative to the cold-exposed non-hibernators, as indicated by increased spermatogenic activity, slightly larger seminiferous tubules, greater organ weights, and the presence of PAS positive, diastase sensitive material. The kidneys of hibernators and non-hibernators, which increased in weight, had PAS positive, diastase resistant material present, with the greatest amount in the hibernators. This material was absent in control kidneys. Brown fat in experimental animals had smaller and more numerous fat droplets per cell than the controls and nuclei which appeared to be more centrally located. Analysis of liver mitochondrial activity revealed the rate of succinate oxidation to be reduced in hibernators and increased in cold-exposed non-hibernators. These results indicate that, early in the hibernation cycle, both physiological and morphological changes occur which distinguish hibernating hamsters from cold-exposed hamsters that failed to hibernate. (Supported by grant GM-13358 from the U.S.P.H.S.)

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G. EDGAR FOLK, JR. MARY A. FOLK, and RICHARD C. SIMMONDS, University of Iowa and Arctic Aeromedical Laboratory.

Early stages in the onset of hibernation in the Arctic ground squirrel.

Some species of mammalian hibernators quickly become dormant for days or weeks when exposed to cold in the fall of the year (13-lined ground squirrel). A few species of mammalian hibernators show gradual test drops in the cold consisting of a lower body temperature every two days during sleep; the temperature drop is increased by about 5°C with each increment (California ground squirrel, Strumwasser). It may take six days to enter hibernation. Twenty-four Arctic ground squirrels were moved from a warm animal room (24 ± 1°C) into a cold room (7 ± 0.5°C). Under these circumstances as many as 50% of 13-lined ground squirrels will quickly and directly enter deep dormancy with a core temperature about one degree above ambient temperature. They do not show maintained intermediate body temperatures. At least six of the twenty-four Arctic ground squirrels did show temporary intermediate body temperatures (test drops) from which they returned to the normothermic state before entering deep dormancy later. These specimens took three days to attain deep dormancy. Eight other animals went into hibernation without showing test drops. The ten other squirrels took from six to forty days to become dormant, or did not hibernate at all. One squirrel showed test drops through two cycles into hibernation, while providing data from an implanted EKG capsule. These drops occurred every night (probably during sleep) or every other night. (Sponsored by the National Science Foundation.)

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PETER M. ANDREWS and J. A. PANUSKA, Georgetown University.

Fractional distribution of cardiac output to the brains of unanesthetized normothermic and hypothermic rats.

An "indicator fractionation technique" (Sparistein, L. A. and G. E. Hanusek, Am. J. Physiol., 193:272, 1958; Sapirstein, L. A., Gastroent 52:365, 1967) was used to determine the fractional distribution of cardiac output to the brains of unanesthetized rats. The right external jugular vein of female albino rats, 230-260 g, was chronically cannulated. Ten animals were cooled without restraint in 20°C air to a colonic temperature of 25°C; ten were studied at a colonic temperature of 37°C. Thirty seconds after external jugular intravenous injection of 5 $\mu$c of $I^{131}$-iodoantipyrine, the rats were decapitated. The brains were removed, weighed, and radioactivity measured in a well-type scintillation counter. A value of 1.18 ± 0.16 (SD)% of $I^{131}$-iodoantipyrine was found in the brains of normothermic rats and a value of 1.10 ± 0.22% in the brains of the hypothermic subjects. This compares closely with values obtained in a study on Pentobarbital sodium anesthetized rats (Physiologist, 10:110, 1967). Using previously reportec cardiac output values (Popovic, V. and K. Kent, Am. J. Physiol., 207:767, 1964; Bullard, R. W., Am. J. Physiol., 196:415, 1959), the following cerebral

blood flow values were calculated: normothermic rats, 0.77 ml/min, and hypothermic rats, 0.38 ml/min. Therefore, although little difference was found between the percentages of cardiac output to the brains of normothermic and hypothermic rats, there was a 50% reduction in cerebral blood flow in response to hypothermia. (Supported in part by DA-49-193-MD-2668 and U.S.P.H.S. ES-00087.)

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DAVID L. CLARK, Michigan State University.

The development of a response of the equilibrium system.

The development of nystagmus of vestibular origin was observed in *Peromyscus leucopus*. The mice received ten trials of constant angular acceleration every other day from day of birth until 35 days of age. Post rotatory eye nystagmus was measured after each trial using electronystagmographic techniques. Both longitudinal and cross-sectional groups of mice were studied, and habituation of the response was also recorded.

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MARY ELIZABETH JACOBS and JAMES EDWARD HEATH, University of Illinois.

Temperature regulation, heat production, and wing beat frequency of the sphinx moth, *Protoparce sexta*.

Spontaneously active moths warmed up prior to activity at the rate of 2.68°C/min (N=72; range 1.38-5.5°) independently of ambient temperatures from 15°C to 26°C. During active periods moths regulated body temperature between a minimum of 33.50°C (N=55; range 30.4-351) and a maximum of 36.88°C (N=131; 34.0-41.5). During flights of 5 or more minutes duration body temperatures were held to constant levels of 36.33°C (N=40; range 33.0-40.0°). These activities required a heat production at a 12°C gradient of 2.49 cal/min for a one gram moth. Wing beat frequency during "warmup" increased with a $Q_{10}$ of 1.38 (N=5; range 1.26-1.44). *Protoparce* regulates its body temperature by adjustments in the heat production and activity of its flight muscles.

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ROBERT R. CAPRANICA, Bell Telephone Laboratories, Incorporated, Murray Hill, New Jersey.

Behavioral and Physiological Correlates of Vocal Communication in Anurans. (Tape recording.)

Within most species of anurans, vocalization serves in species identification. Recent behavioral studies have shown that bullfrogs (*Rana catesbeiana*) and cricket frogs (*Acris crepitans*) will respond selectively to their own mating calls. Bullfrogs will respond only to the mating calls of adult male bullfrogs, but not to the calls of juvenile males. Cricket frogs show a systematic "geographical dialect" in their mating calls: female cricket frogs will respond to the mating calls of males of their own local population, but not to the calls of male cricket frogs of more distant populations. The basis for the high degree of call selectivity in these two species resides in the close relationship betwen the sensitivity of the auditory system and the signal characteristics of the male's call.

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Several genera representing many of the major anuran families are examined regarding their mechanisms of vocalization. Speculations are made concerning the origin and evolutionary divergence of these vocal mechanisms. (Supported by grant GB-4128 from the N.S.F.)

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GERALD A. GREENHOUSE and MAX HAMBURGH, City College of New York and Albert Einstein College of Medicine.

Analysis of embryogenesis of *Rana pipiens* eggs treated with trypan blue.

Attempts of identify the mechanism of the teratogenic action of trypan blue have been complicated by the observation that in mammals the dye does not enter the embryonic tissue. It is therefore of interest to test the teratogenicity of this substance on amphibian eggs where development takes place independent of intervention by the maternal organism.

Fertilized *Rana pipiens* eggs were treated with 0.5% solution of trypan blue in spring water. The stages at which the dye was added and the duration of exposure were varied.

The first signs of aberrant development appear at late stage 13 at which time the neural folds appear abnormal, and elongation of the body axis is inhibited. Subsequent development results in microcephalic larvae with foreshortened trunks and kinked or malproportioned tails. Such embryos never exhibit spontaneous muscular contractions although they may survive for 10-20 days and come to resemble stage 21 larvae.

Microscopic analysis revealed that 1) the notochord was either completely absent or present only in the anterior half of experimental embryos, 2) adjacent to the notochordal abnormalities the lateral mesoderm formed an unsegmented mass whose left and right sides fused beneath the neural tube, and 3) malformations of the forebrain included absence of the diencephalon, optic cups, and cranial ganglia.

Trypan blue was never found within the cells of the embryos.

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LOUIS T. STABLEFORD, Lafayette College.

concentrations also occurred at stage 24 with the 1.2 mg/ml group showing maximum amount of regenerative inhibition. The 1.3 mg/ml group demonstrated the highest toxicity (46%), retention of suckers, and pronounced abnormalities in muscle tissue such as initial tufts extending beyond amputation level, splaying of tissue into several bands, and loss of normal distributional pattern.

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DONALD J. MULCARE and KENYON S. TWEEDELL, The University of Notre Dame.

Renal tumors induced in *Rana palustris* by tumor implants or sub-cellular fractions from *Rana pipiens.*

Renal adenocarcinomas were induced, for the first time, in adult and larval *R. palustris.* The adults were implanted subcutaneously with 0.75 cc of inclusion body renal tumor from *R. pipiens* (Wisconsin). Unlike the Lucké renal adenocarcinoma in *R. pipiens,* the induced tumors had larger acini. The epithelial folds were not closely applied to the stroma, and the stroma itself was more diffuse.

One group of *R. palustris* embryos (Shumway stages 17-21) was inoculated with a suspension of cell components, containing mitochondria, derived from an inclusion body containing renal tumor of *R. pipiens* (Vermont) by cell fractionation (Tweedell, K. S., Am. Zool., 5:711-712, 1965; Cancer Res., in press, 1967). Other *R. palustris* embryos were inoculated with similar fractions derived from normal adult kidneys of *R. pipiens* (Wisconsin). Members of each inoculation group developed renal adenocarcinomas in the late larval period. Certain of the kidneys in both groups developed large vesicles at the approximate site of inoculation, the anterior left mesonephros. These fluid filled vesicles were composed of a single layer of basophilic columnar cells, with numerous mitoses, covered by a connective tissue sheath. This was suggestive of the epithelium of the Lucké renal adenocarcinoma. (Supported by U.S.P.H.S. Grant CA 07849.)

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J. A. SIMONS and E. J. BOELL, Yale University.

δ-Aminolevulinic acid synthetase activity in developing chick liver.

The condensation of glycine and succinyl-CoA to form δ-aminolevulinic acid is the rate-limiting step in the heme biosynthetic pathway (Granick and Urata, J. Biol. Chem., 1963, *238,* 821). The enzyme catalyzing this reaction, δ-aminolevulinic acid synthetase (ALA synthetase), is not present in detectable amounts in liver homogenates of the chick embryo from the ninth day of incubation until just before hatching. At the beginning of the twentieth day the enzyme is likewise not present. However, later in the day when the chick has made a hole in the shell, the enzyme surges to more than four times the adult level (12 $m\mu$ moles ALA/gram/hour). In the newly hatched chick the enzyme varies considerably, ranging from the pre-hatching high to the adult value which is reached a few days after hatching.

An injection of 40 mg 3,5-dicarbethoxy-1,4-dihydrocollidine into the egg results in a massive increase in the activity of the enzyme—up to forty times the adult level.

ALA synthetase can be induced by an oxygen deficient environment. When development occurs under an atmosphere of 14% oxygen, ALA synthetase is present in appreciable amounts on the eleventh and thirteenth days of incubation, but not on the fifteenth. An exposure to the lowered oxygen tension of twenty-four hours is sufficient to induce the enzyme. The continued presence of the inducing stimulus is required for the maintenance of enzyme activity. (Supported by USPHS Training Grant T1-HD-32 and NSF G 24169.)

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WILLIAM H. SOFER and HEINRICH URSPRUNG, The Johns Hopkins University.

Ontogeny of alcohol dehydrogenase in *Drosophila melanogaster.*

At various stages during the development of *Drosophila melanogaster* analyses were made for both alcohol dehydrogenase activity and protein. It was found that in the two strains investigated enzyme activity per unit protein (specific activity) of clarified homogenates was relatively low in eggs and first instar larvae, increased during the second and third instars, dropped during pupation, and rose again shortly before ecdysis. (Supported by N.I.H. Training Grant No. 1-F2-GM-2Y, 115-01 U.S.P.H.S. and N.S.F. GB-4451.)

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RICHARD B. CRAWFORD and M. L. BATCHELER, Trinity College and University of Pennsylvania.

Hexokinase in the developing embryo of *Ambystoma maculatum.* (Introduced by Malcolm Steinberg)

Previous attempts, utilizing the disappearance of glucose or the appearance of lactate in glycolytic systems, have failed to reveal the presence of hexokinase in embryos of *Ambystoma maculatum.* Fluorometric methods were applied in a direct analysis of this enzyme in a 100,000 × *g* supernatant of embryo homogenates. The assay employed the coupling of hexokinase to the glucose-6-phosphate dehydrogenase system, observing the increased fluorescence caused by the reduction of NADP. Due to the increased sensitivity of the method hexokinase activity was demonstrated throughout development. Enzyme levels were extremely low until middle fin bud stages were reached, when a gradual but marked increase was noted. This period of increased activity is coincident with development of embryonic motility and circulation, a time when utilization of free glucose might be anticipated. Specificity of the hexokinase was investigated. Mannose and galactose proved to be appropriate substrates whereas the utilization of fructose was minimal. In late stages the specificity for mannose decreased considerably, reflecting that found in several tissues of the adult. This suggests either two or more hexokinases or the changing specificity reflected in the synthetic patterns of an isoenzyme. Concomitant with these studies, using a similar assay method, glucose-6-phosphate dehydrogenase was found in high levels throughout the entire developmental sequence. These results provide a useful approach to the investigation of the control and synthesis of a specific protein ari-

sing to a high and functional level at a particular stage in embryonic development. (Supported by Grant HD-00519 from the U.S.P.H.S.)

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MARTHA Y. JANNERS and ROBERT L. SEARLS, University of Virginia.

Determination of cartilage in the embryonic chick limb bud.

It has been observed that a block of cells isolated from the chondrogenic area of the limb bud of a stage 22, 23, or 24 chick embryo, and implanted into the wing of an embryo of about the same stage, is incorporated into the host wing and differentiates in accordance with the host wing pattern. (Searls, J. Exp. Zool., in press). The experiments to be described were done to discover if the cells lose this ability to regulate when placed in an ectopic site in a wing bud, and, if so, the stage when the change occurs.

Embryos from stage 23 through stage 27 were labeled with tritiated thymidine. The limb chondrogenic areas were isolated and cut into blocks. These blocks were then implanted through the dorsal surface of the wing of embryos of the same stages. The host embryos were allowed to develop for 48 hours, the host limbs were fixed, and the position of the implanted blocks was determined by autoradiography.

When the donor limb was stage 23 or stage 24, the implanted cells formed cartilage only when they were in the cartilage forming area of the host limb. When the donor limb was stage 25 or older, the implanted cells formed cartilage independently of the cartilage forming area of the host limb. This was true whatever the stage of the host. However, even when the donor was stage 27, some of the cells in the implant were not surrounded by matrix.

The ability of a block of limb chondrogenic cells to regulate to an ectopic site in a host limb seems to be lost between stage 24 and stage 25, but the ability of individual cells to regulate seems to persist until at least stage 27. (Supported by NSF Grant GB 48446.)

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TRYGVE P. STEEN, St. Olaf College.

Stability of chondrocyte differentiation and contribution of muscle to cartilage during limb regeneration in the axolotl *(Siredon mexicanum)*.

In order to determine whether chondrocytes are stable as to type during limb regeneration and whether any cells from limb muscle become chondrocytes in the regenerate, labeled cartilage and muscle were transplanted into limbs which were then allowed to regenerate. Three kinds of label were used to identify transplanted cells after redifferentiation: $H^3$-thymidine, triploidy, or both.

The results of this investigation indicate that limb chondrocytes are intrinsically stable with respect to cell type, for grafts of both triploid and $H^3$-thymidine labeled chondrocytes gave rise to morphologically dedifferentiated blastema cells which almost exclusively differentiated into chondrocytes. A small percentage of non-cartilage cells appeared to be labeled. Although these cells could have arisen from metaplasia, it is more likely that all or most of them arose as spontaneous polyploids or as contaminants of the original grafts. Isotope dilution in $H^3$-thymidine labeled chondrocytes indicated that they had usually divided at least 5 times. Thus, the basis for the intrinsic stability of chondrocytes must be heritable.

Coracoid, scapula, and visceral arch cartilages also contributed chondrocytes to the cartilage of limb regenerates, and the available evidence indicates that cells released from these cartilages are similarly stable as to type. These cartilages did not dedifferentiate and participate in limb regeneration to the same extent as did limb cartilage.

Cells from limb muscle, labeled by the 3 methods used in this study, became chondrocytes during limb regeneration. However, since muscle is a mixture of cell types, the cellular origin of these chondrocytes is uncertain. (Supported by The Danforth Foundation, USPHS Training Grant T01 HD-00032-03, and NSF Grant GB-4265.)

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

The effect of bromodeoxyuridine on chondrocytes *in vitro*.

The thymidine analogue, bromodeoxyuridine (BUDR), induces morphological changes and inhibits synthesis of cartilage matrix in chondrocytes grown *in vitro*.

Six-day cultures of chondrocytes ($5\text{-}8 \times 10^2$ cells/60 mm petri dish), derived from a single chondrocyte, were exposed to 50 $\mu$g BUDR/ml for 9 days. Untreated clones contain closely packed smooth polygonal-shaped cells. Such clones eventually produce a cartilage matrix which stains metachromatically with toluidine blue. Within 24-48 hours after BUDR is introduced, the smooth cell surfaces become disrupted by increasing numbers of cell processes and clones appear more loosely organized. After 9 days, cells are widely spaced and extremely spread out in bizarre shapes. Although shape change is evident by 4 days, there is no significant difference in numbers of cells/clone in treated and untreated cultures. By 9 days BUDR treated clones have fewer cells and no metachromatic matrix is detected.

Microspectrophotometric measurements of BUDR treated cultures show some polyploid values for nuclei 3-4 times larger in diameter than normal. No polyploid values are found in controls. 2N and 4N values are discrete and coincide in controls and treated nuclei suggesting that BUDR does not cause obvious aneuploidy. Limits of the technique preclude detection of small DNA losses.

Preliminary studies indicate that BUDR inhibits chondroitin sulfate synthesis in intact vertebral cartilages. Since DNA synthesis is minimal in these cells, BUDR may act at a level other than gene transcription—possibly at the cell surface.

These studies agree with previous work which correlated specific morphology and intercellular associations of chondrocytes with their ability to synthesize cartilage matrix. (Supported by Grant 1 RO 1 GM 14429-01 from the U.S.P.H.S. and Grant GB-5047X from the N.S.F.)

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ROBERT D. CAHN, MARTHA B. CAHN, and MICHAEL SOLURSH, University of Washing-

ton, Seattle.

Heritability of differentiation *in vitro*: control of expression at the level of synthesis of DNA-like-RNA in cartilage and pigmented retina cell clones.

Chick embryo cartilage and pigmented retina (PR) cells may be grown as clones under conditions allowing expression and transmission of their specialized properties (epiphenotype) during rapid cell division. Alternatively, they may be cultured so they "dedifferentiate" or fail to express their differentiated epiphenotype. At what level of synthesis is "dedifferentiation" controlled?

Second generation differentiated sternal cartilage cell clones derived from picked differentiated clones (CMC) were pulse labeled with tritiated uridine. Labeled RNA was annealed to chick embryo DNA on membrane filters in the presence of increasing amounts of unlabeled competitor DNA from homologous cultures, non-CMC cells derived from CMC cells, embryonic sterna, or differentiated PR clones.

Conclusions: 1) The DNA-RNA annealing-competition procedure is sensitive enough to detect large differences between differentiated and "dedifferentiated" cells derived from the same clone and between different pure cell types. 2) Cold PR-RNA competes only 30% as well as CMC or cartilage RNA against labeled CMC-RNA. 3) Non-CMC-RNA competes only slightly better than PR-RNA in the same system.

Reciprocal experiments were carried out with pulse labeled non-CMC and PR-RNA. All the results support the hypothesis that lack of expression of epiphenotype is due partly to lack of synthesis of appropriate DNA-like-RNA species by cartilage and PR cells. Rate of DNA-like-RNA turnover and length of DNA-like-RNA lifetime are invoked to explain other aspects of the competition curves. (Supported by grants from American Heart Association, 65-G-102; NSF, GB 4649; and NINDB, NBO6761.)

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JOHN PAPACONSTANTINOU and JAMES W. CARR, Biology Division, Oak Ridge National Laboratory.

Crystallin synthesis by lens epithelial cells in tissue culture.

Calf lens epithelial cells maintained in tissue culture for 9 passages (approximately 90 days) maintain the ability to synthesize crystallin proteins, *i.e.*, the tissue specific structural proteins of the lens. $\alpha$-, $\beta$-, and $\gamma$-crystallins synthesized by cells in primary culture and by the same cells after the 9th subculture were compared to those synthesized by uncultured epithelial cells and fiber cells. To determine whether any differences occurred during growth in culture, these cells were incubated with a mixture of $^{14}$C-labeled amino acids, homogenized, and then mixed with $^{3}$H-labeled proteins from a fiber cell homogenate. The $\alpha$-, $\beta$-, and $\gamma$-crystallins of this mixed homogenate were fractionated on DEAE-cellulose by a stepwise elution system, and by a gradient elution system. A comparison was made between the $^{14}$C-labeled, $^{3}$H-labeled, and protein ($O.D_{280}$) profiles to determine whether the crystallins from cultured cells have the same chromatographic properties as do the proteins from the lens fiber cells. By this procedure it was found that all cultured cells (primary and 9th subculture) synthesize $\alpha$- and $\gamma$-crystallins having similar chromatographic properties to the crystallins synthesized in the fiber cells *in vivo*. The $\beta$-crystallins, on the other hand, change their chromatographic properties by the 9th subculture. Further resolution of the $\alpha$- and $\gamma$-crystallins from the 9th subcultures on DEAE-cellulose showed that there are no alterations in their chromatographic properties. These observations are being confirmed by precipitation of the purified $^{14}$C-labeled and $^{3}$H-labeled protein with specific antiserum. In addition, these cells are being analyzed by immunofluorescent techniques. (Sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

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C. H. ELLIS, JR. and R. J. WINTER, Amherst College.

Protein synthesis and skeletal spicule formation in the sea urchin larva.

Investigation into the chemical basis of spicule formation in *Arbacia punctulata* embryogenesis reveals significant incorporation of amino acids into precipitable protein closely associated with the calcareous spicules of the pluteus larval skeleton. Embryos were reared in artificial sea water to an early larval stage, at which time they were incubated for one hour in 2.5 to 5.0 $\mu$c/ml $^{14}$C-protein hydrolysate or $^{3}$H-proline. The larvae were then dissolved in 5 percent sodium hypochlorite to isolate spicules free of cellular and cytoplasmic contamination.

Liquid scintillation counting shows significant incorporation of these amino acids into spicules. Incorporated $^{3}$H-proline is nearly 100 percent recovered in a trichloroacetic acid precipitate. Carbon-14 labeled spicules dissolved in this fashion show, on the other hand, only 20 percent of the carbon-14 counts to be recoverable in precipitated protein. The lost radioactivity can be recovered from the acid supernatant as $Ca^{14}CO_3$.

The results indicate synthesis of a protein intimately associated with spicules at the time of their formation. Preliminary indications that embryos at this stage can convert proline to hydroxyproline support the possibility that they are synthesizing a collagen-like protein. The hypothesis that such a protein serves as a matrix for spicule calcification is discussed. (Supported by NSF Grant GB-5636.)

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HELEN PARK, ANNE ORTMEYER and DWIGHT BLANKENBAKER, National Institutes of Health and the George Washington University.

Mitotic activity in *Hydra pseudoligactis* during re-, generation of apical structures.

Recent reports on mitotic activity in adult hydras (Campbell, Science 148:1231; Shostak, Patel and Burnett, Devel. Biol. 12:434) and in developing buds (Clarkson and Wolpert, Nature 214:780) led us to make mitotic counts on specimens of *H. pseudoligactis* during regeneration of apical structures.

Asexual adults without buds, from cultures maintained in our laboratory, were used. The animals

blood flow values were calculated: normothermic rats, 0.77 ml/min, and hypothermic rats, 0.38 ml/min. Therefore, although little difference was found between the percentages of cardiac output to the brains of normothermic and hypothermic rats, there was a 50% reduction in cerebral blood flow in response to hypothermia. (Supported in part by DA-49-193-MD-2668 and U.S.P.H.S. ES-00087.)

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DAVID L. CLARK, Michigan State University.

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LOUIS T. STABLEFORD, Lafayette College.

The effect of early, limited exposure to 5-fluorouracil on amphibian development.

Blastulae, gastrulae, and neurulae of *Amblystoma punctatum* and *A. jeffersonianum* were exposed for limited periods of development to 100 μg of 5-fluorouracil plus 200 μg of thymidine per ml of 1/10 Holtfreter's solution then transferred to 1/10 Holtfreter's solution for further cultivation. Gastrulation and neurulation were unaffected by the analogue. Embryos exposed during neurulation (stages 13-20) showed an effect that began in early tailbud stage and reached a maximum at hatching (stage 40). Growth of head and tail was retarded; cells of the brain and spinal cord lost cohesion, rounded up, and migrated into the neurocoel and/or surrounding mesenchyme. There was a gradient from anterior to posterior of starting time and intensity of the dedifferentiation. The optic cups became masses of loose, dedifferentiated cells but development of the lenses, nasal structures, and ears was only slightly retarded. After stage 40 redifferentiation began so that by stage 46 (feeding) grey and white matter were forming in the reduced nervous system and small retarded eyes had formed.

It was concluded that messenger RNA synthesized during neurulation is associated with the response of differentiating neural cells to secondary inductors acting between early tailbud and hatching stages. Exposure to 5fluorouracil during neurulation caused formation of a faulty messenger RNA which produced the reported results. (Supported by a grant from the Faculty Research Committee, Lafayette College)

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

Uptake and incorporation of $H^3$-uridine and $C^{14}$-leucine in preimplantation mouse embryos: Effects of inhibitors of RNA and protein synthesis.

Large numbers of synchronously developing preimplantation mouse embryos were obtained through superovulation (Edwards and Gates, '59) and Brinster's mouse embryo culture technique ('63). At twelve hour intervals post-fertilization, the embryos were exposed to $H^3$-uridine (2-10 μc/ml) or $C^{14}$-leucine (0.3-0.6 μc/ml) for a three hour labeling period in the presence or absence of cycloheximide, actinomycin D, or puromycin. Synthesis of RNA and protein was determined by a modified Schmidt-Thannhauser ('45) extraction and liquid scintillation counting.

Both the incorporation and uptake of $H^3$-uridine (cpm/100 embryos) increase sharply after the 4-cell stage. However, the percent incorporation (incorporation/uptake × 100) increases only gradually from the late 2-cell stage (about 10%) to the morula (26-30%). In parallel experiments, the incorporation of $C^{14}$-leucine into protein increases from 46% at the late 2-cell stage to 48% at the morula.

Survival of preimplantation mouse embryos to the blastocyst stage is unaffected by a low concentration of cycloheximide (0.5 μg/ml). This concentration, however, produces about 73% inhibition (relative to controls) of new protein synthesis at the 2-cell stage and approximately 50% inhibition at the 4-cell, 8-cell, and morula stages. In addition, cycloheximide causes increasing inhibition of leucine uptake from the 2-cell stage to the morula (from 25% to 60%). There is no extensive effect of cycloheximide upon RNA synthesis until the morula stage (60% inhibition).

The effects of the inhibitors of RNA and protein synthesis and the permeability of the embryos to these drugs will be discussed. (Supported by NIH Research Grant HD-00827 and NIH Training Grant 1T1-HD-138.)

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MICHAEL D. GARRICK, JAMES P. CHARLTON, and SUSAN RUTLEDGE, University of Virginia.

Effects of inhibitors on hemoglobin synthesis by reticulocytes from human S/A heterozygotes. (Introduced by I. R. Konigsberg).

A promising cell for the study of differentiation is the reticulocyte which devotes more than 90% of its protein biosynthesis to making hemoglobin (Hb). Mutant genes for Hb are apparently translated less well than the wild type (Boyer, Hathaway, and Garrick. Cold Spr. Harbor Symp. Quant. Biol. 29: 333; 1964); for example, in most S/A heterozygotes the ratio Hb S/Hb A = 0.55 to 0.60.

Reticulocytes from S/A individuals have been incubated in the presence of labeled amino acids plus puromycin, cycloheximide, sodium fluoride, or no inhibitor. The incorporation into Hb's S and A and their respective beta chains has been determined after purification according to Huisman and Dozy (J. Chromat. 19, 160, 1965) and Clegg, Naughton, and Weatherall (J. Molec. Biol. 19-91, 1966). Cycloheximide and NaF inhibit incorporation into S and A to approximately equal degrees but incorporation into S is more puromycin sensitive than into A.

Hb S differs from A by the substitution of a valine for a glutamic acid at the sixth position of the beta chain, yet both Hb's contain valine at many other positions. It is tempting, therefore, to speculate that the valyl-T-RNA specified by the sixth position is rate limiting in Hb S synthesis. The implications of this interpretation with respect to occurrence of degenerate codons in mutations affecting development will be discussed further. The methodology of this study should prove of general value when investigating protein synthesis in differentiating systems. (Supported by Grant No. AM 10391 from the National Institutes of Health).

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ROBERT W. ATHERTON and GORDON M. RAMM, Maryland University.

Effects of low level centrifugation on erythrocytes, total hemoglobin, and electrophoretic hemoglobin in the chick embryo (Gordon M. Ramm).

Normal chick embryos (8, 9, 10, 13, 14, 15 days) were analyzed for blood changes following continual centrifugal stress (normal incubation, continual vibration, 2.1g, 3.3g, 5.8g, and 7.4g) for 24 or 72 hours. Determination of erythrocytes per $mm^3$ of whole blood, hemoglobin concentration in grams per 100 ml whole blood, and electrophoretic hemoglobin types were accomplished.

Erythrocytes were counted with a Model B Coulter Counter; hemoglobin was converted to acid hemin and read with a Beckman 340 colorimeter; electrophoresis was accomplished in a vertical E-C Apparatus cell using acrylamide. Statistics used were one-way analysis of variance, with differences between treatment groups detected by Duncan's multiple range test.

Erythrocytes increased during normal development. The youngest embryos with shortest duration of stress indicated a significant ($P = 0.02$) increase in erythocytes, while older embryos with longer duration of stress resulted in decreases of erythrocytes with increased gravity levels.

Hemoglobin concentration remained stable with the embryos studied. Hemoglobin increased significantly ($P = 0.10$) in younger embryos with shorter duration of stress. Decreases occurred in older embryos with longer duration of stress with increased gravity levels.

Electrophoresis of embryos (8 and 9 day) revealed 4 hemoglobin bands. Thirteen day and older embryos had 6 hemoglobin bands ($P = 0.0005$). Then day embryos were intermediate in hemoglobin numbers and younger experimental animals exhibited more bands than controls.

Relationships between metabolism and stress in a developmental system are discussed in relation to (1) genetical mechanisms; (2) homeostatic adaptation; and (3) regulative differentiation. (Supported by U. Maryland, Dept of Zoology.)

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CHRISTOPHER HAWTREY and ERWIN GOLDBERG, Northwestern University.

Lactate dehydrogenase isozymes in mouse testes following experimental cryptorchidism.

The appearance of a unique isozyme of lactate dehydrogenase (LDH) in mature testes of mice (*Mus musculus* has been associated with the onset of spermatogenesis (Goldberg and Hawtrey, 1967, J. Exp. Zool. *164*:309). Experimental unilateral cryptorchidism produced by surgically placing a testis in the abdomen results in a rapid decrease in testis weight and loss of germinal cells from the tubules. The LDH isozymes or the cryptorchid testis measured by polyacrylamide gel electrophoresis were compared to the isozymes of the contralateral testis. During the experimental period no change occurred in the isozyme pattern of the contralateral testis but marked alterations in the cryptorchid testis isozymes were observed. LDH-X, the sperm isozyme, declined from 41% to 16% of the total activity by the eleventh post-operative day. By day 24, no LDH-X activity was detectable. The other isozymes of LDH increased following surgery. LDH-5, which was not present in mature testes, was observable in the 28 day crytorchid testis. Total LDH activity in the cryptorchid testis decreased to 70% of the activity in the contralateral testis. The data suggest a repression of LDH-X subunit synthesis, presumably as a consequence of the elevated temperature of the abdomen as compared to the scrotum. (Supported by grant GB-4663 NSF and NIH Training Grant 5 T1 GM 903).

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MARIA DE ISSEKUTZ WOLSKY, Manhattanville College and ALEXANDER WOLSKY, Marymount College.

Inhibition of tail regeneration in larvae of the leopard frog, *Rana pipiens*, by RNA preparations from the adult liver of the same species.

It was previously reported (Wolsky, Monteil and Wolsky, Am. Zool., 6:614, 1966) that tail regeneration in adult *Triturus (Diemictylus)* salamanders was slightly inhibited by repeated injection of a 0.1 mg per ml RNA preparation (the concentration was given erroneously as 0.1 mg per cent) from liver tissue of the same species. Since then further experiments were made with a different method on another experimental material with the same result. The RNA preparation was made from freshly dissected liver of adult *Rana pipiens*, with a modified method of Kirby and tested on 12 day old larvae of the same species. The larvae were placed, in lots of 15, immediately after removal of half of their tails, in finger bowls containing 200 ml of a 0.12 mg per ml aqueous solution of the preparation. Controls were kept under similar conditions in water. After two days the larvae were killed in Bouin's fixative and the outlines of their regenerating tails drawn with camera lucida. In the controls the average length of the regenerates was $0.6 \pm 0.034$ mm but in the larvae kept in the RNA solution it was only$0.4 \pm 0.030$ mm Standard error of the difference $\pm 0.045$. Studies on the isozyme patterns of regenerates are in progress. The bearing of the results on earlier ones concerning the effect of actinomycin on regeneration (Wolsky and Nguyen Van Doi, Trans. N. Y. Acad. Sci., Ser II, 27:382, 1965) will be discussed. (Supported by the McNaughton Foundation.)

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DONALD J. PROCACCINI and CATHERINE M. DOYLE, Emmanuel College.

The effects of externally applied streptomycin on regeneration time and capacity in *Rana clamitans* larvae.

The tails of 5 day old *R. clamitans* larvae were amputated, the animals were divided into groups and placed in spring water solutions of 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, and 1.3 mg streptomycin sulfate (Squibb)/ml. For experimental purposes the temperature was kept at 15°C to retard but not significantly alter normal development (Needham, 1952).

The results indicate that streptomycin interferes with two principal and related phenomena: 1) the temporal progression from one developmental stage to the next; 2) the processes associated with a specific stage. Time intervals between successive stages in solutions with concentrations between 0.7 and 1.1 mg/ml were not significantly different from the control group. The transition from stage 20 to stage 21 was retarded 2 days and the transition from stage 24 to 25 was retarded 2.5 days in the 1.2 mg/ml and 1.3 mg/ml groups. Normal redifferentiation occurred in 0.7 ml/mg and 0.8 ml/mg groups after stage 22 and theamount of regenerative tissue was not significantly different from that of the control. Redifferentiation began at stage 24 in the 0.9, 1.0, and 1.1 mg/ml groups and produced significantly larger amounts of regenerative tissue over the control group. Redifferentiation in the two highest

concentrations also occurred at stage 24 with the 1.2 mg/ml group showing maximum amount of regenerative inhibition. The 1.3 mg/ml group demonstrated the highest toxicity (46%), retention of suckers, and pronounced abnormalities in muscle tissue such as initial tufts extending beyond amputation level, splaying of tissue into several bands, and loss of normal distributional pattern.

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DONALD J. MULCARE and KENYON S. TWEEDELL, The University of Notre Dame.

Renal tumors induced in *Rana palustris* by tumor implants or sub-cellular fractions from *Rana pipiens.*

Renal adenocarcinomas were induced, for the first time, in adult and larval *R. palustris.* The adults were implanted subcutaneously with 0.75 cc of inclusion body renal tumor from *R. pipiens* (Wisconsin). Unlike the Lucké renal adenocarcinoma in *R. pipiens,* the induced tumors had larger acini. The epithelial folds were not closely applied to the stroma, and the stroma itself was more diffuse.

One group of *R. palustris* embryos (Shumway stages 17-21) was inoculated with a suspension of cell components, containing mitochondria, derived from an inclusion body containing renal tumor of *R. pipiens* (Vermont) by cell fractionation (Tweedell, K. S., Am. Zool., 5:711-712, 1965; Cancer Res., in press, 1967). Other *R. palustris* embryos were inoculated with similar fractions derived from normal adult kidneys of *R. pipiens* (Wisconsin). Members of each inoculation group developed renal adenocarcinomas in the late larval period. Certain of the kidneys in both groups developed large vesicles at the approximate site of inoculation, the anterior left mesonephros. These fluid filled vesicles were composed of a single layer of basophilic columnar cells, with numerous mitoses, covered by a connective tissue sheath. This was suggestive of the epithelium of the Lucké renal adenocarcinoma. (Supported by U.S.P.H.S. Grant CA 07849.)

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J. A. SIMONS and E. J. BOELL, Yale University.

δ-Aminolevulinic acid synthetase activity in developing chick liver.

The condensation of glycine and succinyl-CoA to form δ-aminolevulinic acid is the rate-limiting step in the heme biosynthetic pathway (Granick and Urata, J. Biol. Chem., 1963, *238,* 821). The enzyme catalyzing this reaction, δ-aminolevulinic acid synthetase (ALA synthetase), is not present in detectable amounts in liver homogenates of the chick embryo from the ninth day of incubation until just before hatching. At the beginning of the twentieth day the enzyme is likewise not present. However, later in the day when the chick has made a hole in the shell, the enzyme surges to more than four times the adult level (12 mμ moles ALA/gram/hour). In the newly hatched chick the enzyme varies considerably, ranging from the pre-hatching high to the adult value which is reached a few days after hatching.

An injection of 40 mg 3,5-dicarbethoxy-1,4-dihydrocollidine into the egg results in a massive increase in the activity of the enzyme—up to forty times the adult level.

ALA synthetase can be induced by an oxygen deficient environment. When development occurs under an atmosphere of 14% oxygen, ALA synthetase is present in appreciable amounts on the eleventh and thirteenth days of incubation, but not on the fifteenth. An exposure to the lowered oxygen tension of twenty-four hours is sufficient to induce the enzyme. The continued presence of the inducing stimulus is required for the maintenance of enzyme activity. (Supported by USPHS Training Grant T1-HD-32 and NSF G 24169.)

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WILLIAM H. SOFER and HEINRICH URSPRUNG, The Johns Hopkins University.

Ontogeny of alcohol dehydrogenase in *Drosophila melanogaster.*

At various stages during the development of *Drosophila melanogaster* analyses were made for both alcohol dehydrogenase activity and protein. It was found that in the two strains investigated enzyme activity per unit protein (specific activity) of clarified homogenates was relatively low in eggs and first instar larvae, increased during the second and third instars, dropped during pupation, and rose again shortly before ecdysis. (Supported by N.I.H. Training Grant No. 1-F2-GM-2Y, 115-01 U.S.P.H.S. and N.S.F. GB-4451.)

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RICHARD B. CRAWFORD and M. L. BATCHELER, Trinity College and University of Pennsylvania.

Hexokinase in the developing embryo of *Ambystoma maculatum.* (Introduced by Malcolm Steinberg)

Previous attempts, utilizing the disappearance of glucose or the appearance of lactate in glycolytic systems, have failed to reveal the presence of hexokinase in embryos of *Ambystoma maculatum.* Fluorometric methods were applied in a direct analysis of this enzyme in a 100,000 × g supernatant of embryo homogenates. The assay employed the coupling of hexokinase to the glucose-6-phosphate dehydrogenase system, observing the increased fluorescence caused by the reduction of NADP. Due to the increased sensitivity of the method hexokinase activity was demonstrated throughout development. Enzyme levels were extremely low until middle fin bud stages were reached, when a gradual but marked increase was noted. This period of increased activity is coincident with development of embryonic motility and circulation, a time when utilization of free glucose might be anticipated. Specificity of the hexokinase was investigated. Mannose and galactose proved to be appropriate substrates whereas the utilization of fructose was minimal. In late stages the specificity for mannose decreased considerably, reflecting that found in several tissues of the adult. This suggests either two or more hexokinases or the changing specificity reflected in the synthetic patterns of an isoenzyme. Concomitant with these studies, using a similar assay method, glucose-6-phosphate dehydrogenase was found in high levels throughout the entire developmental sequence. These results provide a useful approach to the investigation of the control and synthesis of a specific protein ari-

sing to a high and functional level at a particular stage in embryonic development. (Supported by Grant HD-00519 from the U.S.P.H.S.)

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MARTHA Y. JANNERS and ROBERT L. SEARLS, University of Virginia.

Determination of cartilage in the embryonic chick limb bud.

It has been observed that a block of cells isolated from the chondrogenic area of the limb bud of a stage 22, 23, or 24 chick embryo, and implanted into the wing of an embryo of about the same stage, is incorporated into the host wing and differentiates in accordance with the host wing pattern. (Searls, J. Exp. Zool., in press). The experiments to be described were done to discover if the cells lose this ability to regulate when placed in an ectopic site in a wing bud, and, if so, the stage when the change occurs.

Embryos from stage 23 through stage 27 were labeled with tritiated thymidine. The limb chondrogenic areas were isolated and cut into blocks. These blocks were then implanted through the dorsal surface of the wing of embryos of the same stages. The host embryos were allowed to develop for 48 hours, the host limbs were fixed, and the position of the implanted blocks was determined by autoradiography.

When the donor limb was stage 23 or stage 24, the implanted cells formed cartilage only when they were in the cartilage forming area of the host limb. When the donor limb was stage 25 or older, the implanted cells formed cartilage independently of the cartilage forming area of the host limb. This was true whatever the stage of the host. However, even when the donor was stage 27, some of the cells in the implant were not surrounded by matrix.

The ability of a block of limb chondrogenic cells to regulate to an ectopic site in a host limb seems to be lost between stage 24 and stage 25, but the ability of individual cells to regulate seems to persist until at least stage 27. (Supported by NSF Grant GB 48446.)

181

TRYGVE P. STEEN, St. Olaf College.

Stability of chondrocyte differentiation and contribution of muscle to cartilage during limb regeneration in the axolotl *(Siredon mexicanum)*.

In order to determine whether chondrocytes are stable as to type during limb regeneration and whether any cells from limb muscle become chondrocytes in the regenerate, labeled cartilage and muscle were transplanted into limbs which were then allowed to regenerate. Three kinds of label were used to identify transplanted cells after redifferentiation: $H^3$-thymidine, triploidy, or both.

The results of this investigation indicate that limb chondrocytes are intrinsically stable with respect to cell type, for grafts of both triploid and $H^3$-thymidine labeled chondrocytes gave rise to morphologically dedifferentiated blastema cells which almost exclusively differentiated into chondrocytes. A small percentage of non-cartilage cells appeared to be labeled. Although these cells could have arisen from metaplasia, it is more likely that all or most of them arose as spontaneous polyploids or as contaminants of the original grafts. Isotope dilution in $H^3$-thymidine labeled chondrocytes indicated that they had usually divided at least 5 times. Thus, the basis for the intrinsic stability of chondrocytes must be heritable.

Coracoid, scapula, and visceral arch cartilages also contributed chondrocytes to the cartilage of limb regenerates, and the available evidence indicates that cells released from these cartilages are similarly stable as to type. These cartilages did not dedifferentiate and participate in limb regeneration to the same extent as did limb cartilage.

Cells from limb muscle, labeled by the 3 methods used in this study, became chondrocytes during limb regeneration. However, since muscle is a mixture of cell types, the cellular origin of these chondrocytes is uncertain. (Supported by The Danforth Foundation, USPHS Training Grant T01 HD-00032-03, and NSF Grant GB-4265.)

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

The effect of bromodeoxyuridine on chondrocytes *in vitro*.

The thymidine analogue, bromodeoxyuridine (BUDR), induces morphological changes and inhibits synthesis of cartilage matrix in chondrocytes grown *in vitro*.

Six-day cultures of chondrocytes ($5\text{-}8 \times 10^2$ cells/60 mm petri dish), derived from a single chondrocyte, were exposed to 50 $\mu$g BUDR/ml for 9 days. Untreated clones contain closely packed smooth polygonal-shaped cells. Such clones eventually produce a cartilage matrix which stains metachromatically with toluidine blue. Within 24-48 hours after BUDR is introduced, the smooth cell surfaces become disrupted by increasing numbers of cell processes and clones appear more loosely organized. After 9 days, cells are widely spaced and extremely spread out in bizarre shapes. Although shape change is evident by 4 days, there is no significant difference in numbers of cells/clone in treated and untreated cultures. By 9 days BUDR treated clones have fewer cells and no metachromatic matrix is detected.

Microspectrophotometric measurements of BUDR treated cultures show some polyploid values for nuclei 3-4 times larger in diameter than normal. No polyploid values are found in controls. 2N and 4N values are discrete and coincide in controls and treated nuclei suggesting that BUDR does not cause obvious aneuploidy. Limits of the technique preclude detection of small DNA losses.

Preliminary studies indicate that BUDR inhibits chondroitin sulfate synthesis in intact vertebral cartilages. Since DNA synthesis is minimal in these cells, BUDR may act at a level other than gene transcription—possibly at the cell surface.

These studies agree with previous work which correlated specific morphology and intercellular associations of chondrocytes with their ability to synthesize cartilage matrix. (Supported by Grant 1 RO 1 GM 14429-01 from the U.S.P.H.S. and Grant GB-5047X from the N.S.F.)

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ROBERT D. CAHN, MARTHA B. CAHN, and MICHAEL SOLURSH, University of Washing-

ton, Seattle.

Heritability of differentiation *in vitro*: control of expression at the level of synthesis of DNA-like-RNA in cartilage and pigmented retina cell clones.

Chick embryo cartilage and pigmented retina (PR) cells may be grown as clones under conditions allowing expression and transmission of their specialized properties (epiphenotype) during rapid cell division. Alternatively, they may be cultured so they "dedifferentiate" or fail to express their differentiated epiphenotype. At what level of synthesis is "dedifferentiation" controlled?

Second generation differentiated sternal cartilage cell clones derived from picked differentiated clones (CMC) were pulse labeled with tritiated uridine. Labeled RNA was annealed to chick embryo DNA on membrane filters in the presence of increasing amounts of unlabeled competitor DNA from homologous cultures, non-CMC cells derived from CMC cells, embryonic sterna, or differentiated PR clones.

Conclusions: 1) The DNA-RNA annealing-competition procedure is sensitive enough to detect large differences between differentiated and "dedifferentiated" cells derived from the same clone and between different pure cell types. 2) Cold PR-RNA competes only 30% as well as CMC or cartilage RNA against labeled CMC-RNA. 3) Non-CMC-RNA competes only slightly better than PR-RNA in the same system.

Reciprocal experiments were carried out with pulse labeled non-CMC and PR-RNA. All the results support the hypothesis that lack of expression of epiphenotype is due partly to lack of synthesis of appropriate DNA-like-RNA species by cartilage and PR cells. Rate of DNA-like-RNA turnover and length of DNA-like-RNA lifetime are invoked to explain other aspects of the competition curves. (Supported by grants from American Heart Association, 65-G-102; NSF, GB 4649; and NINDB, NBO6761.)

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JOHN PAPACONSTANTINOU and JAMES W. CARR, Biology Division, Oak Ridge National Laboratory.

Crystallin synthesis by lens epithelial cells in tissue culture.

Calf lens epithelial cells maintained in tissue culture for 9 passages (approximately 90 days) maintain the ability to synthesize crystallin proteins, *i.e.*, the tissue specific structural proteins of the lens. $\alpha$-, $\beta$-, and $\gamma$-crystallins synthesized by cells in primary culture and by the same cells after the 9th subculture were compared to those synthesized by uncultured epithelial cells and fiber cells. To determine whether any differences occurred during growth in culture, these cells were incubated with a mixture of $^{14}$C-labeled amino acids, homogenized, and then mixed with $^{3}$H-labeled proteins from a fiber cell homogenate. The $\alpha$-, $\beta$-, and $\gamma$-crystallins of this mixed homogenate were fractionated on DEAE-cellulose by a stepwise elution system, and by a gradient elution system. A comparison was made between the $^{14}$C-labeled, $^{3}$H-labeled, and protein ($O.D_{280}$) profiles to determine whether the crystallins from cultured cells have the same chromatographic properties as do the proteins from the lens fiber cells. By this procedure it was found that all cultured cells (primary and 9th subculture) synthesize $\alpha$- and $\gamma$-crystallins having similar chromatographic properties to the crystallins synthesized in the fiber cells *in vivo*. The $\beta$-crystallins, on the other hand, change their chromatographic properties by the 9th subculture. Further resolution of the $\alpha$- and $\gamma$-crystallins from the 9th subcultures on DEAE-cellulose showed that there are no alterations in their chromatographic properties. These observations are being confirmed by precipitation of the purified $^{14}$C-labeled and $^{3}$H-labeled protein with specific antiserum. In addition, these cells are being analyzed by immunofluorescent techniques. (Sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

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C. H. ELLIS, JR. and R. J. WINTER, Amherst College.

Protein synthesis and skeletal spicule formation in the sea urchin larva.

Investigation into the chemical basis of spicule formation in *Arbacia punctulata* embryogenesis reveals significant incorporation of amino acids into precipitable protein closely associated with the calcareous spicules of the pluteus larval skeleton. Embryos were reared in artificial sea water to an early larval stage, at which time they were incubated for one hour in 2.5 to 5.0 $\mu$c/ml $^{14}$C-protein hydrolysate or $^{3}$H-proline. The larvae were then dissolved in 5 percent sodium hypochlorite to isolate spicules free of cellular and cytoplasmic contamination.

Liquid scintillation counting shows significant incorporation of these amino acids into spicules. Incorporated $^{3}$H-proline is nearly 100 percent recovered in a trichloroacetic acid precipitate. Carbon-14 labeled spicules dissolved in this fashion show, on the other hand, only 20 percent of the carbon-14 counts to be recoverable in precipitated protein. The lost radioactivity can be recovered from the acid supernatant as $Ca^{14}CO_3$.

The results indicate synthesis of a protein intimately associated with spicules at the time of their formation. Preliminary indications that embryos at this stage can convert proline to hydroxyproline support the possibility that they are synthesizing a collagen-like protein. The hypothesis that such a protein serves as a matrix for spicule calcification is discussed. (Supported by NSF Grant GB-5636.)

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HELEN PARK, ANNE ORTMEYER and DWIGHT BLANKENBAKER, National Institutes of Health and the George Washington University.

Mitotic activity in *Hydra pseudoligactis* during re-, generation of apical structures.

Recent reports on mitotic activity in adult hydras (Campbell, Science 148:1231; Shostak, Patel and Burnett, Devel. Biol. 12:434) and in developing buds (Clarkson and Wolpert, Nature 214:780) led us to make mitotic counts on specimens of *H. pseudoligactis* during regeneration of apical structures.

Asexual adults without buds, from cultures maintained in our laboratory, were used. The animals

were decapitated proximal to the circle of tentacles, leaving approximately 9/10 of the body for observation. Under conditions in our laboratory, 100% of such segments reconstitute a hypostome and 2-7 short tentacles in 48 hours.

After fixation, staining and clearing, whole animals (controls) and regenerating specimens were bisected longitudinally. Each pair of halves was mounted on a microscope slide such that one half was oriented with its ectoderm uppermost, the other with its endoderm uppermost.

The sections were scanned, at a magnification of 864 × and dividing forms were counted in four kinds of cells: epithelio-muscular and interstitial cells in the sections with the ectoderm uppermost, and digestive and gland cells in those sections with the endoderm uppermost.

Counts on 30 control animals showed that mitotic activity in all four types of cells was broadly distributed in the region of the body from the tentacle bases to the distal border of the peduncle.

Counts on groups of 10 regenerating specimens fixed ½, ¾, 1, 2, 4, 8, 16, 24 and 48 hours after decapitation showed that mitotic activity did not at any time exceed the mitotic activity in the controls. We conclude, therefore, that cell proliferation does not play a major role in the reconstitution of apical structures.

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MICHAEL P. MCGARRY and JOSEPH W. VANABLE, JR., Purdue University.

Mitosis and skin gland development in *Xenopus laevis*.

Several studies have suggested that mitoses are involved in the formation of the multicellular, subepidermal glands that develop in anurans during their thyroxin-directed metamorphosis (Muhse, Am. Jour. Anat. *9*:321, 1909; Helff and Stark, J. Morph. *68*:303, 1941; Vanable, Develop. Biol. *10*:331, 1964). However, the absolute dependency of gland rudiment formation on cellular division has not been shown experimentally. To demonstrate this dependency, and its control by thyroxin, isolated forelimbs of *Xenopus laevis* larvae (hindlimbs 6.0-12.0 mm) were cultured under various experimental conditions in Steinberg's amphibian salts (Carnegie Inst. Wash. Yearbook *56*:347, 1957) supplemented with 1 mg/ml glucose. The epidermis of competent forelimbs cultured in thyroxin-containing medium (0.2 μgm/ml) exhibits a greater mitotic frequency than does such tissue cultured in the absence of thyroxin. Furthermore, the number of glands formed in the skin of competent forelimbs is greater in those cultured in thyroxin medium. Finally, gland rudiment development in the thyroxin supplemented medium is depressed severely when the medium also contains the mitotic inhibitor 5-fluorouridine-2'-deoxyribose (Hartman and Heidelberger, J. Biol. Chem. *236*:3006, 1961, provided by Hoffman-LaRoche, Inc.). Radioautographic experiments with $H^3$ thymidine now in progress should help to clarify further the apparent dependency of skin gland formation on cell division, particularly in its earlier phases. (Supported by USPHS Predoctoral Fellowship 5-F1-GM-32,682-02, and NIH Grant AM-06997.)

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DAVID E. MASLOW, University of Pennsylvania.

The formation of multinucleated striated muscle. (Introduced by R. B. Crawford)

Multinucleate skeletal muscle is formed both *in vivo* and *in vitro* by fusion of uninucleate cells to the ends of advancing protoplasmic masses. The selectivity of this process has been the subject of experimentation. Freshly disaggregated muscle or liver cells from late embryonic mice have been cultured on glass together with similarly treated eleven day chick thigh muscle. Studies were conducted both on histologically detectable differences in nuclear morphology and on radioautographs following tritiated thymidine labeling of the non-chick component. The formation of large multinucleate muscle straps from the myoblasts of differing species origin was observed, but liver nuclei were never incorporated. However, when liver cells were pretreated with actinomycin D and thoroughly rinsed before plating, nuclei from both tissue types were found in close proximity in common cytoplasm, with myofibrils extending around both. A significant reduction in incorporation of labeled uridine and amino acids occurs following pulse treatment with actinomycin D reflecting a reduction in the synthesis of RNA and subsequently protein.

These results suggest that the fusion selectivity normally observed may involve a genomically determined, cytoplasmically mediated recognition factor, perhaps at the cell surface. (Supported by USPHS Grants 5-T1-GM-849-05 and DE-02047.)

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PRENTIS G. COX, Case Western Reserve University.

*In vitro* myogenesis of promuscle cells from the lizard, *Anolis carolinensis*. (Introduced by S. B. Simpson, Jr.)

Recently Simpson and Cox (Science, 1967, in press) succeeded in culturing various components of the regenerating tail of the lizard, *Anolis carolinensis*. Of these components muscle is the most striking in its reaction to culture conditions. Single cell suspensions of presumptive myoblasts from the promuscle aggregates of the regenerating tail were plated at clonal densities. When these cells were plated in either GMII or GMIII at 31°C they underwent rapid proliferation over the next several days. By day five many of the cells had assumed a "rounded up" morphology and by day eight most of the cells in many clones were "rounded up." When these cultures were left in GMIII practically no fusion occurred. On the other hand, when they were either grown continuously in GMII or changed to GMII on day eight or nine they began to fuse, and by day eleven massive networks of myotubes were present.

When subcultured at eight day intervals these cells maintained the above described characteristics through more than 30 cell generations with three subcultures.

Some cultures were changed to GMII containing tritiated thymidine. Autoradiographic studies of these clonal cultures indicate that most of the nuclei which entered myotubes during the first 24-48 hours did not undergo additional synthesis before fusion. However, with the passage of time

more labeled nuclei were found in myotubes. The "rounded up" cells showed a similar sequence of labeling. Indications are that the "rounded up" cells are in a pre-fusion state but cannot fuse unless they are in the permissive medium (GMII). However, since these cells can be suubcultured and caused to undergo many more divisions before fusion they are not in an irreversible post-mitotic condition. (Supported by Grant GM12653 from NIH.)

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E. SUE LUMB, Vassar College and University of Minnesota.

Ultrastructure of differentiating and functioning meso- and metanephric tubules of the chick.

Light and electron microscopic observations were made on mesonephric and metanephric kidneys of chicks ranging in age from four days of incubation to two weeks posthatching. Tests for active transport of phenol red and for accumulation of trypan blue were used to determine excretory capacity of tubules. This material provides information on structure correlated with differentiation and function in two types of kidney tubules of a single species.

Cells of differentiating nephrons display such general embryonic ultrastructural characteristics as: large nucleus with numerous pores in the envelope; large nucleolus; numerous polyribosomes free in the intracellular matrix; patterned aggregates of ribosomes associated with rough endoplasmic reticulum; large and apparently active Golgi apparatus; large mitochondria; and numerous vesicles of various types. Sequential changes occur in cellular components during the differentiation of proximal portions of meso- and metanephric tubules. Of special interest are changes in the following: the apical region, including microvilli, invaginations, canaliculi, and coated vesicles; lateral and basal cell margins; the quantity of free and attached ribosomes; the amount and organization of rough and smooth endoplasmic reticulum; the Golgi apparatus; the number, size, structure, and location of mitochondria, microbodies, lysosomes, and dense bodies resembling protein-absorption droplets. The mesonephric nephron consists of a proximal segment and very short region of attachment to the collecting duct while the metanephric primordium soon forms the diverse segments characteristic of adult birds. The general ultrastructural features of functional embryonic chick tubules resemble those of adults and embryos of species described by other investigators. (Supported by Fellowship F3 HD-919-01 REP from the National Institute of Child Health and Human Development.)

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LEON CANDEUB, Temple University.

Tubulogenesis and the maintenance of tubule morphology by chick metanephrogenic mesenchyme in organ culture. (Introduced by S. R. Hilfer)

Tubulogenesis in the metanephros and the maintenance of tubule morphology has been studied in the past using tissue which was trypsinized to remove the "intact" ureteric component from the metanephrogenic mesenchyme. The possibility exists that in the course of such a separation, isolated ureteric cells or ureteric fragments in close association with developing tubules might remain with and become incorporated into the mesenchymal portion. In these experiments, the intact metanephrogenic ridge of a 5½ day chick embryo is removed and divided into cranial (containing adrenal and vascular contamination), middle, and caudal (containing ureteric bud) thirds. For tissue culture purposes, only the middle and caudal thirds are used.

When metanephrogenic mesenchyme (middle third) is grown transfilter to ureteric bud (caudal third), tubules begin to appear in the mesenchyme approximately eight hours after explanation. In the absence of the ureteric bud component, the mesenchyme of the middle third fails to show tubulogenesis. After two days in culture, the induced mesenchyme may be trypsinized into tubular and single cell components. When the single cells are aggregated into a pellet, and placed on a TH millipore filter, spreading of the cells results. When, on the other hand, the tubular component is placed under similar tissue culture conditions, tubule morphology is retained.

Control of metanephric tubulogenesis and the maintenance of tubule architecture will be discussed in terms of these two mesenchymal components, and their relationship to ureteric bud. The cyto-architecture of these components will be analyzed by means of electron microscopy.

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SIDNEY B. SIMPSON, JR., Case Western Reserve University.

Spinal cord regeneration in the lizard.

This study describes for the first time the detailed composition of the regenerated spinal cord of the lizard. The regenerated cord consists only of ependyma, regenerated descending central fibers and the connective tissue of the menenges. The ependymal portion of the cord is a discrete entity from the surrounding connective tissue and is separated from the latter by a basement lamina. Moreover, the ependymal tube appears to be composed exclusively of a morphologically homogeneous epithelial cell population.

The most interesting observation in this study is the relationship of the ependymal cells to the descending central nerve fibers that have regenerated from the old cord. The majority of the regenerated central fibers are enclosed within the ependymal tube and are fasciculated between adjacent ependymal cells. In the lizard, the regenerating ependymal tube serves as an effective guide for the regenerating central fibers. Much the same mechanism might well account for the precise guidance of regenerating intercentral fibers in urodele cord regeneration. (Supported by grant GM12653 from NIH.)

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WILLIAM H. PRITCHETT, University of Virginia.

The role of size in regenerating limbs of the adult newt. (Introduced by J. N. Dent)

A forelimb and a hind limb were amputated from each animal in groups of large and small male and female specimens of adults of the newt *Triturus viridescens*. Careful observations were made on the

rate of regrowth of each ensuing regenerate. In contradiction to the results obtained by other investigators, both anterior and posterior limbs of large animals were found to regenerate more slowly than those of smaller and presumably younger animals. Evidence is presented to support the view that for a given limb type (forelimb or hind limb) the rate of regeneration is related not so much to total body size as to limb size.

In a second series of animals observations were made on the regeneration rate of the left forelimbs of newts that had been subjected to ovariectomy, sham-ovariectomy, castration, injections of estriol in a carrier solution, or injections of the carrier alone. No significant difference in rate of regeneration of forelimbs was found to be attributable to gonadal hormone effects.

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DAVID L. STOCUM, University of Pennsylvania.

*In vitro* culture of larval urodele limb regenerates. (Introduced by C. E. Wilde, Jr.)

Epidermis-free cone, palette and notch regenerates, both with and without stump, have been cultured for 25 days in a modified L-15 (Leibovitz) medium. Migration of numerous multinucleate giant cells from the explant begins within 24 hrs., followed within a few days by migration of a sheet of fibroblastic cells. The latter cells divide frequently, but no divisions are noted in the nuclei of giant cells.

The *in vivo* cone regenerate is composed of a homogeneous, unorganized population of rapidly dividing cells. Morphological organization of preskeletal rudiments is not detected in sectioned *in vivo* regenerates until the palette stage. The very proximal portions of these rudiments react with methylene blue only after the notch stage is attained. Methylene blue-stained whole amounts of stumpless cultured regenerates of all stages exhibit discrete regions of morphoolgical organization which appear in sectioned explants to be composed of pre-cartilage and connective tissue. Such organization is of approximately equal magnitude in cone and palette cultures, while being somewhat enhanced in notch cultures. The degree of organization for all stages is greater than that of operational controls. Most cone regenerates disappear when cultured with subjacent stump; fewer palettes disappear and notch regenerates rarely do so. The presence of stump does not appear to enhance *in vitro* organization of the regenerate.

These results suggest that completely isolated regenerates of even young undifferentiated stages may grow and undergo an autonomous morphogenesis and differentiation *in vitro*. (Supported by USPHS 5T1-GM-849-05 and DE-02047)

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CHARLES W. GIBLEY, JR., The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas, and the Pennsylvania College of Podiatry, Philadelphia.

Fixatives, buffers, osmolality: their effects on the fine structure of embryonic kidneys.

Recently, a number of papers have appeared on the effects of fixatives, buffers, and osmolality on ultrastructure of cells and tissues. However, few were concerned with embryonic tissues whose high water content and delicate nature complicate the problems of adequate fixation. In an investigation of the fine structure of embryonic kidneys in vertebrates a number of fixatives and buffers with varying osmolalities and temperatures were tested. Kidneys of *Rana pipiens* and chick were used as sources of material. Fixatives included: (1) 1% glutaraldehyde with 1% osmium postfixation; (2) 1% osmium; (3) 1% potassium permanganate; (4) a 1% glutaraldehyde and 1% osmium mixture. Buffers included Millonig's phosphate, s-collidine, and sodium cacodylate. Tissues were fixed in the cold (4°C) or at room temperature (25°C). Conclusions: (1) Embryonic tissues necessitate extreme care in their treatment if good ultrastructural detail is to be preserved. (2) The osmotic properties of fixatives must be controlled carefully within these ranges: chick 260-275 mOsm; frog 180-200 mOsm. (3) Temperature of the fixative is of minor importance, although "membranous whorls" found in glutaraldehyde treated cells are less at 25°C. (4) Buffer vehicles do not substantially alter cellular structure. S-collidine is difficult to handle, but gives comparable results. (5) Glutaraldehyde results in excellent preservation of ground substance and organelles, but causes cell shringage. (6) Osmium-fixed tissue lacks the preservation of ground substance observed in glutaraldehyde fixation. If penetration is sufficient, cell organelles are well preserved. (7) Potassium permanganate is excellent for cell membranes. (8) Fixation in a glutaraldehyde-osmium mixture provides advantages of the individual fixatives and overall preservation is good. (Supported by U.S.P.H.S. institutional (MDAH) grant number FRO-5511-05-IN-46).

196

PATRICIA HARRIS, Oregon State University.

Nucleolus-like bodies in sea urchin eggs.

Eggs of the sea urchin *Strongylocentrotus purpuratus*, fixed for electron microscopy in 1% $OsO_4$ in phosphate buffer at pH 6, or in 3% glutaraldehyde followed by osmium, showed numerous aggregates of densely packed fine granules adhering to the inner surface of the nuclear membrane. These aggregates vary in shape from nearly spherical to lenticular, often occupying smoothly rounded cup-like protrusions of the nucleus, and are not dislodged by centrifugation. Their number varies, but generally each nucleus contains one large body and as many as ten smaller ones. They occur in freshly shed unfertilized eggs and persist until first cleavage division.

Other structures related to the nuclear membrane at this time are the "heavy bodies", RNA containing granular aggregates which adhere to the outside of the nucleus. The difference in granule size between the ribosome-like aggregates on the outside and the much finer dense material on the inside of the nuclear membrane suggests that this relationship does not represent a direct transfer from the nucleus by budding or extrusion. Fixation in a neutral osmium fixative containing sea water, which extracts condensed chromosomes, also removes the nucleolus-like structures, but does not alter the appearance of the heavy bodies, further suggesting their difference in composition. Cytochemical studies for light microscopy were inconclusive due to

the small size of these bodies, their close relationship with the nuclear membrane, and difficulty in distinguishing them from heavy bodies and yolk granules. (Supported by grant GM-12963 from the U.S.P.H.S.)

197

CATHERINE A. VERHEY and FRANK H. MOYER, Washington University.

The role of accessory cells in sea urchin oogenesis.

Ovarian tissue from ripe sea urchins contains accessory cells and cellular debris in addition to oocytes in various stages of development. Tennent and Ito (1941) suggested that accessory cells were degenerating oocytes; another possibility is that they have a supportive function providing nutrients for oogenesis.

Light microscope autoradiography shows incorporation of leucine-$H^3$ and uridine-$H^3$ into growing oocytes but not into accessory cells. This evidence plus the lack of pinocytotic vesicles in the plasma membranes of oocytes shows that RNA and/or protein are not made in the accessory cells and then transported to the oocyte.

Electron microscopy has also revealed that the accessory cell cytoplasm is filled with glycogen-like particles; however, histochemical tests for glycogen were negative and the identity of these particles is undetermined.

The mature oocyte is surrounded by a jelly coat which stains intensely with Alcian blue. Although the oocyte cytoplasm does not show this reaction for acid mucopolysaccharide, localized regions of the accessory cells do. This might suggest that the accessory cells synthesize some component of the jelly coat.

Since large inclusions in the accessory cells stain for protein, RNA, polysaccharide, and lipid, and since their fine structure resembles that of focal cytoplasmic degradation (Hruban, *et al.*, 1963), it is likely that the cells in these areas are degenerating. This possibility should be tested further by enzyme histochemistry.

The data suggest that accessory cells consist of degenerating cells and cell synthesizing acid mucopolysaccharide precursors of the jelly membranes of oocytes. (Supported by NSF Grant GB 5512)

198

VICTOR J. BROOKES and ROGER DEJMAL, Oregon State University.

Deposition of yolk protein in the oocytes of the cockroach, *Leucophae maderae*.

Most of the yolk protein in the mature oocyte of *L. maderae* consists of one large component with a sedimentation coefficient of 27S whereas a second smaller protein, 14S, is present during the early stages of vitellogenesis. The large protein was converted to the smaller one and to even smaller units by mild alkaline conditions *in vitro*. After injection of leucine-U-$C^{14}$ into females with developing eggs, the smaller yolk protein became labelled first and the label was then transferred to the large protein upon prolonged exposure. Leucine-U-$C^{14}$ was also incorporated into a 14S component in the fat body. This component could only be detected by measurement of the isotope and did not build up during prolonged exposure to the labelled amino acid. It was not detected in males or female nymphs or adults with immature eggs. When fat bodies were excised from females during egg development and incubated in a tissue culture medium, the 14S component was detected in the medium. (Supported by NSF grant GB 3818 and a Career Development Award (VJB) from NIH)

199

JOHN B. MORRILL, FRANK O. PERKINS and JANE CROWELL NASTI, College of William and Mary, Virginia Institute of Marine Science, University of Palermo and University of Naples.

Ultrastructure of the cortical region of the egg of *Limnaea palustris* with particular reference to the occurrence of microtubules.

According to Elbers (1959) and Raven (1966) the cortex of the *Limnaea* egg is equivalent to the plasmalemma; cortical morphogenetic factors are bound to this membrane.

In the present study of uncleaved and first cleavage *L. palustris* eggs fixed in glutaraldehyde and post fixed in osmium, the egg's trilaminar plasmalemma is covered with a surface coat material. From the egg's irregular surface scattered microvilli extend obliquely across a narrow perivitelline space and attach to the vitelline membanes by short, bud-like branches. Beneath the plasmalemma is a region of cytoplasm 0.5 to 5.0 microns thick that is relatively free of yolk platelets and mitochondria. Typically this region contains numerous dense particles, probably ribosomes, and irregular vacuoles bounded by a unit membrane.

Microtubules occur in this peripheral region of cytoplasm and run parallel as well as oblique to the egg's surface. Occasionally microtubules appear to branch. Longitudinal and cross-sectional profiles of microtubules occur most frequently in an equatorial girdle. In addition, microtubules are observed in the peripheral regions of cytoplasm at the animal and vegetal poles of the egg and first two blastomeres, as well as in the more central regions of the blastomeres and near the borders of the cleavage cavity. In centrifuged eggs, although microtubules accumulate in the hyaline zone, they are still visible in the region immediately beneath the plasmalemma.

The possible dimensions of the cortex and morphogenetic roles of the microtubules will be discussed. (Supported by grants GB-4393 and GB-5540 from the N.S.F.)

200

JOHN P. WOURMS, Harvard University.

Differentiation of a fish egg chorion composed of extracellular "microtubules".

The drought-resistant, diapausing egg of the Brazilian annual fish, *Cynolebias melanotaenia* Regan possesses an uniquely patterned chorion. The chorion's surface is covered with uniformly spaced, macroscopic, hollow, conical projections which terminate distally as a crown of recurved spikes. Ultrastructural studies show that two concentric components form this chorion. The inner component is of uniformly low electron density and lacks obvious structural differentiation. The outer component is electron dense, highly structured, and is entirely composed of closely packed rods with centers of low density. These extracellular "micro-

tubules" have a diameter of about 250 A which an investing coat of "fuzzy" material increases to 475 A. Twelve to fourteen circular subunits occur in transverse section, while in longitudinal section there appears to be a linear periodicity of slight pitch.

The two chorionic components are derived from two different cell types. During late oogenesis, the inner homogeneous component progresses to its mature form by the disordering of a previously highly order precursor. This structural precursor, consisting of tangentially grouped bundles of interwoven fibrous elements, was produced by the oocyte during mid-cogenesis. The extracellular "microtubules" which constitute the outer, pattern-bearing, chorionic component are secreted by follicle cells during late oogenesis. In advance of this event, within the follicle cells, there are membrane bounded secretion packets of varying size which contain a dense matrix in which the "microtubules" are imbedded. Ordered arrays of "microtubules" retaining some matrix material are present in large vacuoles both within and between follicle cells. These arrays which form a configuration suggestive of the pattern of the mature chorion are aggregated secretion packets which appear to have been extruded from the follicle cell cytoplasm. Follicle cells appear to have evolved a mechanism for selectively depositing extracellular "microtubules" in order to generate a reproducible, species-specific, chorionic pattern. (Supported by a post-doctoral fellowship from the American Cancer Society, PF-355)

201

ROLAND J. LESSEPS, Loyola University, New Orleans.

Developmental changes in lanthanum-staining material external to the cell membrane.

The addition of lanthanum ions to the ordinary permanganate fixative (for technique, see *J. Cell Biology 34*;173, 1967) results in the appearance of an electron-opaque material opproximately 50 A thick external to the cell membrane. This material was found in several embryonic chick tissues of varying ages: heart ventricle and limb bud, 5-day; liver, 6½-day; neural retina, 7-day. During an attempt to study this lanthanum-staining material (LSM) on isolated cell membranes it was discovered that the source of these isolated membranes, adult rat liver, did not have the LSM. Adult chicken liver was also found to be lacking the LSM, whereas 11-day rat embryo liver and heart ventricle possess an LSM similar to that found in early chick embryos. The 11-day-old rat embryos were in approximately the same developmental stage as 4-day-old chick embryos, as judged by the size and shape of the limb bud and the degree of eye pigmentation. Liver from a near-term rat embryo did not have any LSM. Attempts will be made to follow the developmental history of the LSM more exactly in the chick embryo. (Supported in part by NSF grant GB-2363 and by the facilities at the National Cancer Institute, Amsterdam)

202

M. GOLDSMITH, J. A. WESTON, and L. COWELL, Case Western Reserve University.

Crystalline bodies associated with morphogenetic cell death in embryonic sensory ganglia.

Membrane-bound crystalline arrays of granules in basophilic cytoplasm have been observed in electron micrographs of 8-day embryonic chick sensory ganglia treated with NGF *in vitro* (Crain *et al.*, 1964). It was tentatively proposed that these structures are associated with, and hence a marker for, early differentiative growth of neurites from neuroblasts. Similar crystalline structures have been found in PNZ cells of chick embryo limbs (M. Ricklin, personal communication) and in degenerating cells of early chick blastoderms (Bellairs, 1961). Some cells degenerate in all 5-7 day spinal ganglia, but at this time, necrosis is concentrated particularly in thoracic and cervical ganglia, (Hamburger and Levi-Montalcini, 1949). It seemed possible, therefore, that these crystalline organizations of granules were associated with mophogenetic cell death in the sensory ganglia. To test this, brachial, thoracic and lumbosacral ganglia from 6-7 day chick embryos were isolated and prepared for electron microscopy. Grids containing sections of these ganglia (four from each region) were coded for "blindfold" analysis. Coded grids were examined uniformly and scored for the presence of crystalline arrays. One grid containing sections of a lumbosacral ganglion was found to contain crystalline formations, while these structures were seen in three of the four grids of thoracic ganglion. This provides independent evidence consistent with the idea that these structures are related to morphogenetic cell death. Corroborating this, crystalline bodies are also seen in accumulations of necrotic cellular material. We conclude that these crystalline arrays are characteristic of autolysis rather than early neuroblast differentiation in sensory ganglia. (Supported in part by U.S.P.H.S. Grant 8-RO1-HD-03477-03.)

203

ERNEST F. DUBRUL, Department of Biology, Washington University.

Observations on the fine structure of the developing mouse hair. (Introduced by David T. Lindsay.)

The fine structure of the mouse hair in the late anagen (growing) stage has been described (Roth and Helwig, 1964). However, in view of the fact that many mutants involving the morphology of the hair exert their effects during the development of the hair, the fine structure of earlier stages (anagen I-IV) has been investigated.

The epidermal portion of the hair follicle is surrounded by a fibrous adepidermal lamina continuous with that of the surface epidermis. The cells bordering the dermal papilla contain small vesicles associated with this lamina. The collagenous portion of the basement lamella is poorly developed at the ages examined (16d *in utero* to 3d *post partum*). Basal attachment devices are not present. The cells of the hair germ and basal portion of the bulb are relatively undifferentiated. The cells of the internal root sheath are in stages of differentiation along the main axis of the hair. In the basal portions they are filled with numerous fibers emanating from desmosomes. At a higher level, dense trichohyalin droplets appear along the fiber tracts. In the fully differentiated root sheath cells these droplets are absent and the entire cell is filled with a fibrillar material. Concomitantly, the cell membranes thicken and the cells appear tightly

bonded to each other. Melanocytes surround the apical portion of the dermal papilla. These cells contain a prominent Golgi region in their apical half and send numerous processes containing melanin granules up into the shaft of the hair. (Supported by NIH grant 5-T01-HD0001207 and NSF grant GB-5512)

204

MURIEL B. BABCOCK, University of Missouri at St. Louis and University of Virginia.

Developmental relations between ovaries and oviducts in *Drosophila virilis*. (Introduced by Dietrich Bodenstein)

In the course of development of the reproductive system of *Drosophila*, connections are established between structures developing from three spatially separated primordia, the paired ovaries and the genital disc. To elucidate the nature of this system, the development of lateral oviducts was investigated in normal unoperated animals and after larval ovariectomy. Three phases of growth which occur sequentially and are characterized by distinct processes occur in the developing epithelium of the oviducts. Early pupal mitotic activity establishes the imaginal complement of cells as morphogenesis leads to the appearance of the primordia of all imaginal structures, including the paired lateral oviducts. Further growth results from alterations in cellular arrangement. Attachment to the ovaries normally occurs during this phase. A period of cellular enlargement ensues during late pupal life and continues through early adult life.

In ovariectomized flies, a normal sequence of developmental events was found to occur within the oviducts beyond the stage at which ovarian attachment normally would have occurred. On the basis of histological appearance, the oviducts of mature ovariectomized adults are not readily distinguishable from those of unoperated animals. The results of a cytometric analysis, however, revealed that the former fail to undergo cellular enlargement to the same extent as that evident in unoperated specimens. It thus appears that previously reported ovarian influences on oviductal growth do occur but are limited in their effect to the later stages of histodifferentiation which occur during imaginal life. (Supported by N.S.F. Co-op Fellowship and U.S.P.H.S. Predoctoral grant IFI-GM 19-262-1.)

205

F. M. BUTTERWORTH and D. BODENSTEIN, Oakland University and University of Virginia.

A study of the regulatory effect of the ovary on the adult adipose tissue of *Drosophila melanogaster*.

Adipose cells of two-week-old adult males and females are equal in size but differ considerably with respect to the proportions of lipid and glycogen deposits in the cytoplasm. The cells of males contain large amounts of glycogen and low amounts of lipid, whereas the female fat cells contain moderate amounts of glycogen and relatively high amounts of lipid. Adipose cells in males containing a transplanted ovary are feminine in character with respect to glycogen and lipid deposits. Cells of castrated females on the other hand become larger in size, but the amounts of glycogen and lipid remain somewhat feminine. The adipose cells of genetically sterile, *fes*, females are as large as those of castrated females. The amount of lipid per cell in *fes* females is as plentiful as that of normal females and the amount of glycogen per cell in *fes* females is far greater than normal males. The adipose cells of *fes* females containing a transplanted, normal ovary shrink to the same size as normal males and females, and are similar to those of the normal females with respect to amount of glycogen, but are similar to those of normal males with respect to the amount of lipid. The ovary's stimulatory effect on the growth of the larval and repressive effect on the growth of the adult adipose tissue will be discussed. (Supported by grant 1-F2-GM-15,795-01 from the U.S.P.H.S., grants GB-4847X and GB-6144 from the N.S.F., and a faculty research grant from Oakland University.)

206

LYNN M. RIDDIFORD, Harvard University.

Juvenile hormone effects on larval and pupal differentiation in *Hyalophora cecropia*.

A single dose of juvenile hormone applied to *Hyalophora cecropia* eggs during the first half of embryogenesis can interfere with normal larval development of those which hatch. Larvae from eggs treated during early germ band formation showed markedly decreased viability, particularly in the first instar (either immediately or in the molt to the second instar), as compared to either normal or acetone-treated controls. A significant number of survivors underwent an extra larval molt, generally producing a fourth-fifth intermediate. Only a few of those treated between the beginning of appendage formation and blastokinesis showed anomalies in larval development. But at the end of the fifth instar, some could not spin; others spun but could not complete the pupal molt; still others pupated but retained larval tubercles. These same anomalies at the beginning of metamorphosis were the only ones seen among larvae from eggs treated after blastokinesis.

Daily applications of juvenile hormone from the time of hatching also do not affect larval development, but cause retention of larval characteristics by the pupa. This effect can be duplicated by daily applications during the fifth instar. The particular larval characteristics retained depend upon the total dosage given, the most sensitive being the colored dorsal tubercles.

Thus, juvenile hormone can interfere with the programming of genes for future larval or pupal development when applied during embryogenesis. Once larval differentiation begins, the larval genes are no longer susceptible: after that only the future pupal genes may be affected.

207

ELI SHAAYA, University of Virginia.

The significance of the fluctuating ecdysone titer in insects. (Introduced by Howard Hamilton)

A quantitative determination of the ecdysone titer was performed on the tissues of several insect species at various development stages. During the larval or nemphal molt, the maximum hormone titer, occuring at apolysis, was 2 cu/g (Calliphora units/gram) in *Bombyx mori*, and 2.4 cu/g in

*Periplaneta americana*. No ecdysone can be detected before and during ecdysis. It is therefore evident, that ecdysone can play no role in the actual process of ecdysis. Throughout puparium formation there is an elevated hormone level; 5.5 cu/g in *Bombyx mori*, 7.3 cu/g in *Calliphora erythrocephala*, and 6.2 cu/g in *Cerura vinula*. The amount of hormone necessary to initiate equivalent molts is thus quite similar despite the fact that the tested insects are from different families.

The distribution of ecdysone before and during puparium formation has been determined in various tissues of *Calliphora*. The hormone level in the blood remains relatively constant from 20 hours before pupation to 5 hours after pupation. Ecdysone first appears in the integument 8-5 hours before pupation reaching a maximum of 28% of the total hormone content in the body 5 hours after pupation. The titer in the fat body is low until 5 hours after pupation when it rises sharply to 48% of the total body content. No ecdysone is present in the pupae 20 hours after pupation. This is not due to excretion, but to the probable deactivation of the hormone. The prior accumulation of ecdysone in the fat body suggests that this tissue may be the primary site of ecdysone inactivation. (Supported by NSF Grant GB 4847)

208

JOSEPH C. DANIEL, JR., University of Colorado.

Preliminary studies on the diapausing blastocyst of the northern fur seal.

In the northern fur seal, as an example of a mammal that has delayed implantation, the blastocyst enters a "diapause" for about four months. During this period the fur seal blastocyst grows to a size achieved by the rabbit blastocyst in less than 3 days. Studies were performed on these blastocysts *in vitro* in an attempt to delimit the possible causes of the growth inhibition. Of 34 modifications (including serums, energy sources, nucleotides, amino acids, vitamins, hormones, uterine enzymes, histamines and gases) of the culture conditions or medium (Ham's F10) that normally support good growth of rabbit blastocysts, three supported some growth of fur seal blastocysts, namely: media containing lactose, estrone, or ascorbic acid. The blastocysts in these media expanded over a short period (6-12 hours) but mitotic index determinations from acetic-orcein squash preparations showed no significant difference from untreated blastocysts (about 0.5%). It was concluded that the expansion was merely the result of fluid uptake.

When fur seal serum, dialyzed serum, or the dialysate are present in the culture medium, rabbit blastocysts cease growing and collapse. These data suggest the presence of a micromolecular blastocyst-growth-inhibiting factor in fur seal serum. (This research supported by The Arctic Institute of North America)

209

SISTER JEAN WALTER HITZEMAN, Siena Heights College.

Contributions of NADPH-generating enzymes to fetal and prepubertal steroidogenesis.

In the 18-day fetal mouse testis, levels of 17$\beta$-hydroxysteroid dehydrogenase (17$\beta$-SDH) suggest the synthesis of androgens to support the formation of the male genital tract. At the same time the activity of the NADPH-generating malic enzyme has increased significantly ($P<.01$) over that of the 16-day fetal testis, and NADPH-isocitrate dehydrogenase (IDH) activity is elevated over that of the previous day ($P<.05$), although glucose-6-phosphate dehydrogenase (G6PDH) activity is virtually unaffected ($P<.80$). The activity of 17$\beta$-SDH decreases by half during the first 10 days postpartum and remains low thereafter, suggesting no increased androgen synthesis during spermatogenesis, regardless of whether the equilibrium of the enzyme favors the production of androstenedione or testosterone. During the first week postpartum when spermatocytes are proliferating, G6PDH activity increases over 19-fetal days ($P<.001$), whereas IDH increases during the second week postpartum as meiosis begins. Malic enzyme activity decreases during the first meiotic divisions (days 10-20), but during spermatid differentiation (days 20-45) there is heightened activity of this enzyme. Activity of IDH and G6PDH decreases significantly between days 15 and 30. Four hours after the injection of ICSH (ovine, NIH-S11, 50 $\mu$g body weight) into 10-day postpartum mice (prophase I), activities of the malic enzyme ($P<.001$) and 17$\beta$-SDH ($P<.001$) and IDH ($P<.02$) are increased as compared to saline-injected controls. The increase in G6PDH is insignificant ($P<.20$). This suggests that the NADPH-generating malic enzyme and isocitrate dehydrogenase may be more important in contributing the reduced coenzyme for androgen synthesis in the testis than G6PDH which has been implicated in the adrenal gland and ovary. (Supported by USPHS Grant GM-10484)

210

S. MITCHELL HARMAN and GEORGE B. TALBERT, S.U.N.Y. Downstate Medical Center, Brooklyn.

Structural changes in copora lutea and decline of reproductive function in aging female mice.

Most strains of female mice stop reproducing at an age at which they still have a supply of primary oocytes (Jones and Krohn, J. Endocrin., 21:469, 1961) and fertilized ova of aged mice are as viable as those of young mice (Talbert and Krohn, J. Rep. Fert., 11:399, 1966). To evaluate ovulation, fertilization, and luteinization C57Bl/6J female mice aged 4-7 mos. (controls), 10-11 mos., and 12-13 mos. were mated and sacrificed at either 12 hrs. or 7 days post-coitum. Ovaries and uterine tubes were fixed and serially sectioned at 10 mu and corpora lutea and intubated ova were counted. The uterus was examined for implantation sites. At 10 to 11 mos. there was a decline in implantation rate relative to the controls, with fewer implantations per ovulation in most animals. At 12 to 13 mos. most animals had neither implants nor functional appearing corpora lutea at the 7th day post-coitum. At both of these ages, however, there was no decline in ovulations nor in fertilized intubated ova at 12 hrs. post-coitum. Furthermore, there was a high correlation between absence of implantation sites and absence of corpora lutea of pregnancy at 7 days post-coitum, and also a correlation between low rate of implantation and degenerate appearance of luteal cells. It is therefore concluded that onset

of senile infertility in this strain may be related to failure of luteinization, and perhaps specifically to a lack of progesterone. (Supported by N.I.H. Grant HD-021014 and an N.I.H. Predoctoral Fellowship.)

211

JOHN E. MORRIS, The University of Chicago.

Vascular regulation of chick mesonephric regression.

The chick mesonephros completes differentiation by 8 days of incubation, reaches its maximum size and function by about 12 days, and enters the degenerative phase of regression by 16 days. Between 12 and 16 days the first phase of regression, the shrinkage phase, begins (J. E. Morris, *Experientia*, 23:307, 1967). To test the assumption that humoral factors may be responsible for regulating mesonephric regression, grafts of 8-, 12-, and 16-day mesonephric fragments were made to the chorioallantoic membrane (CAM) of chicks which had growing mesonephroi (8 days) or mesonephroi entering regression (13 days). After 4 days the grafts were compared with each other and with normal 8-, 12-, 16-, and 20-day mesonephroi.

Regardless of donor age the grafts on the highly vascular 13-day CAM survived better than those on the developing 8-day CAM. Eight-day grafts did not grow but neither did they regress. Twelve-day grafts often had regions of necrosis, but these did not represent normal regression. Sixteen-day grafts did not regress normally and in many instances had larger lumina and cells than did normal controls. Mitoses were seen in the proximal tubules of 16-day grafts even though they were absent in normal controls.

It was concluded that the action of any humoral factors responsible for controlling mesonephric regression may be modified by changes in the amount of blood supply. One consequence of this effect may be to keep mesonephric regression in phase with the development of the metanephros. (Supported by Fellowship HD-22,042 from the U.S. P.H.S.)

212

JERRY H. CROMER, Vanderbilt University.

The regulation of hepatic xanthine dehydrogenase in the hatching chick embryo. (Introduced by David A. Nunnally)

A study of possible factors regulating the natural critical period of hepatic XDH in developing chick embryos was carried out with experiments designed to induce a precocious increase in tissue levels of the enzyme. Specific activities were determined by the reduction of thiazolyl blue tetrazolium.

New Hampshire red embryos were administered cortisone acetate (two milligrams into the yolk sac) on the sixteenth day of development. This treatment stimulated a marked increase in tissue levels of XDH that was linear for three days, falling off on the fourth day from a twenty-one-fold maximum. Cortisone induction of XDH was inhibited by chloramphenicol and enhanced by actinomycin D. Cortisone, actinomycin D and chloramphenicol administered together yielded results that were about 90% additive of the independent effects of these antibiotics on cortisone induction. Vitamin-free enzymatic casein hydrolysate, and acidic casein hydrolysate to a lesser extent, also induced hepatic XDH synthesis in sixteen-day old embryos, but more transiently than cortisone. Cortisone and enzymatic casein hydrolysate together produced effects approximately 80% additive of their independent actions.

The surge of circulating amino acids which has been implicated in stimulating hepatic purinoneogenesis after glucocorticoid treatment may possibly induce the natural increase (synthesis) of XDH at hatching. Or, cortisone may elicit some other chemical or structural change imparting template activity to inactive polysomes or their preformed precursors. Sensory stimulation of the central nervous system at hatching (Fisher *et al.*, 1967) may place this phenomenon under control of the hypophyseal-adrenal axis.

213

SIDNEY L. BECK, University of Toledo.

Selected response to a teratogen.

Three successive generations (G 3-5) of mice from a four-way outcross involving C3H/HeH, A/Gr, C57BL/Gr and CBA/Gr, mated to maximize outbreeding, have now been selected for susceptibility (CBHA-S) and resistance (CBHA-R) to trypan blue teratogenesis.

Overall frequency of abnormal individuals produced in G 3-5 among treated CBHA-S litters (26 $\pm$ 2%, N = 519) is higher than control (DBHA-C) litters (19 $\pm$ 4%, N = 103); CBHA-R is lowest (9 $\pm$ 2%, N = 210). Most notable is the variation in proportion of treated litters containing affected individuals. In each generation of treatment CBHA-S had a higher proportion of litters affected ($\Sigma$G 3-5 = 81 $\pm$ 4%, N = 101) than the CBHA-C ($\Sigma$G 3-5 = 55 $\pm$ 12%, N = 18); CBHA-R invariably had fewest affected litters ($\Sigma$G 3-5 = 35 $\pm$ 8%, N = 37). The frequency of abnormality within affected litters is not different in the three lines (S = 34 $\pm$ 2%, C = 36 $\pm$ 6%, R = 29 $\pm$ 5%).

A within litter comparison among CBHA-S shows that matings between two affected individuals (Ss) resulted in a higher overall proportion of abnormal progeny (32 $\pm$ 4%, N = 194) than in litters of normal dams and abnormal sires (Sw) (24 $\pm$ 4%, N = 151); matings of two normal parents (Sr) also produced a low proportion of abnormal progeny (22 $\pm$ 4%, N = 174). In this breakdown the difference appears to be in the proportion of abnormal individuals within affected litters.

Treated CBHA-S litters contain about 0.5 fewer young at weaning than either CBHA-C or CBHA-R; all mean litter sizes are markedly smaller than untreated CBHA-C. Among CBHA-S, all-normal litters are smaller than litters with abnormals. The reverse appears true in CBHA-C and CBHA-R.

Between-litter selection in a four-way outcross for susceptibility and resistance to the teratogenic effects of trypan blue appears successful based on the parameter of amount of abnormality induced by the treatment. The mechanism whereby this differential response is obtained appears to be by way of a larger number of litters which fail to respond at all in the resistant line; although, within the susceptible line, a within-litter comparison reveals consistent variation in the amount of abnormality depending upon whether both, one, or neither parent was abnormal. (Supported by USPHS Research Grant HD-02653.)

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DAVID A. WRIGHT, Washington University.

Genetic control of lactate dehydrogenase patterns in early frog development. (Introduced by F. H. Moyer)

Electrophoretic analysis of lactate dehydrogenase (LDH) in *Rana pipiens pipiens, R.p. sphenocephala, R. palustris,* and reciprocal hybrids between them suggested that a gene controlling the B subunit was different in each species. In $F_1$ hybrid embryos paternal and hybrid LDH bands appeared by heart beat (stage 19) although the maternal contribution to the pattern remained disproportionately high until after feeding (stage 25) (Wright and Moyer, J. Exp. Zool. 163:215-230, 1966).

A female frog was obtained whose LDH pattern suggested she was heterozygous for a gene for the B subunit usually found in *R.p. sphenocephala* and another B subunit not yet found in the homozygous condition. Starch gel electrophoresis of individual embryos from a backcross of this female to a normal *R.p. sphenocephala* male and an outcross to a *R.p. pipiens* male provides evidence that the B subunits of frog LDH are controlled by alleles at a single genetic locus.

Among those offspring whose genotype does not include genes specifying all the subunits in the maternal pattern, maternal isozymes found in the unfertilized egg persist until eleven days past feeding (stage 25) although the expression of zygote genotype began at heart beat (stage 19). This persistence of maternal isozymes must be due to stable enzyme protein and not to continued enzyme synthesis directed by stable maternal messenger RNA since certain LDH bands theoretically possible in the latter case are not found. (Supported by NIH Fellowship 5-F 1-GM 29, 130-03 and NSF Grant GB 5512.)

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ELIZABETH A. KOCH and ROBERT C. KING, Northwestern University.

Evidence for differential properties of the plasma membranes of certain *Drosophila* cystocytes.

Each developing egg chamber in *Drosophila melanogaster* contains 16 interconnected cystocytes. Previous studies (J. Morph. *121*:55-70) show that fourth generation cystocytes 1e and 2e behave differently from the rest (3e-16e) during germarial development. Only 1e and 2e (the pro-oocytes) form synaptinemal complexes. Since all 16 cells arise through four consecutive divisions of a single cell, mitosis should insure their chromosomal equivalence, and the particulate, cytoplasmic components should be distributed randomly. Since the nucleus and the cytoplasmic organelles are eliminated as the source of the cue for pro-oocyte differentiation, we searched for differences in the cystocyte plasma membranes. Are the membranes of the pro-oocytes different from those of the other 14 cells?

With this question in mind we made an electron microscopic study of serially sectioned, dividing cystocytes. While early mitoses are followed by the formation of cleavage furrows, plasma membranes between nuclei formed at later divisions arise through the fusion of plaques of membrane bound vesicles. A ring of membranous material is synthesized around the spindle, and when this dissolves a canal remains between the daughter cells. Each fourth generation cystocyte can contain 1, 2, 3, or 4 ring canals. A given rim marks the inner limits of a membranous area of specific age, composition, and size. Cells 1e and 2e alone contain 4 canals, each surrounded by a rim of unique morphology. Consequently, the plasmalemma of cell 1e constitutes a mosaic of membranous areas. The pattern of this mosaic is a mirror image of 2e but different from 3e-16e. Thus a unique plasmalemmal pattern possessed by two of the 16 sister cells is accompanied by a unique type of nuclear synthesis. (Supported by Grants GB4891 and E408 from the NSF and ACS, respectively.)

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SR. DOROTHY KLINGELE and HARVEY A. BENDER, University of Notre Dame.

Phenogenetics of two *lozenge* alleles of *Drosophila melanogaster.*

The pleiotropic manifestations associated with the *lozenge* complex includes the impairment of the reproductive capacity. A statistical study, comparing and contrasting the reproductive capacities of the $lz^D$ (reported as dominant by Novitski, 1949) and the $lz^{61}$ (described as fertile by Burdick, 1963) alleles was undertaken. Fertility, fecundity and viability of $lz^D$, $lz^{61}$ homozygotes and Basc heterozygotes were tabulated for a 14-day period. All three factors were found to be markedly reduced in the homozygotes, with the $lz^D$ allele showing the more pronounced effect. Similar studies utilizing the fourth chromosome enhancer, ($spa^{on-1z}$), depressed the fecundity of $lz^D$ heterozygotes.

Histological investigations were performed on seven-day-old $lz^D$, $lz^{61}$ and control females to study abnormalities of the reproductive system and the possible inter-relationship of the imaginal corpus allatum upon the reproductive capacity of *lozenge* females. Pathologies were evident in the homozygous and heterozygous females. The pathologies were more marked whenever the $lz^D$ allele was involved. No apparent differences were noted in the corpus allatum of the *lozenge* females and the control genotypes.

Recombinational studies suggest that $lz^D$ and $lz^{61}$ are heteroalleles and that $lz^D$ is probably located at the $lz^1$ sub-site; $lz^{61}$ is likely a homoallele of $lz^{60e}$, located at the $lz^e$ sub-site. (Supported by USPHS Grant GM-06897-05 and AEC Contract AT (11-1)-38.)

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WILLIAM S. KLUG, D. BODENSTEIN and R. C. KING, Northwestern University and University of Virginia.

Determination of the site of action of the *su²-Hw* gene of *Drosophila melanogaster* through ovarian transplantations.

Females homozygous for the recessive gene, *suppressor² of Hairy-wing (su²-Hw),* show a characteristic ovarian pathology (Am. Zool., 6:512). The chromatin of the endopolyploid nurse cells instead of being dispersed normally throughout each nucleus forms one small and five large condensed

masses. The nucleoli are abnormal in morphology, and the amount of cytoplasmic RNA is reduced. These observations suggest that in the absence of the + allele of *su²-Hw*, the nurse cell nuclei undergo abnormal nucleic acid metabolism.

Various ovarian transplants were performed to rule out the possibility that the ovarian phenotype results from the effect of the mutant gene on other tissues which influence the ovary secondarily.

Ovaries from newly enclosed, adult females homozygous for the *su²-Hw* gene were implanted into host abdomens. The host females were either wild type or homozygous for the gene, *female-sterile* (*fes*, 2-5±). It has been previously shown that ovaries from wild type flies undergo normal development in the abdomen of *fes* flies, and since such hosts are genetically ovariectomized there is no competition between the implant and the host ovary. Ovaries from wild type adults were implanted into flies homozygous for *su²-Hw*. At the end of four, eight, or eleven days, both the donor and host ovaries were recovered and observed as Feulgen-stained whole mounts.

Both the host and donor ovaries developed autonomously in all experiments. Therefore *su²-Hw* ovaries cannot undergo normal development in an abdomen where + ovaries develop to maturity and + ovaries can develop normally in the abdomen of *su²-Hw* females. Thus, the ovarian pathology characteristic of the mutant is due to the genotype of the ovarian cells themselves. (Supported by USPHS grant 5T1GM903)

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ELIZABETH GATEFF and HOWARD A. SCHNEIDERMAN, Western Reserve University.

Developmental studies of a new mutant of *Drosophila melanogaster*: lethal malignant brain tumor (*1* (2) *gl⁴*).

We recently uncovered in our laboratory a mutant of *Drosophila melanogaster* characterized by spectacularly large and transparent third instar larvae. E. B. Lewis has mapped the new lethal mutant and designated it *1* (2) *gl⁴*. It is allelic to *1* (2) *gl²*, lethal giant larva.

To investigate the development of the mutant we used a method devised by Ernst Hadorn, in which we culture fragments of imaginal discs in adults and then examine the developmental capacities of clones derived from these fragments. These clones usually maintain their determination or firm biases for hundreds of cell generations. The results revealed that this mutant has several novel attributes useful in analyzing control systems that regulate development.

We have established clones from mutant imaginal discs and brains. The mutant imaginal discs grow phenomenally, but no longer respond to metamorphosis hormones. One line derived from a mutant disc has been cultured *in vivo* for 21 generations, and repeatedly tested for its ability to metamorphose, without success. Apparently, the mutation permits the cells to grow, but prevents them from responding to metamorphosis hormones.

The mutant brain lobes are enlarged, and mutant brain cells grow rapidly and invasively and kill their hosts. These mutant brain cells have been cultured for 35 transfer generations (about 200 divisions) and clones have been derived from a single cell. It behaves like a malignant tumor. It appears to be the first transplantable non-melanotic malignant tumor found in insects. Its control by a specific gene makes it of special interest.

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DANIEL H. KOHL and FRANK H. MOYER, Washington University.

Electron spin resonance measurements of genetic variation in mouse melanins. (Introduced by Viktor Hamburger)

Melanins are thought to be indolequinone-semiquinone polymers (Swan, 1963). Each semiquinone monomer has an unpaired electron associated with it. Thus melanins are free radicals and, since they are extremely stable free radicals (Commoner, *et al.*, 1957), are ideally suited for study with electron spin resonance spectroscopy (ESR). This technique is extremely sensitive, here detecting $10^{-3}$ $\mu$moles of unpaired electrons at signal:noise = 10.1. While it is difficult to measure the number of unpaired electrons absolutely, the relative number may be reliably determined, often to within ± 10%. ESR can also reveal differences in the molecular environment of the unpaired electrons.

Free radical signals generated by hair from inbred strains of black and brown mice exhibited qualitatively similar spectra. The black hair had 4 times more unpaired electrons/mg hair than did the brown. The spectra from yellow hair were qualitatively and quantitatively different from black hair, having 8 times fewer unpaired electrons/mg hair. The presence of more than one free radical species and/or differences in polymer structure could account for the qualitative differences of the spectra generated by yellow hair.

Melanin granules were prepared by acid hydrolysis of black and brown hair. These granules had approximately the same number of unpaired electrons/gm granule. Thus, the data suggest that the black hair has about 4 times the amount of pigment as does the brown hair. This is of the same order as the ratio of pigment volume between these genotypes obtained by direct measurement (E. S. Russell, 1948). (Supported by NSF grants GB 5512, GB 4595.)

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FRANK H. MOYER and DANIEL H. KOHL, Washington University.

The occurrence of melanin pigment in albino mice.

An investigation of the electron spin resonance (ESR) spectra of hair from inbred albino mice revealed signals similar to those attributable to phaeomelanin in the hair of yellow animals. Prolonged digestion of albino hair in hot concentrated HCl yielded a black pigment which had an ESR spectrum similar to eumelanin. To test the possibility that this pigment was the result of autoxidation of melanin precursors during extraction, pure DOPA was subjected to the extraction conditions, but no pigment was formed.

"Albino" melanin is less dense than "black" or "brown" melanin. Furthermore ESR measurements show that it has about 10 times fewer unpaired electrons per gram HCl digest than "black" or "brown" melanin. Either there are fewer semiquinone residues in "albino" melanin per quinone

monomer or the HCl digest contains a greater proportion of some substance which doesn't contribute to the ESR spectrum.

The fine structure of "albino" melanin as seen in the electron microscope has been compared to that of "black" melanin. The pigment from the albinos consists of small particles, 0.1 $\mu$ or less in diameter, which are often clumped together. The pigment from black animals consists of typical black melanin granules, approximately 1.4 $\times$ 0.5 $\mu$ in size (see Moyer, 1966 Am. Zool. 6:43-66).

These data suggest that phaeomelanin is a precursor of eumelanin and could be taken to suggest that the albino locus controls an enzyme acting later than tyrosinase on the pathway leading to melanin synthesis. (Supported by NSF grants GB 5512 and GB 4595.)

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

Atypical pigmentation in fishes of the genus *Xiphophorus*.

The macromelanophore genes of *X. maculatus* control specific patterns in circumscribed areas. When introduced into other species of *Xiphophorus* they often give rise to abnormal pigmentation resulting in melanosis and melanomas. In hybrids the pigmentation is not only heavier in the primary sites, but other areas are also affected. The $Sd^1$ (spotted dorsal fin) gene, for example, in a *hellerii* genotype also affects the lips, pectoral, anal and caudal fins and most of the body. Often macromelanophores appear at the secondary sites at the same time as (or even before) they are seen in the dorsal fin. In old *maculatus* maintained for one or two years a few macromelanophores develop in the caudal fin, the site at which often the largest secondary tumors arise in *hellerii* hybrids. Enhanced pigmentation may also occur within *X. maculatus* when the pigment genes of one population are introduced into another. In intraspecific hybrids patterns are intensified less than in interspecific hybrids, but the same areas are involved. In the $F_1$ of *maculatus* $\times$ *couchianus* the Sd gene of the Hp stocks produces a heavy melanosis that often covers the entire body from the level of the operculum into the caudal fin. While grafts are made in young fish of potentially melanotic dorsal and caudal fins from Sd donors into wild type hosts, all grafts develop heavy pigmentation and macromelanophores appear in the host tissue immediately adjacent to the graft. No secondary sites of macromelanophore pigmentation are ever formed. These observations suggest that atypical pigmentation at the secondary sites is not due to metastasis. During early ontogenetic stages propigment cells may migrate to many different sites of the body. In *maculatus* the macromelanophore gene within its own gene pool controls primarily the site and time at which these cells differentiate into melanophores. In hybrids there is a loss of control leading to an acceleration of melanophore differentiation in many parts of the body usually free of them. (Supported by PHS grant Ca-06665.)

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SURINDER K. AGGARWAL, ROBERT C. KING, and URMILA AGGARWAL, Michigan State University and Northwestern University.

Developmental morphology of the female *Drosophila* reproductive system.

Light and electron microscopic investigations of ovaries from *Drosophila melanogaster* collected at various times during the larval and pupal period were conducted. The larval ovary contains large populations of small mitotically active cells at its anterior and posterior poles which are separated from each other by a central population containing both small and large cells. The ovaries of middle third instar larvae also contain terminal filaments which are short and lie side by side below the apical cells. The cells at the leading edge of the apical population are able to lay down the tunica membrane. The cells of the basal population produce the basal stalks and cells of the calyx of the oviduct. The formation of the ovarioles is probably accomplished in part by the basipetal migration of an apical population of cells under the restraining influence of the tunica membrane. The advancing sheet of cells flows about the terminal filaments and come to enclose the germ cells and future follicle cells in each terminal filament-capped tube. The number of terminal filaments thus determine the number of ovarioles. Large flattened cells adhering to the outer surface of the ovary seem to lay down the outer membrane. (Supported by grants GB 4891 from NSF and E 408 from American Cancer Society.)

223

JUDITH SHULMAN WEIS, New York University.

Experimental studies on nerve growth factor in teleost fishes. (Introduced by Alfred Perlmutter)

The nerve growth factor (NGF) found in some tumors, snake venom, mouse salivary glands, and embryonic spinal axes, stimulates growth and development of sensory and sympathetic ganglia of mammalian and chick embryos. An antiserum against this protein decreases the size of sympathetic ganglia of young mammals.

To determine if NGF is present in teleosts, spinal axes of a veriety of species were homogenized and tested in an *in vitro* bioassay using spinal ganglia from seven-day chick embryos. The outgrowth of neurites from explanted ganglia indicated the presence of NGF in homogenates from many species tested.

The effect of purified NGF from mouse submaxillary glands was tested on the zebrafish, *Brachydanio rerio*. In this species spinal ganglia do not form until a few weeks after hatching: until this time the sensory function of the nervous system is fulfilled by Rohon-Beard cells. Once formed, the spinal ganglia continue growing in proportion to the size of the individual animal by hyperplasia as well as hypertrophy. Triweekly intracoelomic injections of 0.1 microliter of NGF (100 biological units/microliter) for three weeks caused a significant size increase in spinal ganglia over litter-mate controls, as determined by paper reconstructions from serial sections. Hyperplasia and hypertrophy of neurons caused this increase. Sympathetic ganglia were also enlarged. Similar injections of bovine anti-NGF serum significantly diminished the size of spinal ganglia, due to a reduction in cell number.

An effect of antiserum has been previously noted in other animals only on sympathetic ganglia.

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CHARLES E. WILDE, JR., RICHARD B. CRAWFORD and F. J. HENDLER, University of Pennsylvania, Trinity College, S.U.N.Y. Downstate Medical Center and the Mount Desert Island Biological Laboratory.

Further studies on the relations between the time of RNA synthesis and morphogenesis in *Fundulus*.

In the embryo of *Fundulus heteroclitus*, a serial order of essential morphogenetic stimuli has been demonstrated related to time dependent synthesis of RNA. RNA synthesis is itself completely dependent upon aerobic metabolism. Incubation anaerobically or in cyanide reversibly inhibits RNA synthesis, protein synthesis and morphogenesis. These findings were used further to analyze this problem. Double pulse experiments using cyanide (95% RNA synthesis inhibition, reversible) and actinomycin D (50% RNA synthesis inhibition, irreversible) were carried out. Cyanide incubation was interrupted with a one hour period of incubation in actinomycin D. Cognate experiments where 50% sea water incubation either preceded or followed actinomycin D were also carried out. Thus, at any particular time an irreversible block could be given, temporally. Actinomycin D pulse initiation was begun at fertilization and every minute thereafter for the first hour. Results were scored in terms of normal or defective morphogenesis *in vivo* and by histology. These confirm a serial order of morphogenetic messages, presumably related to RNA synthesis and its dependent protein synthesis but emphasize that these begin during the first minutes following fertilization. Particularly sensitive periods related to axiation occur between the second and fourth minutes and 14th to 16th minutes. Opening these periods results in more normal morphogenesis. These events can be postponed until after high blastula by continuous preincubation in cyanide. At this time the same serial order can be demonstrated anew by similar appropriate experiments. (Supported by grants DE-02047 and HD-00519 from the U.S.P.H.S.)

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F. J. HENDLER, RICHARD B. CRAWFORD and CHARLES E. WILDE, JR., S.U.N.Y. Downstate Medical Center, Trinity College, University of Pennsylvania, and the Mount Desert Island Biological Laboratory. (Introduced by J. Wendell Burger)

Studies on time dependent protein synthesis in *Fundulus* embryos.

Previous studies have demonstrated the pattern of RNA synthesis and the effects of cyanide and actinomycin D on embryogenesis in *Fundulus heteroclitus*. To further elucidate aspects of morphogenetic control, studies on protein synthesis have been conducted. Embryos of varying age were incubated with labeled amino acids for two hours and the extent of incorporation was determined in the hot trichloroacetic acid insoluble fraction. The pattern of incorporation appeared to vary with the amino acid and the developmental stage. A marked increase in incorporation at mid-blastula was found which continually increased with further morphogenesis. Cyanide caused virtually complete inhibition of amino acid incorporation following cleavage. During earlier stages of development the inhibitory effect of cyanide was dependent on the amino acid utilized and the time elapsed after fertilization. Under the conditions of assay, in post cleavage embryos, actinomycin D caused moderate but variable inhibition. Puromycin and pactamycin inhibited amino acid incorporation approximately 90% whereas cycloheximide had no effect. Due to the more marked action of cyanide on protein synthesis when compared with more specific inhibitors, attempts were made to dissociate permeability from metabolic effects. Appropriate experiments have demonstrated that cyanide inhibited intracellularly the incorporation of amino acid into protein. Experiments have been initiated to determine amino acid incorporation during the immediate post fertilization period. From these studies it is clear that frequent changes in amino acid utilization occur during very early embryogenesis. These observations are in agreement with earlier experiments demonstrating the relationship of morphogenesis to RNA synthesis. (Supported by grants DE-02047 and HD-00519 from the U.S.P.H.S.)

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B. A. FLAXMAN and P.F.A. MADERSON, Harvard Medical School.

Organ culture of the lizard epidermis as a system for the study of cyclic protein synthesis and cell pluripotentiality. (Introduced by G. Szabo)

Morphological studies have shown that cyclic skin-shedding in snakes and lizards involves the formation and loss of "epidermal generations". These have a constant specific structure consisting of 6 (and sometimes more) different cell types which arise from an apparently morphologically homogeneous germinal population, which undergoes three periods of intense mitotic activity giving rise to the component parts of each generation.

*In vivo* studies have suggested that the duration of the cycle may be influenced by hormones, but have shed no light on the nature of the mechanisms involved in the production and maturation of the different cell types.

We have grown explants of intact lizard integument and isolated epidermis as organ cultures on Millipore filters.

Our results indicate that considerable cyclic division and maturation of epidermal cells continues to occur *in vitro*. The comparative histology and ultrastructure of control and experimental material are described.

These observations suggest that the potential for cyclic cell division and maturation is intrinsic to the epidermis, perhaps involving a feed-back mechanism within the organ system, while only the duration of individual cycles is regulated by external factors.

It is suggested that this system affords a unique model for the study of epidermal cell pluripotentiality. (Sponsored by U.S.P.H.S. Grant 5 ROI CA 5401-07.)

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BRUCE M. CARLSON, University of Michigan.

The effect of actinomycin D upon epidermal-mesodermal interactions in limb regeneration.

Although the necessity of epidermal-mesodermal contact in normal limb regeneration has been repeatedly demonstrated, the nature of this interaction has remained obscure. In experiments designed to explore the nature of this interaction, the skin was removed from both forelimbs of axolotls. Skin from right limbs was soaked for 3 hours in actinomycin D (5.0 or 10.0 μg/ml 0.6% NaCl). For controls skin from left limbs was soaked in 0.6% NaCl. Each piece of skin was orthotopically replanted, and both limbs were amputated through the treated skin, proximal to the elbow. After an initial healing period, the control limbs regenerated normally. Except for a slightly paler color, limbs bearing actinomycin-treated skin were indistinguishable from the controls, both grossly and histologically, during the first week following amputation. While the control limbs formed early blastemas, no grossly visible evidence of regeneration was apparent in the experimental limbs, but histologically some dedifferentiation was occurring. Normally 3-4 digits were seen in the control regenerates before blastemas appeared in the experimental limbs. By 35-40 days blastemas on some experimental limbs began to develop very rapidly, and within a week some of them attained levels of development close to the controls. Regeneration in others was permanently suppressed. Actinomycin D appears to temporarily suppress the ability of the wound epidermis to cause aggregation of dedifferentiated cells into a blastema, and when the effect wears off, these cells quickly organize into a blastema and begin to differentiate. (Supported by a Rackham Grant, University of Michigan.)

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L. E. DeLANNEY,*† M. K. BLACKLER,† and K. V. PRAHLAD,* Wabash* and Ithaca† Colleges.

The relationship of age to allograph and tumor rejection in Mexican axolotls.

The literature on amphibian tolerance deals predominantly with anurans and signifies tolerance induction in larval forms. Reported here for the Mexican axolotl, a slowly developing, neotenic urodele, are data inconsistent with the viewpoint that tolerance is the rule in all young larvae. Allografts of integument, limbs, and a strain-specific lymphosarcoma generally regressed at 20°C. By contrast, adult axolotls challenged with growing, entire, newly hatched C-strain larvae permit the growth of donors to adulthood as heterochronic parabionts. These hosts will now "tolerate" the proliferation of the strain-specific lymphosarcoma. Young animals received allografts as follows for age-range and number (in brackets): *first set skin*—40-100 (44); *entire sinble limbs*—10-100 (44); lymphosarcoma—281107 (271); *skin as second set after tumor regression*—(34). The generalizations are (1) Skin, limb, or tumor all show signs of rejection but vary from each other. (2) Limbs, rapidly growing, heterogeneous tissue populations, are "tolerated" longest. (3) Limbs gradually resemble host's or are chimeras. (4) The sum of onset and overt total rejection of skin falls in the same span of time and shows little correlation with time of planting. (5) Tumor masses greatly exceeding limb masses are regressed rapidly after onset of rejection. (6) Subsequent plants of C-strain skin after C-strain tumor regression are rejected at accelerated "second set" rates. The Mexican axolotl mounts rejection against allografts of several kinds when challenged as early as 28 days after spawning. (Supported by grants GM 05619 and GM 15363.)

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NICHOLAS COHEN, UCLA School of Medicine.

The maturation of transplantation immunity in the salamander, *Ambystoma tigrinum*.

*Ambystoma tigrinum*'s capacity to recognize as foreign and therefore totally destroy skin allografts appears to mature quite late in larval life. Larvae raised and continually maintained at 25 ± 0.5°C, were challenged 30, 40, 50, 60 and 80 days after they hatched with skin allografts from non-siblings of the same chronological age. Skin grafts on mature adults served as controls.

All 35 adult grafts exhibited a progressive loss of viability with 95% of them being destroyed totally (complete melanophore death). By contrast, about 50% of all the grafts made at 30-50 days post hatching retained full viability (no vasodilation or melanophore fragmentation) for at least 100 days following their transplantation (the duration of each experimental series). Of the grafts in the 30-50 day post hatching group that underwent partial rejection, only 11-17% were totally destroyed; the rest remained 50-95% viable. Although a significantly lower percentage (20%) of the grafts placed on larvae at 60 days post hatching were fully viable for 100 days, only 14% of those exhibiting partial melanophore fragmentation were eventually scored as zeros. The fate of transplants on 80 day post hatching animals was most similar to that of adult grafts. All of these transplants showed some loss of viability and of these about 70% were totally destroyed. In summary, whereas mammals are immunologically competent at the perinatal period, comparable responsiveness in this species apparently develops only after an unusually long period of active larval life. (Supported by grant 1F 2 Ca 30-748-02 from the U.S.P.H.S.).

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NORMAN S. KERR and SYLVIA J. KERR, Univ. of Minnesota.

A new hypothesis concerning plasmodium formation in the true slime mold, *Didymium nigripes*.

Two lines of evidence indicate that amoebae of our strain of *D. nigripes* differentiate into plasmodia without the fusion of either cytoplasm or nuclei. First, time lapse films showed plasmodium formation without ameboid fusion (Exp. Cell Research 45:646, 1967). Second, cytological examination of chromosomes in metaphase from amoebae and plasmodia showed no difference in ploidy. (Several ploidy levels were present in any population of amoebae or nuclei from a single plasmodium.)

The presence of glucose in the absence of other nutrients inhibits the differentiation of amoebae into plasmodia whereas $Ca^{++}$ or $Sr^{++}$ stimulate the differentiation. Evidence bearing on the hypothesis that active glycolysis favors continued ameboid growth instead of plasmodial differentiation will be presented. $Ca^{++}$ may stimulate the differentiation

by inhibiting certain glycolytic enzymes. (Supported by grant AI-05521 from U.S.P.H.S.)

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E. B. EDNEY, University of California, Riverside.

The transition from water to land in the Crustacea Isopoda.

Marine ancestors of the Suborder Oniscoidea were preadapted for transition to land by reason of their reptant locomotion, flat shape, intenal fertilization and brood pouches. Subsequent adaptations in terrestrial forms include reduction of transpiration, modification of the pleopods and the development of sensory and behavioral mechanisms appropriate to cryptozoic land life.

Recent work has been concerned with several aspects of the biology of the group. Population structure and dynamics in relation to controlling and limiting factors have been investigated. The effects of photoperiod and of temperature regimes in relation to breeding and metabolic rate have been demonstrated. Work on the extent and control of vertical and lateral movements suggests that feeding necessitates nightly excursions in some situations, in others the need to lose water by transpiration has been implicated.

Hygroreceptors have probably been identified; olfaction has been proposed as a means of shelter seeking and thigmokinesis as an aggregation mechanism. Microclimatic conditions relevant to life in a xeric biotope have been measured and acclimation to temperature in respect of lethal limits and of other parameters has been found in some species but not in others.

Excretion of gaseous ammonia has been demonstrated. Depression of body temperature by transpiration occurs in species which transpire rapidly, low transpiration rates have been found in xeric species and evidence for a transition effect in the temperature/transpiration relation has been found in at least one desert species. There is still a dearth of information about the effects of desication on osmotic and ionic concentrations in the haemolymph.

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DESMOND E. HURLEY, New Zealand Oceanographic Institute.

The transition from water to land in the Crustacea Amphipoda.

Truly terrestrial Amphipoda are known only in the Family Talitridae, the only family also found extensively in the supralittoral. Species are known from salt marshlands to high mountainsides. Commonly, they are cryptozoic inhabitants of the leafmould of Indo-Pacific forests, from Japan to the Subantarctic Islands, as well as India, Australia and South Africa. Except as recent introductions they are absent from Europe and America. Distribution patterns of other animals would lead one to expect occurrence in South America; evidence is so far against this.

Success in the Indo-Pacific is considered due to invasion of leafmould directly from supralittoral debris; in the Pacific these frequently merge. Leafmold provides an insulated niche with sufficient food and moisture for colonisation with least modification. Apart from a tendency to loss of pleopods, the evidence suggests that adaptation merely continues trends already present in littoral species, e.g., larger and fewer eggs.

Evolutionary history is lacking as fossils are unknown, and, since leafmould species are derived from cosmopolitan littoral genera, they may have arisen independently in different countries. Semicosmopolitan species, e.g., *Talitrus sylvaticus*, may have been partly distributed with plants by man; accidental transplantation from New Zealand to England has been shown. Within New Zealand, present-day distributions imply that some species pre-date the last Glacial Maximum (20,000 years ago).

The Amphipoda have not achieved the terrestrial independence of the Isopoda; they are restricted to a narrow niche. Some species have colonised grasslands but this is not environmentally very different from leafmould. (Supported by the New Zealand Department of Scientific and Industrial Research and by symposium grant GB-6613 from the N.S.F.)

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DOROTHY E. BLISS, The American Museum of Natural History.

The transition from water to land in the Crustacea Decapoda.

Consequent upon their migration from the sea into the intertidal zone and farther inland, eight families of decapod Crustacea (3 in the Macrura Nephropidea, 1 in the Anomura Paguridea, 4 in the Brachyura Brachyrhyncha) have overcome several major hurdles. They have succeeded in (1) adapting to a greater range of environmental temperatures than those of the marine environment; (2) maintaining an adequate respiratory exchange through gills and epithelial membranes even though the medium is air rather than water; (3) maintaining a normal balance of salts and water, which in some instances has required rigid conservation of water; (4) maintaining intra- and inter-specific relationships, including those of courtship, mating, rearing the young, and defending oneself against enemies within and outside of the social group, if such a group exists.

Perhaps the most extensive adaptations are those concerned with a normal balance of salts and water. Involved are the uptake of salts, mainly by certain of the gills, and as a direct consequence, the uptake of water by these same gills. Also involved are the excretion of excess salts, mainly by the antennal glands, and as a direct consequence, the excretion of water. In regions where water is in short supply, its uptake and conservation has required morphological, physiological, and behavioral modifications. Among the most significant are those involving the gut in the temporary storage of water. In a species of gecarcinid, movement of water from the hemolymph into the gut takes place under neuroendocrine control at ecdysis. When such redistribution of water is prevented, the animal displays an abnormal "profile" after ecdysis. (Supported by grants GB-4380 and GB-6388 from the N.S.F.)

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JOHN D. COSTLOW, JR., and C. G. BOOKHOUT, Duke University.

The effect of environmental factors on larval development of the land crab *Cardisoma guanhumi*.

Larvae of the land crab *Cardisoma guanhumi* were reared in twenty-four salinity-temperature combinations to determine how these factors affect survival and development of the larvae. The effect of salinity on post-larval molting and growth was also studied to determine when crabs of this species become adapted to the terrestrial environment in which the adults are normally found.

Although a small number of zoeae survived to megalops in salinities of 25-40 p.p.t., 20°C, none completed metamorphosis. At 25°C, some survival to the first crab was observed (10-26%) in salinities of 15-40 p.p.t.; at 10 p.p.t., larvae did not develop beyond the second zoea and at 45 p.p.t., only 1% completed development. At 30°C, there was a higher percentage of survival in salinities of 25-35 p.p.t., than observed at 25°C, but none of the larvae completed development at 10 p.p.t. At 25° and 30° C, a higher percentage of survival was observed in the megalops stage than in the zoeae.

Duration of the larval stages was affected by certain salinities. At any one temperature, rate of development was similar in salinities of 25-35 p.p.t. As the salinity was increased or decreased, the time required for development to the crab was lengthened.

Increase in size of crabs through the first seven post-larval molts was similar in salinities of 5-35 p.p.t., 25°C. In fresh water, size increments were reduced but the frequency of molting was significantly higher.

The results are considered as they may apply to adaptation of the adult crabs. (Supported by grant GB-5711 from the N.S.F.)

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PAUL P. RUDY, JR., University of Lancaster.

Sodium and water balance of *Potamon edule*.

*Potamon edule* is a freshwater brachyuran crab which lives along streams draining the Appenines of Italy. This crab is semi-terrestrial, living in burrows and beneath mats of shrubbery which line the streams.

A study of the sodium and water balance of *Potamon edule* is reported. This study consists of measurements of total water and sodium flux rates, urine production, drinking, and water losses to dry air. It also includes a description of the sodium uptake curve with values given for half-saturation and for maximum uptake rate.

A comparison is drawn between the salt and water balance of this freshwater semi-terrestrial species and the better known marine semi-terrestrial species. (Supported by NIH Fellowship 5-F2-GM-15, 819.).

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D. EUGENE COPELAND, Tulane University.

Fine structure of salt and water uptake in *Gecarcinus lateralis*.

Each pericardial sac of *Gecarcinus lateralis* has a slight bulge into the posterior region of the branchial cavity and closely underlies the gills in that region. When living on moist sand, the animal absorbs water by capillary attraction to the setae of the lower surface of the body, then through channels at the base of the legs into the branchial cavity, and the water is, presumably, then finally absorbed by the surface of the pericardial sac. Electron microscopy lends little support to the theory of water absorption by the pericardial sac. The wall has a heavy membrane with no signs of glandular activity. Absorption, if it occurs, must be by simple osmosis. There are, however, highly developed, complicated cellular areas in the respiratory lamellae of those gills overlying the pericardial sac. The cells form a single layer with extensive and multitudinous lateral interdigitations. The bases of the cells, adjacent to the cuticle, are thrown into narrow folds at the bottom of which pinocytosis occurs on a large scale. The cells are densely populated with mitochondria. It is hypothesized that the ground water reaching the surface of the pericardial sac is transferred to the gill lamellae and that the cellular mechanism actively absorbs salt from the water. The water is then, in turn, absorbed. A possible structural basis for Diamond's theory of water transport by local osmosis is discussed. (Supported by grant GM-06836 from the N.I.H. and grant GB-676 from the N.S.F.)

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LINDA HABAS MANTEL, The American Museum of Natural History.

The foregut of *Gecarcinus lateralis* as an organ of water balance.

The permeability of the foregut of the land crab *Gecarcinus lateralis* to $H^3$, $Na^{22}$, and $Cl^{36}$ was studied *in vitro* at various stages of the molt cycle. During anecdysis, or intermolt, the foregut is lined with a chitinous layer and is almost completely impermeable to water and salts, both in the direction hemolymph → lumen and lumen → hemolymph. Early in proecdysis the chitinous lining detaches from the underlying epidermis. Since the unlined epidermis is fragile, no viable *in vitro* preparation can be made. Immediately following ecdysis, before the new cuticle hardens, the foregut of animals with eyestalks is very permeable to water and salts in the direction hemolymph → lumen, while that of eyestalkless animals is impermeable.

When an extract of the thoracic ganglionic mass of *G. lateralis* is added to the hemolymph side of the foregut *in vitro*, there is immediately a large increase in permeability to water and salts. This occurs in the foregut of crabs with eyestalks, whether anecdysial or immediately post-ecdysial, and also in eyestalkless crabs immediately after ecdysis. Boiling the extract destroys its activity. If unboiled extract is applied to a preparation that has been treated with boiled extract, there is no increase in permeability.

These and other findings indicate that the land crab *G. lateralis* moves water and salts into its foregut at ecdysis. This water remains in the foregut for several days, the period when the crab is vulnerable to desiccation. This entire process, which is under neuroendocrine control, may be an adaptation to terrestrial life. (Supported by Postdoctoral Fellowship HD-23,985-01A1 from the U.S.P.H.S. and by grants GB-4380 and GB-6388 from the N.S.F.)

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W. B. VERNBERG and F. J. VERNBERG, Duke University.

Respiratory adaptations in Crustacea from different habitats.

A number of studies have shown that the respiratory rate at both whole animal and tissue level is greater in terrestrial and semi-terrestrial Crustacea than in aquatic forms.

An enzyme system that can react directly with molecular oxygen is cytochrome *c* oxidase. As a general rule, "the idea has been that, the more aerobic the cells or tissue, the more fully developed will be the cytochrome system contained therein" (Nielands and Stumpf, 1958). It would be logical to assume, then, that as an animal evolved toward terrestrial life, the cytochrome *c* oxidase activity would increase.

Accordingly, cytochrome *c* oxidase activity has been assayed spectrophotometrically in tissues of three species of crabs: the spider crab *Libinia emarginata*, which is a relatively deep water species, the blue crab *Callinectes sapidus*, a shallow water species, and the ghost crab *Ocypode quadratus*, which is largely terrestrial and is found on open beaches. Since there is some evidence that cold enhances cytochrome *c* oxidase activity, tissues from both cold-acclimated (10°C) and warm-acclimated (25°C) crabs were used. Three tissues from each species of crab were assayed; mid-gut gland, gill, and muscle from the walking legs. These assays were made at 10°C, 20°C, 30°C, and 40°C.

Among the three species, tissues from both cold- and warm-acclimated *L. emarginata* tended to have the lowest rate of activity, tissues from *O. quadratus* the highest. Of the three tissues, muscle had the highest activity in all three species. The pattern of response to cold-acclimation tended to reflect the thermal regime of the different habitats. (Supported by grant GB-4600 from the N.S.F.)

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DON CURTIS MILLER and F. JOHN VERNBERG, Union College and Duke University Marine Laboratory.

Some thermal requirements of temperate and tropical zone fiddler crabs influencing geographic distribution.

Two tropical species of fiddler crabs, *Uca rapax* and *U. thayeri*, may occur as far north as St. Augustine, Florida, following a few years with mild winters, or they may be rare north of Cape Kennedy following a severe winter, such as occurred in 1957-58. As the $L.D._{50}$ at 10°C for 18°C acclimated *U. rapax* occurs in 4.5 days, it is apparent that winter kill can serve to limit the northward distribution of tropical *Uca* in Florida. This response is in dramatic contrast to the thermal adaptation of the temperate zone species inhabiting the same region, which can cold acclimate and hence survive low winter temperatures.

Factors limiting the range of two temperate zone species, *U. minax* and *U. pugnax*, in central Florida were not identified. The shallow lagoons of this region experience extreme temperatures and salinities. Yet in the laboratory, over 66% of the crabs could tolerate 32-36° in salinities of 6 or 66%, excepting *U. minax* in 66%.

Passano's work (Biol. Bull. 118:129-136) on inhibition of molting at 20° and below in *U. pugnax* suggests one way *Uca* may be limited north of Cape Cod. This is in agreement with the observed distribution pattern and regional temperature data. Repetition of Passano's study yielded similar results with *U. pugnax, U. pugilator*, as well as tropical *U. rapax*. It is hypothesized that a shift in the thermodynamics of molting has not occurred in temperate zone *Uca*, although they evolved other thermal adaptations, which permit survival at temperatures well below the 20° "minimum" of the tropics. (Supported by grants G5577 and G8788 from the N.S.F.)

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JAMES R. REDMOND, Iowa State University.

Oxygen transport by the blood of the land crab *Gecarcinus lateralis*.

Conditions relating to oxygen transport were measured in the pericardial blood and venous outflow from the first walking leg of *Gecarcinus*. Oxygen-dissociation curves for *Gecarcinus* hemocyanin were found to be sigmoid and, at 27°C and pH 7.45, have a $p_{50}$ of about 17 mm Hg oxygen. Average oxygen partial-pressures as measured by oxygen electrode indicated a $pO_2$ of 32 mm Hg in pericardial blood and 9 mm in the venous samples. Van Slyke analyses of similar samples gave an average of 2.17 volumes per cent oxygen capacity for whole blood, 1.45 volumes per cent for pericardial blood, and 0.61 volumes per cent for venous blood. Estimates based on the Van Slyke analyses indicated an average $pO_2$ of 28 and 14 mm Hg in pericardial and venous samples respectively, these values agreeing fairly well with the oxygen electrode measurements. Of the oxygen delivered to the tissues, about 94% is carried as oxyhemocyanin and about 6% is carried in physical solution in the blood. As the blood passes through the gills, the hemocyanin, on an average, becomes 80-85% saturated with oxygen and returns from the tissues 20-40% oxygen saturated. Compared with the relatively few decapod crustaceans for which similar data are available, the blood of *Gecarcinus* has a relatively high oxygen capacity and transports more oxygen to the tissues per unit volume of blood. (Supported in part by grant GM-11199 from the U.S.P.H.S.)

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LEONARD STUTMAN and MARILYN DOLLIVER, St. Vincent's Hospital, New York, N. Y.

Coagulation mechanism in the hemolymph of *Gecarcinus lateralis*. (Motion picture)

Hemolymph of *Gecarcinus lateralis*, maintained on a constant diet and 50% sea water, was drawn from a limb with the use of a saline-washed syringe and 4%, 12.5%, and 25% sodium citrate, or ethylene diamine tetra acetic acid (EDTA), or heparin. Only 12.5% and 25% sodium citrate or EDTA were satisfactory anticoagulants. All clotting tests were performed at the ambient temperature of 25°C. Examination of hemolymph under phase microscopy reveals a paucity of cellular elements (less than 1% by volume of cells as compared to more than 40% cells by volume in most mammalian systems). Spontaneously clotting fluid demonstrated thick, branching, fibrinous strands of material that is non-antigenic to rabbit anti-human fibrinogen and fibrin anti-sera. The most striking finding about clotting times was their extreme variability:

average 8 minutes, range 15 sec to infinity, intermolt; average 3 minutes, similar range, premolt. Recalcification was obtained with M/7 and M/14 $CaCl_2$ revealing dependency on calcium ion. However, when *G. lateralis* serum or plasma was used in any complex system including prothrombin determinations, partial thromboplastin times, or thromboplastin generation times, there was no effect. Protein content, which ranges between 7-13 gm per 100 ml, does not play an important role in the coagulation mechanism. Attempts to identify the fibrinous material by cellulose acetate electrophoresis and analytical ultracentrifugation revealed 4 peaks by electrophoresis and 3 peaks in the Spinco Model E. All were globulin-like; the 3 peaks in the ultracentrifuge were an 18 S, 14 S, and a 7 S protein. The fibrin-like protein was not definitely identified. (Supported by grants from the Ripple and Polachek Foundations.)

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WOLFGANG WIESER, Zoologisches Institut der Universität, Innsbruck.

Uptake of food and digestion in terrestrial crustaceans.

The basic metabolic adaptation to terrestrial life is a shift of entrance pathways for some nutrients from gills and external body surfaces to gut. For example, the more terrestrial species of littoral crustaceans switch to food as the main source of water and salts. Thus, the intertidal amphipod *Orchestia platensis* may cover its salt demands from algae on which it feeds, but it is also dependent upon the salinity of the substratum. The more terrestrial *Ligia oceanica,* on the other hand, has divorced itself entirely from the restrictions of substrate salinity.

No serious difficulties accompany uptake of mobile cations from food, but a shift from aqueous solutions to plant material as the source of nutrients poses special problems in assimilation of heavier metals. Some important heavy metal ions are soluble in water but form stable organic complexes in plant cells. This poses serious adaptive problems for marine animals with terrestrial ambitions.

There is evidence for the following adaptive trends: Increase in storage capacity of hepatopancreas, particularly for Cu and Zn; tighter regulation of movements of Cu and Zn to reduce losses; more efficient compartmentalization of hepatopancreas and participation of blood and cell proteins as transport vehicles in order to reduce toxicity of Cu and Zn; and, most important, close cooperation of herbivorous crustaceans with microorganisms, which render Cu (and perhaps Zn) available for resorption. (Supported in part by Symposium Grant GB-6613 from the N. S. F.)

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ROY HARTENSTEIN, SU College of Forestry at Syracuse University.

Nitrogen metabolism in *Oniscus asellus.*

Various particulate fractions and the soluble fraction of the body wall, midgut, and hepatopancreas of the above isopod were assayed for adenine aminohydrolase, xanthine oxidase, uricase, allantoinase, allantoicase, urease, and glyoxalase to determine the major end-products of purine catabolism. Similar enzymatic fractions were assayed for carbamoyl phosphate synthetase, ornithine carbamoyltransferase, argininosuccinate synthetase, argininosuccinic acid lyase, and arginase to determine whether the required enzymes of the urea cycle are present. The results of these assays are discussed in light of overall nitrogen metabolism and with reference to the production of ammonia, known to be a volatile end-product in terrestrial isopods.

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CHARLES A. GIFFORD, Alfred University.

Uric acid deposition in the land crab *Cardisoma guanhumi.*

Nitrogen excretion in terrestrial decapod Crustacea has not been extensively studied. Aquatic forms secrete mainly ammonia. Other terrestrial arthropods dispose of waste N by forming uric acid (insects) or guanine (arachnids), which is stored in accumulation organs or excreted.

During the course of another study in which a large number of *C. guanhumi* were dissected, it was noticed that variable amounts of insoluble white particulate matter occurred throughout the hemocoel. It is particularly abundant around the stomach and hepatopancreas, in the cardiac pouches, the space surrounding the hindgut in the abdomen, the lower lateral margin of the epithelial gill chamber lining, and in the bottom of the cephalothorax. The thoracic ganglion may be completely embedded in it. It also occurs in the joints of the appendages.

Peaks of infrared absorption spectra of samples taken from around the hindgut and rinsed off the outside of the hepatopancreas, then washed with water and alcohol, match those of reagent grade uric acid, although they are slightly less well defined. Flame photometric analyses of water-washed samples showed only traces of Na, K, Ca, and Mg. The substance is thought to be uric acid.

Although reproducible quantitative estimates of total uric acid have not been achieved, freshly captured crabs, judged by other criteria to have molted recently, have contained little or none of the substance. Crabs held in the laboratory for several months without molting have contained relatively large amounts. Blood uric acid concentrations estimated by several methods have ranged from 160 to 330 $\mu$gm/ml, similar to values for other uricotelic animals. (Supported by Grant GB-941 from the N. S. F.)

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JOHN D. O'CONNOR and LAWRENCE I. GILBERT, Northwestern University.

Alterations in crustacean lipid metabolism during the molt cycle.

Investigations concerning the control of crustacean lipid metabolism were performed using the land crab *Gecarcinus lateralis* and the fresh water crayfish *Orconectes virilis.* Both *in vivo* and *in vitro* experiments utilizing $^{14}$C-1-acetate as substrate indicate that lipid synthesis by the hepatopancreas of *G. lateralis* is significantly altered by destalking, the destalked crabs synthesizing lipid at a much higher rate than their normal counterparts. Further *in vivo* experiments showed that the most actively synthesized lipid component of destalked animals is phospholipid, the specific activity of which

is always significantly higher than in normal crabs. Experiments with *O. virilis* using glucose differentially labelled in the 1 ($^{14}$C-1-glucose) and 6 position ($^{14}$C-6-glucose) indicate that the rate of lipid synthesis in isolated hepatopancreas decreases as the gastrolith/carapace (G/C) ratio increases. The data suggest that the rate-limiting step in lipid synthesis in the premolt crustacean occurs prior to the formation of acetate. (Supported by Grant AM 02818 from the U.S.P.H.S. and Training Grant 5T1GM 903 from the U.S.P.H.S.)

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MICHAEL R. WARBURG, Israel Institute for Biological Research, Ness-Ziona.

Behavioral adaptations in terrestrial isopods.

The Oniscoidea are the only group of Crustacea inhabiting all terrestrial environments including the desert region. Accordingly their response to environmental factors such as temperature, humidity, and light indicates a gradual change in pattern related to the habitat. Thus in isopods from the littoral zone, temperature is less significant than is the response to moisture; similarly photoreaction is of secondary significance. In isopods from mesic habitats *(Oniscus, Porcellio, Armadillidium)* the response to light is not of major significance, whereas both hygroreaction and thermoreaction are important. These isopods indicate a strong positive thermoactive response whereby speed increases with temperature (Warburg 1964, Anim. Behav. 12:175).

The response of isopods from xeric habitats in semi-arid regions (*Armadillo officinalis* from Israel, *Buddelundia albinogrisescens* from South Australia) is mostly photonegative. In this respect they are similar to some desert isopods (*Venezillo arizonicus* from southwestern United States and *Armadillo albomarginatus* from the Negev Desert, Israel), where the negative photoreaction is of great ecological significance and does not change with temperature or humidity. These desert isopods are strongly negatively thermoactive and their activity in fact drops when temperature rises. Another desert isopod *Hemilepistus reaumurii* is an exception in that it is negatively photokinetic only at higher temperatures whereas at moderate temperatures it is positively photokinetic. Indeed in winter and spring on windless days it can be seen on the surface when temperatures do not exceed 30°C. (Supported in part by Symposium Grant GB-6613 from the N. S. F.)

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K. RANGA RAO, Andhra University, Visakhapatnam, India.

The pericardial sacs of *Ocypode* with relation to water conservation, molting, and behavior.

The crabs *Ocypode macrocera* and *O. platytarsis* are found in the intertidal region of the Visakhapatnam beach, but *O. cordimana* is semi-terrestrial and can be found living up to 200 yards away from the shore. The surface area of the pericardial sacs in *O. cordimana* is larger than that of *O. platytarsis* and *O. macrocera*. The specimens of *O. cordimana* have been maintained in good condition for periods up to ten months on sand dampened with tap water. Specimens of *O. platytarsis* and *O. macrocera* did not survive under these circumstances.

The pericardial sacs of the three species of *Ocypode* swell in relation to the molt cycle. However, the phase of the intermolt cycle in which swelling occurs varies with the species. In *O. cordimana* the pericardial sacs swell during the terminal plateau of proecdysis if the specimens are maintained on sand moistened with tap water, and this plays a vital role in the conservation of water. In contrast, the pericardial sacs of *O. macrocera* and *O. platytarsis* swell only during the active phase of ecdysis and obviously play no significant role in the proecdysial uptake and retention of water.

Histochemical and chemical analysis showed deposits of uric acid in the epithelium of the pericardial sacs of *O. cordimana,* while similar deposits were not encountered in the pericardial sacs of *O. macrocera* and *O. platytarsis.* These results indicate that among the species of *Ocypode,* the role of the pericardial sacs varies in accordance with the necessities of water conservation of each species in the natural environment. (Supported by the Ministry of Education of the Government of India and by Symposium Grant GB-6613 of the N. S. F.)

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FRANKLIN H. BARNWELL, Northwestern University.

The role of rhythmic systems in the terrestrial adaptation of fiddler crabs.

The fiddler crabs are semi-terrestrial and, hence, intermediary in the transition of decapod crustaceans from the sea to the land. Many species inhabit the intertidal zone where they function as terrestrial creatures at low tide but pass the time of high tide in underwater burrows. In these crabs tidal rhythmicity is an important feature of their normal terrestrial activity. At the same time the crabs may show specific diurnal and nocturnal habits and so are influenced by the day-night cycle.

Serving to adapt the fiddler crabs to the daily and tidal periodicities of the environment are persistent rhythms in their physiological and behavioral processes. These rhythms contain both daily and tidal components. In natural populations the tidal component is subject to extensive modification, through interaction with the daily component, and in ways related to tidal conditions in the local habitat. Examples of these latter modifications have been obtained in recent studies of the overt rhythms in metabolic rate and locomotor activity in fiddler crabs from North and Central America. These ecologically significant adjustments include the following: 1) differences in the form of the tidal component in crabs from two levels of the same beach; 2) absence of the overt tidal rhythm in crabs from non-tidal habitats and its induction by geographic transplantation of the crabs to the intertidal zone; and 3) in crabs from a region of mixed tides, changes in the form of the tidal component reflecting the complex changes in the form of the tidal cycle. (Supported by O.N.R. Contract 1228-30 and N.S.F. Grant GB 3481.)

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WILLIAM HERRNKIND, University of Miami.

Development of celestial orientation during ontogeny in the sand fiddler crab *Uca pugilator.*

Adult sand fiddlers on the lower beach or in shallow water exhibited a landward directed escape response when disturbed. Both field releases and tests in artificial situations revealed that this response was guided visually by the sun or polarized sky-light, although prominent landmarks modified the preferred direction. The landmark influence was especially strong when crabs became desiccated.

The celestial orientation so obvious in the adult was not apparent in early crab stages. Responses of crabs over the full range of ontogeny were tested by placing from 1-5 in a 30 cm diameter, clear plastic pan set in a sloping base that permitted only a view of the sky. Positions were recorded every 15-30 seconds for 5-20 minutes. Crabs less than 6 weeks old, collected from the field or reared in the laboratory from the egg, exhibited indistinct (but not random) preferences in variable directions. Crabs 2-5 months old from the field oriented more strongly but only occasionally landward. At 6-8 months of age, definite landward preferences appeared and became consistent at about 10-12 months of age. During the stages prior to this, crabs responded readily to artificial landmarks (plastic screens) that presented areas of optical contrast. Test crabs 1-5 months old generally travelled toward the landmark independent of its position relative to the sun or plane of sky polarization. (Supported in part by a NASA predoctoral fellowship.)

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

Aesthetasc structure in three decapod Crustacea—a comparative study.

A comparative study of the thin-walled aesthetasc pegs on the antennules of three genera of crabs has revealed differences in structure that may be correlated with the environment in which the animals live. *Coenobita* spp. are terrestrial hermit crabs and *Pagurus samuelis* is a marine one (tribe Anomura); *Cancer* spp. (tribe Brachyura) are marine true crabs. Despite their relative phylogenetic separation, the two marine crabs *Cancer* and *Pagurus* have similar aesthetascs, whereas those of *Coenobita* differ radically, even from its relative *Pagurus*.

The aesthetasc hairs consist of sheaths of cuticle surrounding the ciliary dendrites of bipolar neurons, whose cell bodies are grouped beneath the bases of each hair. Those of *Pagurus* and *Cancer* are long and slender, with the basal bodies arising within the aesthetascs themselves. The cuticle distal to the basal bodies is uniformly thin and permeable. The aesthetascs of *Coenobita* are short and blunt and partially recumbent, so that one surface is exposed and the other protected. The thin cuticle is limited to the exposed surface. The dendrite branches lie immediately beneath it, the rest of the lumen of the hairs being occupied by sheath cells. Cilia, basal bodies, and all more proximal elements lie well beneath the bases of the hairs.

The limited exposure of the dendritic tissue in the short asymmetrical *Coenobita* aesthetasc suggests that water loss may be an important problem in the terrestrial organ but not in its marine homologues.

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HOWARD O. WRIGHT, University of Houston.

Visual displays in brachyuran crabs. (Motion picture)

Studies of nine genera in the families Grapsidae and Gecarcinidae are presented, with preliminary observations in several other families of Brachyura. There are two major groups of displays of the chelae, the Lateral Merus and Chela Forward groups. The genera *Uca* and *Hemigrapsus* also use other displays, which are possibly derived from the Lateral Merus.

The Lateral Merus is used by all crabs studied, except the commensals and some cryptic species. I have seen the Chela Forward only in two families of the Grapsidae and in the genus *Ocypode*, but it is uncertain if it has evolved more than once. All crabs which use the Chela Forward also use the Lateral Merus. In some species the Chela Forward is used in most agonistic behavior and in courtship, but in others the Chela Forward may be confined to courtship.

Both Chela Forward and Lateral Merus have several subtypes. These subtypes may be used in very specific situations, such as the Reverse Lateral Merus used in burrow defense by *Cardisoma crassum*. The subtypes may also correspond to different intensities of motivation. (Supported by an NSF Science Faculty Fellowship and a Smithsonian Visiting Research Associateship.)

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MICHAEL SALMON and SAMUEL ATSAIDES, University of Illinois and University of Maryland.

Visual and acoustic signaling during courtship of fiddler crabs. (Motion picture)

Male fiddler crabs (Genus *Uca*) employ both visual and acoustic signals to attract females to their burrows for mating. The complexity of the interplay between these two signal modalities is discussed and attributed to a semi-terrestrial existence, as well as to a colonial social organization. The latter has promoted frequent intra- and interspecific contacts in a variety of behavior contexts, and the evolution of species-typical displays used during courtship, territorial defense and fighting.

The majority of species found along the Atlantic and Gulf coast of the United States have similar types of courtship systems. A quantitative description of the signals is offered, and the principal differences between closely related species discussed. Some preliminary data is given on the absolute intensity levels of sounds produced by males in the field, and thresholds of response to pure tones in the laboratory. Some further conclusions concerning the evolution of courtship in *Uca* will be discussed. (Supported by grant GB-3430 from the N.S.F.)

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HERMANN SCHÖNE, Max-Planck-Institut, Seewiesen.

Agonistic, sexual, and cleaning activities in semi-terrestrial ocypodids and grapsids. (Motion picture)

The mechanisms of social communication and sex recognition are based mainly on the chemical

and tactile senses in most aquatic brachyrhynchs, but optical and vibratory cues play an important role in the communication systems of the semi-terrestrial groups of the ocypodids and grapsids. In addition to the well-known work on fiddler crabs, observations have been reported on about 15 other species of ocypodids and 7 species of grapsids. They show striking movements of the chelipeds and other optically conspicuous behavior patterns that seem to have a communicative function. Some of these patterns have been interpreted as agonistic behavior, in others the movements may function in courtship.

The agonistic behavior of *Ocypode quadrata* and *Goniopsis cruentata* and the courtship behavior of *Goniopsis* will be described in connection with films (Numbers E 897, E 900, and E 899 of the Encyclopaedia cinematographica, Institut für den wissenshaftlichen Film, Göttingen).

In addition I will report on a behavior of *Goniopsis*, in which the animal covers itself with a frothy mass and acts as if cleaning (Schöne, E 1054). (Supported by Deutsche Forschungsgemeinschaft and by symposium grant GB-6613 from the N.S.F.)

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M. PATRICIA MORSE, Northeastern University.

Functional morphology of the digestive system of the nudibranch mollusc, *Acanthodoris pilosa*.

The digestive system consists of a buccal cavity for food reception, esophagus for conduction of food, stomach and digestive glands for food distribution, digestion and absorption and cecum and intestine for feces formation. Various sections of the digestive tract show modifications correlated with suction feeding including a muscular buccal pump, a thin-walled crop and a reduced cecum. The digestive gland epithelium consists of two cell types, the pyramidal-shaped crypt cells and the more numerous tubule cells with their variants A and B. The epithelium of the buccal cavity, esophagus, stomach and intestine is ciliated and a variety of gland cells supply secretions to all regions. Movement of food is primarily by ciliated currents and intracellular digestion is shown to occur in the digestive gland epithelium.

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JOHN MAXWELL ANDERSON, Cornell University.

Some details of the digestive system in a sea-star, *Oreaster reticulatus*.

The large cardiac stomach of *Oreaster* consists of one median and two lateral pouches in each ray. It communicates broadly with the overlying pyloric portion, which is deeply divided by 5 pairs of ducts radiating to the pyloric caeca. The constriction in the stomach is ringed by a fibrous girdle, joined by stout branches of the extrinsic retractors in each ray. Other branches provide web-like intrinsic retractors inserting on the median pouches. The lateral pouches receive still other branches which bifurcate repeatedly, attach to the walls of the pouches, run down and eventually terminate as very numerous end-branches along a line of insertions near the esophageal end of the stomach. These fibers correspond to the "Class 1" intrinsic retractors described for *Asterias* and *Patiria* (Anderson, 1954, 1959; Biol. Bull. 107, 117). Additional bundles of fibers, resembling the "Class 3" fibers of *Patiria*, run vertically outside the peritoneum low in the stomach. *Oreaster* is frequently observed (Thomas, 1960, Quart. J. Fla. Acad. Sci. 23) with stomach everted against the sandy bottom; the supplementary retractor elements presumably function in relation to this habit. The walls of the pouches are traversed on their inner faces by branching systems of flagellated gutters, corresponding to the patterns of the Class 1 retractors on the outer surface.

In each pyloric caecum, the median duct is deep and branches alternately right and left, forming small, narrow pouches beneath the glandular bags making up the aboral part of the organ. Secretory stripes on opposite faces of the pouches, not permanently fused, separate a series of flagellated channels. These features differ in detail from corresponding structures in previously studied species belonging to different families.

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ROBERT F. BROWNING and CHARLES F. LYTLE, The Pennsylvania State University.

*Hydractinia* as a food collecting device for hermit crabs. (Motion picture)

The association of the colonial hydroid, *Hydractinia*, with hermit crabs is a frequently cited example of symbiosis although previous workers have found that the relationship is not obligatory and that *Hydractinia* planulae do not settle or develop preferentially on shells occupied by hermit crabs. The hydroid may benefit from the association by obtaining a constant supply of oxygen and perhaps by sharing in the food of the hermit crab but the benefits to the hermit crab have been unclear.

We have studied this association and the feeding behavior of the hermit crab, *Pagurus*, with the aid of cinematography. Analysis of these films and direct observations have indicated that the hermit crab is able to utilize food captured by the hydroid. When a few brine shrimp nauplii are deposited near the hermit crab, the crab makes ineffectual efforts to feed on the small darvae. When an abundant supply of nauplii are provided, large numbers are captured by the tentacles of *Hydractinia*. The hermit crab then seizes clusters of the nauplii which have been immobilized and concentrated by the hydroid. As the supply of nauplii captured by the hydroids within reach of the chelipeds is depleted, additional nauplii are gathered from more distant portions of the hydroid colony by raking movements of the second and third pereiopods. It therefore appears that in the presence of abundant zooplankton, the hermit crab is able to feed on small organisms captured by *Hydractinia* and thus extend the size range of food particles (organisms) which it can utilize.

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THEODORA PAULSON and R. S. SCHELTEMA, Woods Hole Oceanographic Institution.

Selective feeding on algal cells by the veliger larvae of the gastropod *Nassarius obsoletus* (Say).

The planktonic larvae of the prosobranch gastropod, *Nassarius obsoletus* (Say), are able to feed selectively upon several species of phytoplankton.

When given the opportunity to feed upon a mixture of *Dunaliella* sp., *Phaeodactylum tricornutum*, and *Cyclotella nana*, the veliger larvae exhibit a definite order of preference.

Several thousand larvae of the same age were allowed to feed in experiment jars containing one to one-and-a-half liter mixtures of the algae. Control jars contained the food mixture without larvae. After ten to fourteen hours the concentrations of the algae in the experiment jars and in the control jars were determined using a blood-cell counter (haemocytometer). It was to be expected that if the veligers fed at random, the relative proportions of the three species would be the same as those of the control. However, if the veligers showed preference, then the proportions of the different algae would be significantly altered from those of the control.

In all sixteen trials, the proportions of the three species of algae in the experiment jars did differ significantly from that of the control. The concentration of *Cyclotella nana* declined the most, relative to the control, in a majority of the trials, while *Phaeodactylum* ranked second and *Dunaliella* third in order of depletion. As the sixteen trials were distributed among three arbitrary veliger size categories, certain trends could be observed with respect to veliger age. *Dunaliella* was clearly the last choice for veligers of all sizes. However, differential selection for *Phaeodactylum* and *Cyclotella* diminished with increasing veliger size.

The mechanism of selective feeding in *Nassarius obsoletus* veligers remains unknown. The possible advantage of such selection to the veliger must be determined by subsequent growth experiments testing the nutritive value of each of the three species of algae.

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R. VON BURG and W. C. CORNING, Fordham University.

Central nervous system regulation of cardiac rhythms in *Limulus polyphemus*.

Extensive physiological studies have been conducted on both the heart and the eye of *Limulus polyphemus* but the neurophysiology of the central nervous system has not received much attention. This investigation attempted to extend the earlier findings of Carlson (Am. J. Physiol. 1903-1909) and Pax and Sanborn (Biol. Bull. 126:133-140, 1964), concerning central regulation of cardiac rhythms.

Using the anatomical findings of Patten and Redenbaugh (J. Morphol. 16:1-91, 1900) and Hanstrom (Acta Univ. Lund. Aud. 22:1-79, 1926) as a guide, and lesioning and stimulation as the principal techniques, the forebrain, circumesophageal ganglia and the ventral cord were investigated for controlling influences on the heart.

The findings indicate that the forebrain has a strong contralateral tonic influence on the heart. This is mediated by both the accessory brain nerves and the ventral cord. At the level of the hindbrain there is also a tonic control that is mainly inhibitory. In the ventral cord the influences are both acceleratory and inhibitory and there exists a diminishing hierarchy of influence starting with the first abdominal ganglion and extending through the fourth. Though the main effect of the intact cord is inhibitory, severing contralateral pathways exposes acceleratory functions. These effects are mediated principally by the anterior roots of the abdominal ganglia.

A model is presented that fits the data of the present investigation. The main features of this model is the redundancy of function throughout the central nervous system of this animal and the contralateral driving of inhibitors by accelerator centers as is seen at both the forebrain and ventral cord levels. Each area, depending upon its state and position of dominance, can supersede or be superseded by another area governing that same function.

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WILLIAM G. MEREDITH and RAYMOND G. STROSS, University of Maryland.

Niche differentiation in the crayfishes, *Cambarus b. bartoni* (Fabricius) and *Orconectes limosus* (Rafinesque).

The geographic range of the crayfishes, *Cambarus b. bartoni* (Fabricius) and *Orconectes limosus* (Rafinesque) overlap in central Maryland. In this region, large populations of *C. bartoni* are found only in mountain streams, although scattered individuals usually occur downstream. *O. limosus* inhabits larger streams in the valleys, but is absent above the point where the stream gradient consistently exceeds ten feet per mile.

Numerous factors were investigated to evaluate the niches and explain the distributions of the two species. Both had similar dawn and dusk periods of activity. *C. bartoni* was dominant in tension contacts, indicating a strong competitive advantage. The habitat preference of *C. bartoni* varied from avoidance of moving water when on a sand substratum to slight preference for moving water on gravel or rock; no preference was manifested for vegetation. *O. limosus* avoided moving water on all substrata and showed strong preference for vegetation. *O. limosus* was excluded from favored areas when both species were tested together. *C. bartoni* always attempted to burrow when deprived of water, while *O. limosus* did not.

It was concluded that *C. bartoni* is probably restricted to mountain streams by its requirement for rocky stream bottoms in which its particular kind of burrow can be constructed. *O. limosus* is excluded from mountain streams by its inability to withstand rapid currents, its failure to burrow, and competitive exclusion by *C. bartoni*. (Supported by grant WP 00101 from the U.S.P.H.S.)

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THOMAS C. SHENG, ERIK RIFKIN, and HERBERT W. F. YEE, University of Hawaii.

The role of certain parenchymal cells of *Terpios zeteki* (Porifera: Demospongiae) in phagocytosis and elimination of foreign particles.

In an earlier paper (Cheng, Yee, and Rifkin, Pac. Sci., in press), the quantitative and qualitative distribution of five types of free parenchymal cells of *Terpios zeteki* was reported. It is known that the parenchymal cells (= amoebocytes) of sponges are capable of phagocytizing a variety of homologous and heterologous materials; however, little is known about their role as internal defense mechanisms against foreign materials, biotic or abiotic,

In order to determine if one or more types of amoebocytes are capable of phagocytizing foreign particles and to determine the fate of introduced particles, 0.2 cc of sea water suspensions of India ink and carmine particles were injected into a series of *T. zeteki* maintained at a constant salinity and temperature. These were examined histologically at time intervals. It was found that both ink and carmine particles formed clumps in the mesoglea soon after injection. These clumps were gradually reduced as the result of phagocytic uptake primarily by archaeocytes although a few collencytes were also involved. Ink- and carmine-laden archaeocytes migrated through the mesoglea and exited via excurrent canals. No such cells were found in incurrent canals or in flagellated chambers. The tissues of all sponges were cleared of ink by the 96th hour and of carmine by the 24th hour.

Quantitative differential counts revealed a slight initial increase in the number of collencytes and archaeocytes which was supported by the finding of cells of these types dividing in smears and sections examined at the 6th and 12th hours post-injection.

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IDELISA BONNELLY de CALVENTI, JOSEFINA PEREZ, and SOPHIE JAKOWSKA, Universidad Autonoma de Santo Domingo, Dominican Republic.

Antimicrobial substances from *Codakia orbicularis* and *Crassostrea rhizophorae*.

*Codakia orbicularis*, a marine bivalve (salinity 30-34%) and *Crassostrea rhizophorae*, an estuarine oyster (salinity 3-7%) are commonly eaten raw in the Dominican Republic. Preliminary analysis indicates that the former species contains more total nitrogen, protein and calcium than the oyster, but less water and fats, with equivalent amounts of phosphates.

The animals collected in Spring and early Summer measured approximately (shell length): *Codakia*, 5.1-5.7 cm, and *Crassostrea*, 9.4-13.8 cm. Supernatants of aqueous extracts of homogenized animals were prepared according to Li, 1960. These, and the fluid drained from the molluscs ("juice") were tested separately by paper disc method, under standardized conditions, for growth inhibition in *Micrococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*. In general, the "juice" of both species produced greater growth inhibition than the supernatant, and no inhibition resulted with the precipitate alone. Activity was retained by the liquids stored frozen for 2 months. Larger inhibition zones resulted by concentrating 25 ml aliquots in a water bath at 95°C for up to 4 hrs. Prolonged heating during concentration of larger volumes (50 ml) seemed to destroy some of the active principles, especially in the supernatant.

Materials from both species were effective against Gram-negative forms and *Candida albicans*. Products from *Crassostrea* inhibited growth of *M. aureus*, while with *Codakia* the results were variable. However, independent paper disc tests using frozen and ethanol-precipitated "juice" from identical samples of *Codakia* produced inhibition zones on streaks of *M. aureus, Sarcina lutea, Streptococcus faecalis* and *M. pyogenes*, in decreasing order of magnitude.

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JOHN S. PEARSE, American University in Cairo and Kerckhoff Marine Laboratory.

Reproductive periodicities of five Indo-Pacific echinoids in the Gulf of Suez. (Introduced by R. E. Carpenter)

Reproductive activity was followed histologically in populations of the echinoids *Prionocidaris baculosa* and *Lovenia elongata* near the head of the Gulf of Suez, *Tripneustes gratilla* in the Red Sea near the mouth of the Gulf, and *Diadema setosum* and *Echinometra mathaei* near both the Gulf's head and mouth. These are all widespread tropical species, yet the Gulf of Suez is temperate in character: sea temperatures fluctuate seasonally from about 15°C in the winter to about 30°C in the summer.

Each species had a different reproductive pattern. Gametogenesis was in fairly close synchrony among individuals of all the populations except in those of *Echinometra* from near the mouth of the Gulf of Suez. Spawning occurred briefly in mid-summer in *Prionocidaris*, and from early spring through late fall in *Lovenia*. *Tripneustes* was reproductively active only during the winter. Spawning occurred in the summer in *Diadema* near both the head and mouth of the Gulf. In *Echinometra*, however, restricted summer spawning occurred only in the Gulf of Suez, and individuals in the adjacent Red Sea were reproductively active throughout the year.

These reproductive periodicities are compared with those of some of the same species in more tropical areas, and their possible relations to temperature and other seasonal environmental fluctuations are discussed. (Supported by Contract N62558-5022 (NR 104-889) from the Office of Naval Research.)

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JOHN J. McDERMOTT, Franklin and Marshall College.

The oyster crab, *Pinnotheres ostreum*, as a host for a parasitic nemertean.

In a previous report (Am. Zool. 6:331, 1966) *Pinnotheres ostreum* was listed as a host for the same species of nemertean found in *Pinnixa chaetopterana*. Worms are similarly located in the branchial chambers of female crabs. Female worms cement their egg sacs to the pleopods of the ovigerous host, and their is near synchronous development to hatching of nemertean larvae and crab zoeae. They reach the pleopods by extending their sheaths through a foramen at the abdominal junction or by perforating an anterior sternal suture.

During the summer of 1967 at Beaufort, N. C., 33 of 125 crabs were infested. The crab population consisted of 86 mature females (5.5 to 13.6 mm carapace width) and 39 immatures, 7 of which were males. Thirty-two (37.2%) of the mature crabs were infested while only one immature female harbored a single immature worm. Over 50% of the infested crabs had nemerteans in both chambers, with a maximum of 21 worms in one crab, 9 of which were mature red females. Large numbers of egg sacs are produced under these conditions.

A single female worm in a gill chamber causes comparatively little disturbance to the gills. In heavy infestations, however, all of the gills may be covered with the worms and their sheaths, re-

sulting in deformed and sometimes abortive gills. The latter may be due to an interference with normal molting. No doubt gaseous exchange is inhibited to some extent, but water circulation continues as the scaphognathite and epipodites are not immobilized by the worms. (Supported by grant B-959 from the N.S.F., and N.S.F. Postdoctoral Award at Duke University Marine Laboratory.)

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ROBERT J. SHIELDS, City College of New York.

Effect of salinity on the parasitic copepod, *Lernaea cyprinacea*.

The common killifish, *Fundulus heteroclitus*, naturally frequents a wide range of salinities in estuarine habitats. Experimental infestation of this host provided an opportunity to observe the effect of salinity on the parasitic copepod, *Lernaea cyprinacea*. Fifth and sixth stage copepodid larvae, reared on goldfish, were transferred to fresh water acclimated *F. heteroclitus*. Within 3 to 5 days penetration was observed. Control fish were maintained in fresh-water, while experimental fish were transferred to test salinities. Maximum exposure was to full strength sea water (32 p.p.t.).

Increased adult parasite mortality was first observed, when hosts were exposed to salinities above 50% of sea water. However, through the range of 60 to 80% of sea water some parasites on host remained active up to 10 days. All parasites on a fish were killed in 24 hours upon exposure to 90% sea water. Viable eggs were only produced in 5, 10, and 15% sea water solutions, but empty transparent egg sacs continued to be produced at higher salinities. Although copepodid larvae on a host were capable of surviving several days at salinities up to 40% of sea water, completion of the life history only was possible in 5 to 10% solutions. However, at 10%, the time necessary for full development was increased and the number of surviving parasites reduced. Parasites reared in salt solutions failed to indicate any degree of acclimation.

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WILLIAM B. MUCHMORE, University of Rochester.

Phoresy in American pseudoscorpions.

It is widely known that some pseudoscorpions attach themselves to other, larger animals and are thereby transported from place to place. Reviews of the subject by Vachon (1940, Ann. Soc. Ent. France, 109:1-18) and Beier (1948, Österr. Zool. Zeit., 1:441-497) have dealt mainly with European pseudoscorpions and with African, Asian and South American material in European collections. The present paper summarizes the records of pseudoscorpion phoresy in Central and North America as presented by Beier and as published since 1948. Additional records are presented, based upon material in the author's collections. The utility of phoretic behavior in pseudoscorpions is discussed, with special reference to certain cave-dwelling forms and to those which are found under the elytra of large beetles. (This work was supported in part by grant GB5299 from the N.S.F.)

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DENNIS O'BRIAN and CAROLYN BECKMAN, Seton Hall University.

Parental age effects on survival and fecundity of isogenic and non-isogenic lines of *Drosophila melanogaster*.

*Drosophila melanogaster* (Oregon R) adults were raised on standard agar-molasses medium, at 25°C, under a 12 hour light 12 hour dark regime. Two isogenic lines were obtained from the Oregon R. The non-isogenic stocks were permitted to interbreed for a period of six months prior to initiation of the experiment.

Offspring from young parents, 3 to 5 days old, exhibited no differences in survival from those obtained from old parents, 22 to 28 days of age, and survivorship curves appeared to be similar in all cases studied. Variability in fecundity (eggs laid and number of eggs hatched per female) was approximately the same in the isogenic and non-isogenic lines. However, egg hatchability was characteristic for each line.

Preliminary interpretation of results seems to indicate that aging is a multiple process.

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HANS ELIAS, HERBERT HAUG, WINFRIED LANGE and DAVID SCHWARTZ, Chicago Medical School, University of Hamburg and University of Mainz.

Cerebro-cortical surface areas of some mammals.

The surface areas of the cerebral cortex of some mammals were determined by stereological methods. A few representatives of three groups widely spaced from each other as far as taxonomy is concerned were examined: Man (Primates) served as the center of reference because the anatomy of the human brain is known best. Of Marsupialia we selected the North American opossum (*Didelphys virginiana*) and the black faced kangaroo (*Macropus melanops*), since these two forms are taxonomically very distant from one another within the order of Marsupialia and yet of comparable body size. The Cetacea are represented in this sample by the Delphinid's *Tursiops truncatus* (bottle-nosed dolphin); *Grampidelphis griseus* (Risso's dolphin); *Globicephala macrorhyncha* (pilot whale) and *Pseudorca crassidens* (false killer). These latter forms are closely related but of different body sizes.

The brains were hardened for 4-6 months in 20% formalin solution and then cut into slices of thickness $t$ with an electric meat slicer. For surface determination, a pattern of parallel straight lines, one centimeter apart, engraved on a plastic sheet is superimposed upon the slices; and the points of intersection of these lines with the cortex-pia boundary are counted on all slices.

Hennig's formula, $S = 2 \cdot P \cdot t$, yields directly the surface area S measured in square centimeters, if counts are accumulated over all slices. P is the number of points counted. $t$ can be determined accurately by dividing the measured length of the hemispheres by the number of slices. (Supported by grant NB 07104 from the U.S.P.H.S.)

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ROBERT W. GILLEAN and LAWRENCE S. DILLON, Texas A&M University.

Variation in brain morphology associated with ecological adaptation in Sciurinae.

A comparative study of brain morphology of selected species of Sciurinae was conducted to determine to what degree, if any, variation in gross structure might be correlated to ecological adaptation. Two arboreal species (*Sciurus carolinensis* and *S. niger*), two semi-arboreal forms (*Tamias striatus* and *Citellus variegatus*) and one strictly ground-dwelling species (*C. mexicanus*) were the subjects. Aside from deviations in general shape among the several categories, the cerebrum was found to be more highly arched in *Sciurus* than in the others; in the tree squirrels, too, the olfactory bulbs were shorter and more ovoid. Along with *Tamias*, the arboreal forms had more prominent optic chiasmata than the *Citellus* species. The greatest distinctions centered in the cerebellum. In *Sciurus* all vermian lobes except the lingula were more highly developed than in the others. For example, the nodulus consisted of a single lobule in *Tamias*, of two in *Citellus*, and of three in *Sciurus*. Furthermore, in both tree squirrels the paraflocculus exhibited more extensive subdivision than the others, with the lobules arranged in a characteristic fashion. As *C. variegatus* is comparable in body size to the tree squirrels, these distinctions are most probably related either to ecology or evolutionary development. In contrast, the flocculus was comparably developed in all five forms.

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RICHARD L. BOROWSKY, Yale University.

The bodyspot polymorphism of *Xiphophorus variatus*: its maintenance by habitat diversity. (Introduced by G. Evelyn Hutchinson)

The idea is advanced that the bodyspots (macromelanophore patterns) of the Variable Platyfish, *Xiphophorus variatus* serve as cryptic coloration. The fishes live in several distinct types of habitats and there is a strong correlation between local habitat type and the phenotype frequencies of the population. Differences in substrate preference exist between fishes of the various phenotypes and these differences have been observed in field studies and in laboratory experiments.

The observations support the hypothesis that the main factor in the maintenance of the genetic diversity is habitat diversity. Heterozygote advantage, if it exists at all here, probably plays only a minor role. (Supported by grant GB 5653 from the N.S.F.)

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JOHN W. THORNTON and MILTON R. CURD, Oklahoma State University.

Functional morphology of *Percina caprodes* intraocular muscles.

Electron and light micrographs of sections of the eye of *Percina caprodes*, a freshwater percid fish, show that there are four intraocular smooth muscles rather than a single muscle as was previously assumed. Two of these muscles insert on the lens; one is associated with the iris and the other is near the margin of the cornea. The anatomy and functional significance of these muscles in accommodation is described. (Supported by Grant 5 RO1 NB06353 VIS of U.S.P.H.S.)

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RICHARD J. KREJSA, Columbia University, College of Physicians and Surgeons.

On the supposed participation of the ectoderm in the ontogenesis of Teleost scales.

Our current knowledge of scale ontogenesis is, for the most part, based on concepts available in the early German literature. Most modern investigators have accepted a strictly mesodermal origin of the scale. The few early workers who supposed an ectodermal origin or contribution were discredited. The present investigation characterizes the histology and histochemistry of embryonic fish skin through normal stages of scale development in the medaka (*Oryzias latipes*), the brook trout (*Salvelinus fontinalis*), and the guppy (*Poecilia reticulata*). Re-interpretation of past and present findings in the light of recent concepts of epithelial-mesenchymal interactions suggests a possible role for the epidermis in normal scale development.

Under light microscopy, the embryonic epidermis generally consists of a single layer of simple squamous and occasional cuboidal cells on a basement membrane. The only layer of the dermis visible is the corium (stratum compactum) which lies immediately subjacent, and imperceptibly bound, to the basement membrane. As development proceeds, cytodifferentiation is pronounced in definite areas of the trunk, especially in the lateral line region where the epidermis becomes multi-layered and scleroblasts aggregate below the basement membrane to form the first scale anlage. Within this two-layered papilla a thin disc (focus) of calcifiable protein-mucopolysaccharide matrix forms which becomes calcified later in the developmental sequence. This "bony layer", heretofore thought to be homogeneous, is now shown to possess two layers distinguishable by tinctorial properties and polarized light. This study, along with supporting data from other disciplines, leads the author to reopen the question of ectodermal participation. (Supported by U.S.P.H.S. training grant 5-TO1-DE-132-04, National Institute of Dental Research.)

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ROBERT C. STEIN, State University College, Buffalo.

Anatomy and sound in oscine birds.

Analysis of the "songs" of some oscine birds, particularly warblers (Parulidae), indicates that two sounds may be produced simultaneously, and may be modulated independently. As these sounds pass out the vocal tract they may be modified further. Data are presented to show how various details in the physical structure of a "song" may be related to the action of individual components in the syrinx. Experimental data are presented to show the effect of resonance in the trachea.

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RAYA SOBOL and ELAZAR KOCHVA, Tel Aviv University.

Distribution and secretion of some histochemically detectable enzymes in venom glands of viperid snakes.

Two exidative enzymes, succinate dehydrogenase and monoamine oxidase, and some hydrolytic en-

zymes, leucine aminopeptidase (LNA) and non-specific phosphatases, were examined in the venom glands and in the extracted venom of 12 species belonging to 8 viperid genera.

The oxidative enzymes could be visualized in the gland epithelium, but not in the secreted venom. Of the hydrolytic enzymes a positive reaction for LNA was found in lysosome-like organelles and in the apex of the secretory cells. Several electrophoretic bands were stained in the zymograms of extracted venom suggesting the presence of several enzymes capable of hydrolyzing the histochemical substrate (1-leucyl-4-methoxy-β-naphthyl amide). Non-specific phosphatases were detected at the cell apex, in the intraluminal secretion and in the extracted venom of some genera (*Atheris, Bitis, Pseudocerastes, Crotalus*). In venom zymograms of *Pseudocerastes* two cathodic electrophoretic fractions showed alkaline and acid phosphatase activity; in *Atractaspis* there was a strong reaction in the cells and in three anodic fractions of the venom, with most of the reactive material remaining at the application slot.

The results of this study point to the possibility of following certain enzymes from the site of their synthesis in the secretory epithelium of the glands, through the gland lumina and into the extracted venom. (Supported by NSF grant GB-4032.)

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HERBERT I. ROSENBERG, Cornell University.

The venom apparatus of some elapid snakes.

Histological, histochemical, and electrophysiological studies of the venom apparatus of some elapid snakes yield points of possible interest beyond the description of structural patterns. (1) They permit characterization of some of the products secreted by the glandular portions of the venom apparatus. (2) They provide preliminary information on the mechanism of venom expulsion. (3) They enable comparison of the venom apparatus of elapid and viperid snakes.

The accessory gland completely surrounds the duct of the main venom gland and secretes an acid mucosubstance (PAS and alcian blue positive) which is characterized as a sialomucin (sections treated with sialidase). This mucosubstance is added, apparently during injection, to the main venom gland secretion which contains proteins (mercury bromphenol blue positive) and neutral mucosubstances (PAS positive and alcian blue negative).

Electrical stimulation of the main compressor muscle of the venom gland (M. adductor externus superficialis) of *Bungarus caeruleus* indicates that venom expulsion occurs by straightening and drawing mediad of the curved gland. Sudden compression of the gland decreases the volume within the capsule and thereby increases intraglandular pressure. The antagonistic action of the dorsal and ventral portions of the compressor must not be interpreted as a simple squeezing of the gland or tightening of a ring of muscle.

The comparative study of the venom apparatus demonstrates differences in the arrangement of the tubules within the gland, muscles associated with the venom gland, and organization of glandular components. The venom apparatus of elapids and viperids are clearly non-homologous in many details.

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BERNARD GOLDSTEIN, University of California, Davis.

Burrowing mechanisms in some fossorial mammals. (Introduced by M. Hildebrand)

An investigation of the burrowing mechanisms of three species of ground squirrel (*Spermophilus*), one genus of gopher (*Thomomys*), and one genus of mole (*Scapanus*) is in progress. Variations in the proportions of white, red, and intermediate muscle fibers within the pectoral muscles are being determined. Flexor and extensor muscles of the wrist, elbow, and shoulder are being tested by the Periodic Acid-Schiff Reaction to determine the relative amounts of glycogen present in individual cells. Diameter of cells and location of nuclei are also being compared.

Parallel fibered and pinnate muscles are being compared to determine the effects of architecture on force. Forces developed by protraction and retraction of the pectoral appendage in digging are being determined by attaching the arm of an anesthetized animal to a transducer connected to an isometric stylus system of a physiograph. The skin is removed and muscles are stimulated to tetanus by a method developed by the author and Dr. Ted Goslow. Proportional differences of the relevant bony levers will also be determined.

Soil analyses have been made to determine the resistance offered to the digging animals. A penetrometer has been developed to measure the relative hardness of tractable soils. Resistance is compared on an arbitrary scale ranging from that of pure sand (1) to that of hardpan (10). Finally, the design of a man-made trenching rig called the backhoe will be used as a model illustrating mechanical principles applicable to burrowing mechanisms. (Supported by USPHS. Grant GM36345-01.)

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MILTON HILDEBRAND, University of California, Davis Campus.

Research report: symmetrical gaits of dogs, and mechanical analysis of several bone-muscle systems.

This paper will be an informal report of such of the author's academic activities in the past year as may interest colleagues in vertebrate morphology. Emphasis will be on two studies: (1) Analysis of the symmetrical gaits of about 40 breeds of dogs shows that long-legged and short-legged dogs tend to walk differently in ways that were predicted following study of gaits selected by wild animals of different body builds. (2) Several bone-ligament-tendon systems will be analyzed with particular attention given to (a) a unique (?) force-multiplier system in a digital abductor of the springhaas, *Pedetes*, and (b) the ligamentous mechanism for altering the effective lever arm of the avian biceps femoris muscle as the knee joint is flexed.

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WALTER J. BOCK, Columbia University.

Consequences of pinnateness of the M. pseudotemporalis superficialis in passerine birds.

Measurements were made of the length and angle

of pinnateness of muscle fibers on each side of the central tendon every millimeter along the tendon of the three major subdivisions of the two-joint, multipinnate M. pseudotemporalis superficialis (right and left sides) of twenty *Passer domesticus*. Pinnateness ranges from 5° to 55°, the average being 25° and most measurements falling between 15° and 35°. Fiber length ranges from .3 mm to 2.4 mm, with most lengths falling between .5 mm and 1.5 mm. Because this muscle contracts from a stretched to resting length, as it adducts the mandible, the stretched length of each fiber was calculated for a .5 mm tendon excursion, and the ratio of stretched to resting length computed.

Pinnateness was plotted against fiber length, position along the tendon and stretched/resting length ratio; no correlations could be ascertained. Hence I concluded that pinnateness and fiber length correlate with architectural parameters of the muscle.

The stretched/resting fiber length ratio was usually well over 150% for a .5 mm tendon excursion which is greatly excessive. Because of the structural relationships of this two-joint muscle and the movement of the quadrate, I concluded that the muscle remains close to resting length as the mandible is depressed. Consequently, the M. ps. superficialis would contract isometrically when adducting the mandible. Because the fibers undergo little excursion, they can become very short and thereby permit a great increase in the number of fibers and hence the maximum force of the M. ps. superficialis. (Supported by grant G.B. 3802 from the N.S.F.)

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ANNE M. WHITING, The Pennsylvania State University.

Amphisbaenian cloacal glands. (Introduced by A. M. Taub)

The cloacal regions and tails of representatives of ten amphisbaenian species have been studied as a part of comparative study of the reptilian cloacal glands. There are four different glands in the amphisbaenians.

1). Precloacal (preanal) glands are present in all but one of the species examined. These are elongate glands located within the dermis. Each opens via a short duct to the external body surface at a precloacal pore. The basal layers of the gland are composed of cuboidal epithelium, but the cells become squamous as they approach the lumen. These glands vary from four to eight in number in the different species.

2). Paired alveolar or tubuloalveolar glands, lined with mucous cells and located dorsal or dorsolateral to the proctodaeum, are present in all examined species. They appear histologically very similar to the dorsal glands observed in other squamates.

3). A mentral gland was found only in *Blanus cinereus*. This gland consists of a series of adjacent lobes, and forms a semicircle around the ventral side of the proctodaeum. The ventral glands and the precloacal glands appear to form holocrine secretions.

4). In males of some species, a tubulous gland was observed at the apex of the retracted hemipenis. This is probably homologous with the *Penisdrüse* reported for *Anguis* by Leydig. (Supported by NSF Grant GB 6521X.)

303

P. F. A. MADERSON and C. GANS, Harvard Medical School and S.U.N.Y., Buffalo.

Some observations on integumentary sound producing mechanisms in the Eublepharine gekkonid *Teratoscincus* and other squamates. (Motion picture)

Although the potential of the squamate integument to give rise to a variety of specialized structures has been largely ignored until recently, the epidermally derived rattle of the Croataline tail has been studied from morphological and behavioral aspects. In this paper attention is drawn to other, les widely-known integumentary sound-producing organs.

The dorsal integument of the tail of *Teratoscincus* differs from the usual gekkonid pattern. It bears rows of wide overlapping scales (the number and distribution varying according to the species) the histological structure of which is quite unlike the typical tuberculate scales seen in other gekkonids. The morphology of the superficial portions of the epidermis of the overlapping scales shows further modifications in apparent association with sound production. These modifications are described and discussed and compared with that of other sound-producing squamate integumentary systems.

As Werner (Senck. Biol. 48:117-124) has suggested, the importance of this caudal warning mechanism in the life of *Teratoscincus* is emphasized by the fact that when the tail regenerates, new specialized scales are formed. This is a sharp contrast to the normal gekkonid situation where the scale pattern of the regenerated tail differs from that of the original.

The general problem of the functional significance of such specialized sound-producing systems is discussed. (Supported by U.S.P.H.S. Grant 5 RO1 CA 5401-07, and N.S.F. Grant GB 65-21.)

304

H. J. DE JONGH, Harvard University.

Reorganization of the cranial muscles in *Rana temporaria*, during metamorphosis. (Introduced by Carl Gans)

The mandibular and hyoid muscles of the frog are gradually reorganized during metamorphosis in connection with changes in the mode of feeding and respiration. With the exception of the musculus mandibulolabialis all larval muscles are retained in the adult, while only one muscle develops first during metamorphosis.

During metamorphic climax (Kopsch stages 26-30) the larval muscles obtain new places of attachment, or extend their areas of attachment while extensive fusions occur in the depressor muscles of the hyoid and of the jaw. During this process the larval fibers degenerate: they are replaced by new muscle fibres, which develop from myoblasts originating in the perimysium and endomysium. Larval muscle fibers and new fibers are morphologically distinct at all stages of metamorphosis. The degenerative changes of the larval muscle fibers are comparable to those of the tail musculature.

The histological reorganization is not simultaneous in all of the mandibular and hyoid muscles; it is correlated with the sequence of changes in at-

tachments. It is possible that the described pattern of reorganization occurs in all those striated muscles that are retained from larval to adult condition, rather than muscles alone. These obsevations raise some interesting questions about the possibility of homologies between larval and adult conditions. (Supported in part by Milton Fund of Harvard University.)

305

MARVIN H. CANTOR, PHILLIP SHEELER, JOSEPH MOORE and ROGER E. BOHMAN San Fernando Valley State College.

Effects of environment on growth characteristics of *Polytomella agilis.*

The flagellate, *Polytomella agilis,* was grown in batch culture at 25°C on complex medium and on Cramer-Myers medium. Cells were also grown synchronously on these media using a repetitive temperature cycle. Population densities and the distributions of cell sizes were determined at various stages of growth using a Coulter electronic particle counter (Model B). The protein, RNA and carbohydrate contents of the cells were determined chemically.

In batch culture on either medium there was a marked decrease in the mean cell volume (MCV) during the lag phase. This change was associated with an increase in cell number preceding the period of exponential growth. The MCV remained essentially unchanged during the logarithmic phase but showed a marked increase during the stationary phase. Cells grown on complex medium reached a higher peak population in the stationary phase than cells grown on Cramer-Myers medium. Under conditions of synchronous growth, the MCV increased during the cold period (22 hours at 9°C). A reduction in cell size occurred during the warm period which followed (2 hours at 25°C) and was correlated with the division of these cells.

The protein and RNA per cell decreased during the exponential phase of growth in batch cultures and remained at these low levels through the stationary phase. Carbohydrate, found mainly in the form of cytoplasmic granules, increased during logarithmic growth. The changes in the carbohydrate content of the cells were also followed cytochemically and electron microscopically. (Supported by Grant AI-07926 from the U.S.P.H.S.)

306

DANIEL M. LILLY, CHARLES J. KOVACS and FRANK M. CALASCIBETTA, St. John's University, N. Y.

Incorporation of tritium into the ribonucleic acid growth factor produced by *Colpidium.*

A particulate factor (probiotic) produced by *Colpidium campylum* and released into the culture medium has been found to increase the growth rate of *Tetrahymena pyriformis* and other ciliates. Purified preparations of the active material in concentrations as low as 40 $\mu$g per ml stimulated growth of Tetrahymena from 50 to 100 per cent above control cultures. Chemical and physical evidence has indicated that the active material is ribonucleic acid (RNA), probably in the form of ribosomes. Recent experiments with uridine-$^3$H showed that most of the tritium was incorporated into the growth promoting particles. These were easily separated from the *Colpidium* culture medium by a membrane filter of 0.1 $\mu$ porosity. After the particles were introduced into *Tetrahymena* cultures the radioactive label was recovered in several fractions. The RNA fraction of the *Tetrahymena* accounted for most of the radioactivity, but significant amounts of tritium were present in other fractions. The distribution may possibly be explained by enzymic breakdown of the factor in the culture medium as well as in the ciliates. (Supported by Grant GB-6582X from the NSF.)

307

LOIS M. DOUGHERTY and V. P. MESKILL Long Island University.

A technique to assess the effects of bacterial products and other substances on the growth of *Tetrahymena pyriformis.*

The use of turbidometric procedures to assess the effects of various bacteria on the growth of protozoa has many limitations. Attempts were made, because of this, to grow *Tetrahymena pyriformis* axenically on an agar medium so that additional techniques could be utilized. Recently, *T. pyriformis* has successfully been cultivated on a 0.6% agar medium in 100 × 15 mm Petri dishes. This semi-solid medium contained yeast extract, proteose peptone and dextrose. An even, uniform layer of *T. pyriformis* cells was consistently obtained when the original inoculum of ciliate culture was properly plated and incubated at 24°C. The effects of *Staphylococcus aureus* and *Staphylococcus epidermidis* on the growth of *T. pyriformis* was studied by aseptically placing cellulose acetate disc filters, impregnated with the bacteria or their culture filtrates, in the center of each Petri dish containing the plated ciliate. A sterile, 10 cc, center-tip, Leur lock hypodermic syringe with 13 mm filter paper discs of various porosities in a Swinny adapter was utilized for the preparation of the test discs. Immobilization, lysis of the *Tetrahymena,* as well as zones of clearing could be observed surrounding the discs after 24 hours of incubation. In preliminary studies it was possible to differentiate between the effects of *S. aureus* and *S. epidermidis* on the growth of *T. pyriformis.* These findings appear to indicate that the technique utilized in this study could quite easily be modified so that qualitative and quantitative observations could be made regarding the effects of various bacteria, bacterial products, or other substances on the growth of certain protozoans.

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BURDETTE W. ERICKSON, JR., SANFORD H. VERNICK, Georgetown University, and VICTOR SPRAGUE, Chesapeake Biological Laboratory.

Observations on spores of *Glugea* sp. using shadow casting and electron microscopy.

*Glugea* cysts were obtained from four spined sticklebacks, *Apeltes quadracus,* netted in the Zostera beds fronting the Chesapeake Biological Laboratory at Solomons, Maryland. The cysts were punctured with a scalpel, their contents washed into an ammonium succinate holding solution and the resulting suspension was refrigerated until needed. Two drops of the spore suspension were deposited on previously carboned #200 copper grids

and allowed to air dry. The grids were then placed in a Mikros VE10 vacuum evaporator and shadowed with chromium at a height to shadow ratio of 1:4. Observations were made in an RCA EMU-2D electron microscope.

After the treatment described above some filaments were found to be extruded. The distal end of some unbroken filaments terminated in a cup-shaped structure which averaged 1.3 × 1.0 microns for 6 spores. This may represent one of the various kinds of terminal enlargements observed by earlier works using light microscopy. Structurally, it appears to be the funnel-shaped transition zone between filament and nuclear vesicle ("posterior vacuole") which Sprague and Vernick (J. Protozool. 14 (Suppl.): 29)postulated. These authors (manuscript) think that the funnel which they postulated in the intact spore contains the genome of the spore and may function to conduct this material through the everting filament.

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BROWER R. BURCHILL, Los Alamos Scientific Laboratory, University of California.

Conjugation in *Stentor coeruleus*.

Unlike some other ciliates, organisms of the genus *Stentor* have only infrequently been observed in sexual reproduction, and no means has been found by which this process can be induced or predicted. The present study of mating in *S. coeruleus* was occasioned by the discovery of a high incidence and persistence of conjugation in all cultures of this ciliate in the author's laboratory. Mating was first observed in all nine cultures within a 38-day interval in the fall of 1966 and has subsequently been repeatedly observed in all cultures with varying degrees of frequency. Many of the observations made have confirmed those of other workers, and several new observations have been added, including the ability of well fed cells to mate, the ability of a high percentage of the cells in a culture to mate at one time, and a more precise description of the region of cell attachment (an interposition of two protruding areas of each cell in the region of the frontal field). Several photomicrographs of conjugant pairs will be shown, and a discussion of possible factors responsible for the unusually high persistence of mating in these cultures will be presented. (This work was performed under the auspices of the U. S. Atomic Energy Commission.)

310

LESZEK KUZNICKI, University of California, Los Angeles and M. NENCKI, Institute of Experimental Biology, Warsaw.

Studies on geotaxis of four species of *Paramecium*. (Introduced by Theodore L. Jahn)

During 80 years of investigations on negative geotaxis in protozoa, establishing the position of the center of gravity in Paramecium has been the key problem. Until the present time only dead fixed specimens or models were used in observations on the sinking of ciliates and each author obtained divergent results as to the position of the center of gravity. It is well known that $Ni^{++}$ blocks the effective work of the ciliature. This immobilizing technique was used to determine the orientation of sinking, motionless but living specimens without any significant alterations in size and body shape. The organisms studied were *P. caudatum, P. aurelia, P. multimicronucleatum* and *P. bursaria*. Ciliates were rinsed in 1 mM $CaCl_2$ solutions and specimens were collected from the upperpart of flasks (i.e., negatively geotactic). $NiCl_2$ solutions (0.1-0.4 mM) were then applied. Sinking immobilized specimens were examined in capillary tubes (100 mm long, 1.8 mm diameter) which were filled with the experimental solutions. Behavior of the sinking paramecia was examined for the full length of the tube. The velocity of sinking was measured 40-80 mm below the top. In all species, the center of gravity was not exactly determined. Sinking specimens oriented in various positions with the slight majority—*P. caudatum*, posterior end downward; *P. aurelia*, random; *P. multimicronucleatum*, anterior end downward; *P. bursaria*, horizontal positions. The velocity of sinking was: *P. caudatum* (15-94 $\mu$/sec), *P. aurelia* (18-96 $\mu$/sec), *P. multimicronucleatum* (48-176 $\mu$/sec), and *P. bursaria* (78-221 $\mu$/sec).

These results cast considerable doubt on the theory that negative geotaxis of Paramecium is an effect of a posterior center of gravity. (Supported by grant 5T1 AI 70-09 from the N.I.H.)

311

HAROLD E. FINLEY and JAMES J. FREEMAN, Howard University.

A medium for axenic and non-axenic propagation of colonial and solitary peritrichs.

In 1959, Finley *et al.* described a hydrolysate medium for the cultivation of *Vorticella microstoma* (J. Protozool. 6:201-205). That medium has been modified and supplemented with a vitamin mix composed of L-isoleucine, DL-methionine, DL-phenylalanine, DL-serine, DL-threonine, DL-tryptophan and DL-valine; when supplemented and appropriately diluted the medium supported colonial and solitary peritrichs under axenic and also non-axenic conditions.

Simplified procedures for preparing the medium and results obtained from *Opercularia* and three *Vorticella* species will be described. Supported by Grant AI-00800 from U.S.P.H.S.)

312

RICHARD B. TERRY, University of North Carolina.

Fine structure of pocks produced in the chorioallantoic membrane of the chick after infection with Rous sarcoma virus.

Pocks were obtained by infecting the chorioalantoic membranes (CAMs) of 11 day chicks with two different strains of Rous sarcoma virus (RSV). After seven days, selected pock-containing regions and CAMs from 18 day uninfected chicks were prepared for electron microscopy.

The control ectoderm contains cells in several stages of keratinization. Condensation of chromatin against the inner lamella of nuclear membranes occurs in most ectodermal cell nuclei. The granular endoplasmic reticulum (RER) is sparse. In the peripheral cytoplasm some clusters of non-membrane bound ribosomes are interspersed with accumulations of keratinoid fibrils and mitochondria with abundant cristae. A single layer of chorionic fibroblasts underlies the ectoderm.

A majority of the proliferating cells originate from chorionic ectoderm. At the surface of the discoidal tumors are a few cells in advanced stages of keratinization. The nuclei of remaining cells show limited chromatin condensation and have prominent nucleoli. Their cytoplasm contains RER and frequent disorganized smooth membrane systems. Non-membrane bound ribosomes are abundant, but accumulations of keratinoid fibrils are infrequent. Mitochondria often appear in aggregates and sometimes seem fused. Many mitochondria contain whorls of membranes, some associated with a small lipoid deposit, and mitochondrial cristae are sparse and disorganized. Cell processes are more tenuous than those for control cells, and budding virus is observed in these regions. All pocks contain viral particles, found in the extracellular spaces without collagen in RSV morph$^r$ infected CAMs, but also in the collagen matrix in RSV morph$^r$ infected CAMs. (Aided by a grant from the American Cancer Society.)

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SANFORD H. VERNICK, KALMAN PERK, BOLIVAR J. LLOYD, JR., and KIRBY L. SMITH, National Cancer Institute, National Institutes of Health.

Separation of young and old mouse RBC's by means of electron microscopy.

Previous investigators using electron microscopy of RBC ghost membranes distinguished between young and old erythrocytes in humans (Danon. D., and Y. Marikovsky, C. R. Soc. Biol., CLV:12-18, 1961), and domestic animals (Danon, D., and K. Perk, J. Cell. and Comp. Physiol. 59: (2), 117-128, 1962).

For electron microscopy, the mouse red blood cells were prepared by gradual hemolysis according to the method of Danon *et al.* (Bull. Research Council of Israel 6E, 36, 1956). One drop of the final suspension was placed on carboned #200 grids, shadowed with chromium and viewed in an RCA EMU-3G electron microscope.

The results of the study confirm observations of the previous workers. In the mouse the membrane of young cells appears granular and relatively "thick." The old cell membranes are "smooth," and "thin" with smaller granules, possibly representing loss of biologic activity with increasing cell age. Counts of 500 cells for each animal revealed approximately ⅓ old cells. Similarities and differences between RBC ghosts obtained from virus induced murine leukemias and the normal will be discussed.

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EVA LURIE WEINREB and STANLEY WEINREB, New York University and Rutgers—The State University (New Jersey).

A comparative study of endocytosis.

Hematological response of a teleost, *Carassius auratus*, to experimental endocytosis was studied to determine blood cell functions and analogies to mammalian cell types. Goldfish were injected with Thorotrast to stimulate endocytosis and provide a marker. Peripheral blood samples were collected, at intervals, over a 26 hour period. Stained smears were examined for quantitative and qualitative changes, and effects of phagocytosis. Differential leukocyte counts were made on control and experimental fish; mean ± standard error and 95% range were determined.

Lymphopenia and neutrophilia noted during the first hour persisted for more than six hours. Mature and immature neutrophils, demonstrating abundant granulation, phagocytosed material, and vacuolization, were conspicuous. Medium to large lymphocytes prevailed, with a paucity of small cells. A variety of blast cells, particularly lymphoblasts, were prominent at three hours. All neutrophilic maturation stages were noted. Mature neutrophils predominated at 12 hours, with increasing numbers of lymphocytes. The blood picture began to normalize about 18 hours, with evidence of lymphocytic development and marked decrease of phagocytosis, returning to normal about 24 hours.

Responsive elements, based on morphological and statistical evidence, are neutrophils, lymphocytes, and blast cells. Both mature and immature cells are active phagocytes. Increase in lymphoblasts and developing neutrophils, with decrease in small lymphocytes, indicates a shift toward stem and phagocytic cells. In goldfish, references to "mononuclear" phagocytes include stem cells and lymphocytes; monocytes are not found, and normally rare macrophages are not significantly involved in the reaction. (Supported by grants NSF-GU-894 from the N.S.F. and T1-GM-505 from the U.S.P.H.S.)

315

EDMUND GUTTES and S. GUTTES, Loyola University of Chicago, and R. A. ELLIS, Brown University.

Electron microscope study of mitosis in *Physarum polycephalum*.

In the slime mold, *Physarum polycephalum* the nuclear membrane remains intact during mitosis until late anaphase. At prophase, the nucleolus moves close to the nuclear membrane and gradually breaks down into smaller fragments which remain identifiable by groups of electron-dense granules. The mitotic spindle is formed within the nuclear membrane and the first sign of its development is the appearance of a small plaque of fibrous material near the disintegrating nucleolus. During the separation of the daughter chromosomes at anaphase the nucleus has an irregular shape suggesting vigorous movement of the nuclear membrane. While the daughter plates move toward the poles, material is left in the middle of the interzone which consists of spindle fibers and of seemingly homogenous clumps of unknown (nucleolar?) origin. The nucleolar remnants remain closely attached to the chromosomes and they move with them to the poles. At late anaphase, parts of the nuclear membrane break down, first at the poles of the spindle and a little later in the area which surrounds the interzone between the daughter plates. The remaining parts of the nuclear membrane are large enough to provide the initial membrane for the telophase nuclei. The nucleolar remnants are included in the reconstituting daughter nucli. During a short period following telophase, increasing numbers of electron-dense granules appear between the chromosomes forming many small pronucleoli which gradually coalesce into one central nucleolus. (Supported by grant GM 11949 from the U.S.P.H.S.)

316

DANIEL MALAMUD, Temple University School of Medicine.

Association of glycogen and DNA synthesis in isoproterenol-stimulated salivary glands after Dactinomycin.

The catecholamine isoproterenol (IPR) stimulates DNA synthesis in mouse salivary glands. This stimulation occurs after a lag period of about 20 hours and is followed by a single wave of mitoses.

Salivary gland glycogen concentration decreases to about 50% of control level after injection of IPR. This initial decrease is followed by a marked increase reaching a peak of 600% of the control level at 18 hours after IPR. As DNA synthesis increases the glycogen concentration falls. In addition to a temporal relationship between glycogen and DNA synthesis, any treatment which decreases the glycogen level also decreases DNA synthesis. Thus an injection of epinephrine or a second injection of IPR 17 hours after the first injection decreases both glycogen concentration and DNA synthesis.

It has been demonstrated that stimulation of DNA synthesis by IPR is sensitive to Dactinomycin (Exptl. Cell Res. 46:571). At low doses (0.016 μg/g body weight), Dactinomycin injected 30 minutes before IPR delays the stimulation of DNA synthesis for an additional 5 hours. In addition to this delay, DNA specific activity after Dactinomycin plus IPR is decreased to 50% of the specific activity after IPR alone. After Dactinomycin plus IPR salivary gland glycogen concentration increases slower than after IPR alone, reaching a peak at 24 hours after IPR. These results suggest a close relationship between glycogen concentration and DNA synthesis in IPR-stimulated salivary glands.

317

MARY A. ROCHE, C.N.D., and G. M. MATEYKO, New York University.

A study of nucleolar number and sex chromatin in cells of adult *Rana pipiens*.

An investigation was made of the number of nucleoli and of the possible presence of a sex chromatin in squashes or smears of brain, liver, kidney, pancreas and epidermis tissue of adult *Rana pipiens* stained with methyl green-pyronin Y.

All five types had a single nucleolus in approximately 90% of the nuclei. Most of the other cells had two nucleoli, with a few scattered instances of three or four nucleoli.

The ratio of the nuclear area to the nucleolar area was derived for mono-nucleolar and bi-nucleolar nuclei of brain and liver cells. There was no significant difference between the mean ratios of the two types of brain cells, but liver cells had a significantly higher ratio in bi-nucleolate nuclei than in mono-nucleolate. The nuclear size was constant, so it was the nucleolar size which varied in the liver after fusion.

Most frog cells contain several large chromocenters similar in appearance to the mammalian sex chromatin. The range of number of chromocenters in cerebral cells of both sexes was one to ten, with a mode of four. Distribution curves for the two sexes are almost identical, indicating no sexual dimorphism.

The percentage of female nuclei containing chromocenters adjacent to the nuclear membrane, the most common position, was slightly higher than in males, but the range from ten female frogs was 27% to 63%, while the range from ten male frogs was 23% to 63%. No sex determination could be made on this basis. (Supported by National Science Foundation Graduate Fellowship.)

318

WINIFRED W. DOANE, Yale University.

Cytogenetic and biochemical studies of amylases in *Drosophila melanogaster*.

An attempt was made to place the *Amylase* locus on the salivary chromosome map of *D. melanogaster*. Females homozygous for *w*; *c* $Amy^{1,6}$ $adp^{60}$ were crossed to males carrying a lethal deficiency on the X chromosome plus a covering duplication inserted at band 52F on chromosome 2 (*y* $w^{a}$ $N^{52G1}$/*Y*; *Dp(1;2R)*$w^{+51b7}$ $Amy^{1}/Amy^{1}$). $F_1$ females were test-crossed to males of the parental female type. Linkage data indicates *Amy* lies to the right of band 52F.

Evidence suggests that *Amy* 'alleles' characterized by more than one major amylase band upon electrophoresis reflect closely linked duplications of the *Amy region* (Bahn, in press). (Thus periods are replaced by commas in symbols for these strains.) Supporting this is an experiment in which $F_1$ hybrids of the genotype $Amy^{1}$ $adp^{60}/Amy^{2,6}$ $+^{adp}$ were introduced into a population cage. At $F_{45}$, the cage sample (N = 769) contained 5 exceptional phenotypes indicative of crossing-over within the *Amy* region: $Amy^{1,6}$ $+^{adp}$ (2 females; 2 males) and $Amy^{6}$ $+^{adp}$ (1 female). The first type favors Bahn's view that the locus for band #6 lies to the right of that for #1 or #2. The second suggests that crossing-over may also occur left of the $Amy^{1}$ locus.

Activity in amylase bands from different strains was analyzed quantitatively following disc electrophoresis. The pH optimum proved similar for all; all were susceptible to α-amylase inhibitors. They differed, however, in their relative sensitivity to heat and certain inhibitors. Band #1 was more stable than #4 and #6. Band #4 in $Amy^{1,4}$ and $Amy^{4,6}$ showed similar sensitivity, but band #3 from $Amy^{1,3}$ proved more stable than the same band in $Amy^{3,6}$. A parallel to the latter situation was found when band #2 was compared in $Amy^{2,6}$ and $Amy^{1,2}$. Thus, although strains producing two bands may reflect a duplication of the *Amy* region, amylases with the same electrophoretic mobility but found in different strains need not represent the same duplication. (Supported by grant GB 1718 from the N.S.F.)

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G. JUNE MARSHALL, University of Southern California School of Medicine.

Thymic Dysplasia in ICR/Ha Mice.

Inbreeding ICR/Ha Swiss mice has yielded mutant mice with thymic dysplasia. These mutants are retarded in growth; assume a characteristic hunched posture; and have shortened life spans which do not extend to the reproductive period. The similarity of the life history of these mice to that of mice with wasting and runting diseases led to an attempt to breed for this mutant. Sib matings and backcrosses have increased the incidence of the

mutant and allowed for a number of studies.

Gross dissection of the mutants showed wasted animals with poorly developed musculature and subcutaneous tissue and negligible body fat. The thymi were small, measuring 2-3 mm in length, with distinct lobes which do not overlap at the midline. Other major gross findings were enlarged gallbladders, 6-8 mm in length filled with clear, yellow bile; thin livers with leaf-like lobes; and in 50% of the animals depleted marrow cavities.

Microscopic studies of the lymphatic tissues revealed well formed lymphatic nodules in the spleens, nodes, and in the lamina propria of the gastrointestinal tract. The major lymphoid disturbance, exclusive of the thymus, appears to be a depletion of small lymphocytes. The thymi showed a number of changes including a reversal of medullary and cortical tissue and depletion of small lymphocytes. Immunoelectrophoretic studies have revealed a decrease in globulins, especially in the beta region.

This mutant appears to have a primarily thymic defect which retards growth and leads to early death. The pattern of inheritance and the immunologic competency of these mutants will be discussed. (Supported by a grant from A.C.S.)

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WERNER G. HEIM, The Colorado College.

Similarities between rat slow alpha-2 globulin and alpha-2 macroglobulins of other species.

Rat slow alpha-2 globulin ($SA_2G$), also termed $\alpha_2$-acute phase globulin, is essentially absent from the serum of healthy, non-pregnant adult animals. It appears in the serum, under control of the adrenal cortex, during pregnancy and lactation, in early life, during episodes of regenerative and neoplastic growth, in inflammatory states and after the administration of certain heavy metal salts. On the other hand, there is present, at all times, in man and other mammals, an alpha-2 macroglobulin whose molecular weight is similar to that of $SA_2G$ and whose concentration increases in many or all of the conditions under which $SA_2G$ appears in rat serum.

Evidence will be presented to show that $SA_2G$ and alpha-2 macroglobulin may be closely related. It is postulated that $SA_2G$ is the equivalent, in the rat, of the alpha-2 macroglobulins of other mammals but that the range of concentrations found in the rat under various conditions is wider than in other mammals and normally includes concentrations close to or at zero. (Supported by N.S.F. grants GB 5685 and GB 6639.)

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W. ST. AMAND, University of Mississippi.

Radiation-induced radioresistance in the MClM mouse ascites sarcoma.

Both take frequency and growth rate response after irradiation of MClM tumor cell populations with a history of exposure to X-rays indicate increased radioresistance. Populations irradiated with sixteen 2000 r increments and allowed to grow for three transfer generations without futher challenge show a persistent change in cell size distribution. The resistant population is less variable than control and shows a larger proportion of cells in smaller cell-size classes.

The resistance cannot be ascribed to interrelationships of tumor and tumor bed. The progression of change mitigates against an explanation based on mutational events and suggests that selection is a responsible factor.

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RAMON S. GRILLO and GLORY ZEIDMAN, Adelphi University.

An autoradiographic evaluation of S-phase in *Triturus viridescens*.

Adult newts, *Triturus viridescens*, of approximately 2 gms body weight, each received a single intraperitoneal pulse injection of one of the following four doses of tritiated thymidine (1.9 C/mM): 1.0 $\mu$c/gm wt; 0.1 $\mu$c/gm wt; 0.05 $\mu$c/gm wt; 0.01 $\mu$c/gm wt. 0.1 $\mu$c/gm wt; 0.05 $\mu$c/gm wt; 0.01 $\mu$c/gm wt. Newts were killed by decapitation, several pieces of intestine and liver were fixed in Bouin's fluid, processed according to the standard Paraffin-embedding technique, serially sectioned, stained by the Feulgen technique and autoradiograms prepared using NTB-2. Autoradiograms were scored for changes in (1) grain count reduction and (2) per cent labelled mitoses.

Mean grain counts for the intestinal nest cells and liver capsule cells demonstrate an erratic pattern of peaks and troughs with wide variation between experimental points in each of the four dose groups. There is no obvious period of plateau or grain count halving and therefore, one is unable to estimate length of S-phase or cell cycle time in the newt with this technique.

According to the technique of Quastler and Sherman (1959), changes in percent labelled mitoses with time result in a bimodal curve from which the various segments of the nuclear cycle and total cell cycle time can be estimated. In the newt intestine and liver capsule, mitoses are labelled by 12 to 16 hours after pulse injection and remain so for a considerable period of time. The data thus far indicate an S-phase of at least 40 to 50 hours with a cycle time, at least for the intestine, of approximately 80 hours. (Supported in part by grant AT (30-1)-3900 from the A.E.C.)

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

Attempts to modify the skin reaction of *Pasteurella pseudotuberculosis* toxin (PPT) with various chemical substances.

*Pasteurella pseudotuberculosis* toxin (PPT) was produced by the method described by Schar and Thal (1955) and each lot was standardized by protein assay. The lethal effect was quantitatively estimated in Bagg strain mice (25-35 grams) by the method of Reed and Muench (1938). The $LD_{50}$ calculated on the basis of 24 hours was 2.4 $\mu$g/gram and on the basis of 72 hours it was 0.6 $\mu$g/gram.

Attempts were made to modify the skin reaction of (PPT) in rabbits (dose 100 $\mu$g/0.1 cc, intradermally) with quinacrine (50 mg/kg), chloroquine (5 mg/kg), chlorpromazine (5 mg/kg), reserpine (1 mg/kg), alpha methyl DOPA (100 mg/kg), dibenamine (15 mg/kg), pyribenzamine (10 mg/kg) and beta-histine (30 mg/kg).

One (1) cc of 1% Evan's blue dye was injected intravenously for permeability determinations at 4,

24, and 48 hours. The permeability phase occurred early (1-4 hours). When dye was injected immediately after (PPT) and animals sacrificed 4 hours later, chlorpromazine, reserpine and alpha methyl DOPA remarkably reduced the rate of infiltration of the dye. In animals treated with chloroquine or beta-histine the rate of dye infiltration appeared to be greater. When dye was injected 24 hours after toxin, quinacrine, chloroquine, and chlorpromazine still showed dye infiltration at the site of injection whereas the controls and all other treated animals showed no dye infiltration. In addition the intensity of dye infiltration with chlorpromazine was much less than that with chloroquine or quinacrine.

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WILLIE M. REAMS, JR., and ROBERT E. SHERVETTE, Medical College of Virginia and University of Richmond.

Sulfhydryl content of the normal and X-irradiated PET mouse gastrocnemius.

It has been recognized that hyperpigmentation of the skin is the result of eliminating the –SH inhibition of melanogenesis when treatment is by X-irradiation. The results of a study by Reams and Schaeffer (1967) showed a progressive increase of melanocytes in the skin of PET mice with an increasing dosage of X-irradiation. However, it was noted also that the X-irradiation of the gastrocnemius resulted in a decrease in the number of intramuscular melanocytes.

The present study was designed to determine if the changes in the X-irradiated muscle is related to possible changes in –SH concentration. The technique employed was a quantitative measurement of moles –SH by azo-aryl mercaptide coupling in the normal and the X-irradiated muscle. The data show that the melanocyte population of the right and left legs of normal PET mice, and the left non-irradiated leg muscle of the experimental animals were essentially identical for the developmental period studied. On the other hand, the melanocyte population of the right irradiated leg muscle showed an ultimate decrease in comparison to the controls. Contrary to expectation, on the basis of the –SH determinations for the normal and the experimental animals, there was no apparent relation between the moles –SH and the radiation induced changes in melanocyte population. (Assisted by a grant from the University of Richmond Research Fund.)

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HOLLIS M. FLINT and E. L. KRESSIN, Metabolism and Radiation Research Laboratory, USDA.

Gamma Irradiation of the Tobacco Budworm.

Both sexes of adult tobacco budworms, *Heliothis virescens* (F.), were completely sterilized by 45 kilorads (krad) of gamma irradiation: 35 krad resulted in 99% sterility. At a dose of 45 krad, irradiated females laid about ⅔rds as many eggs as the control, and longevity of both sexes was reduced 0-10%.

The mating ability of males sterilized as adults or pupae was not significantly less than that of the control males in most tests, and females sterilized as adults mated as frequently as the controls. Normally, neither sex mates more than once a night as determined by transfer of spermatophores.

Males sterilized as pupae or adults were not fully competitive with untreated males as measured by egg hatch from ratio tests with untreated males and females. However, in a test in which the mating of sterile males was determined by labeling spermatophores with $C^{14}$, males were found to be fully competitive in mating females.

Females alternately mated to sterile and untreated males usually remained fertile after mating with an untreated male followed by a sterile male or became fertile about 50% of the time if mated with a sterile male followed by an untreated male. Thus when mating to both types of males occurred, the female was more likely to be fertile than sterile. It was also observed that when second matings altered fertility, the alteration was complete — the result of sperm displacement, not sperm mixing.

Sterile males mated to untreated females resulted in about 25% fewer eggs per female than controls.

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W. L. FRENCH, Louisiana State University and J. B. KRITZMILLER, University of Illinois.

The formation of molecular hybrids *in situ* on the chromosomes of *Drosophila melanogaster*.

The technique of molecular hybridization of the nucleic acids has been used extensively in molecular biology.

It is well documented that the specificity of the molecular binding of the hybridized nucleic acids resides in the complimentation of their nucleotide sequences. The present work reports the formation of such molecular complexes *in situ* on the salivary gland chromosomes of *Drosophila melanogaster*.

Slide preparation involves the spreading and denaturation of the DNA on the chromosomes in 50% acetic acid buffered with sodium acetate at pH 2.2 to avoid depurination and breakage of the phosphodiester bonds. The basic proteins which can result in nospecific binding of the nucleic acids are removed with 1.0 N sulfuric acid at —2°C. RNA and polysaccharides are removed by enzymatic digestion.

The chromosomal slide preparations with the DNA denatured and interfering basic proteins, RNA and polysaccharides removed are thoroughly dried. The slides are then incubated for 6 to 10 hours at 60-65°C with an isolated purified radioactive ($P^{32}$ or $H^3$) nucleic acid.

Molecular complexes between the DNA *in situ* on the chromosomes and the introduced radioactive nucleic acid are detected by autoradiography.

The present work reports specifically the formation of DNA-DNA complexes *in situ* on the chromosomes of *Drosophila melanogaster* with homologous DNA.

Preliminary work in conjunction with Dr. D. Steffenson indicates the usefulness of this hybridization procedure for the specific chromosomal site localization of the various isolated fractions of RNA.

Additional works indicate the feasibility of applying this procedure to a quantitative and qualitative molecular genetic taxonomy.

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CHRISTINA L. MOYER, D. J. REITINGER, and H. MAGALHAES, Bucknell University.

Comparative study of golden hamster renal and testicular cells cultured *in vitro*.

Golden hamsters, of the Bucknell Cream strain, were sacrificed at 10, 15, 20, and 30 days of age. Kidneys and testes removed from the same animal were washed in sterile balanced salt solution and treated with 0.5% trypsin. The minced trypsinated tissues were stirred for ten minutes and then centrifuged at 2500 rpm for 15 minutes. Cell pellets were resuspended in MEM - 10% calf serum and cultured in Falcon flasks at 37 C. The development of cell cultures and monolayers was followed with phase contrast microscopy and photographs were made.

Kidney cell cultures derived from 10 day old animals showed monolayer formation with two apparently different cell types. Testicular cells from animals of the same age did not show monolayer formation. Cell cultures prepared from organs of 15 day old animals showed fibroblasts as the dominant cell type in kidney culture and both fibroblasts and epithelioid cells in testis culture. Cell cultures derived from animals at 20 days of age again showed differences depending on the origin of the cells. Monolayers did not form as promptly in the kidney cell cultures, but the testicular cells did produce monolayers with two cell types. Cell cultures derived from animals 30 days of age were characterized by cells that were apparently smaller than those previously observed.

Although golden hamster kidney cells are commonly used in virology, little work has been done on the characteristics of the cells themselves. (Supported in part by grant GY-2926 from the N. S. F.).

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DONALD E. NELSON, University of South Dakota.

Histoquantitative analysis of the neurosecretory system of *Rana pipiens* larvae. (Introduced by Donald G. Dunlap).

Laboratory-reared *R. pipiens* were prepared for microscopic examination by standard histological techniques and the sections were stained according to Dawson's aldehyde fuchsin method (Anat. Rec. 115:63, 1953). Measurements were made of the average size of the preoptic neurosecretory cell nucleus as based on camera lucida drawings (Saxon, Acta Path. Microbiol. Scand. 48:341, 1960), the relative size of the neural lobe, the maximum thickness of the median eminence, and the percentage of thyroid epithelium according to the technique of Uotila and Kannas (in Saxon *et al.*, Ann. Zool. Soc. Vanamo. 18:1, 1957).

Neurosecretion was observed along the infundibular floor at the stage of operculum formation and before the development of a definite neural lobe or a cytologically differentiated adenohypophysis.

The relative size of the neural lobe had no apparent correlation with the percentage thyroid epithelium but a highly significant Pearson-r value with total length and stage of development ($P > 0.01$). No significant relationship was noted between the relative growth of the neural lobe and total length. The percentage thyroid epithelium showed a low positive relationship to the median eminence thickness and a low negative r-value with the size of the neurosecretory cell nucleus. The latter could have resulted from a negative feedback from the thyroid to the hypothalamus.

A small number of larvae exhibited aldehyde fuchsinstaining granules in the intermediate lobe. No constant relationship was noted between this finding and the degree of pigmentation. (Supported by a Title IV National Defense Education Act Fellowship.)

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WILLIAM M. SCHAFFER, Princeton University.

The role of behavior in the evolution of the sheep and goats (Introduced by Charles A. Reed).

The evolution of the sheep-goat line has been marked, if not dominated, by profound specialization of the skull and horns. Previously, it has been suggested (Reed and Schaffer, 1966, *Amer. Zool.* 6(4):565) that these developments are adaptive within the framework of agonistic behavior peculiar to the group. Thus males require strong horns and sturdy skulls to contend successfully with each other during the rut. Analysis of the fighting behavior of *Capra* and *Ovis* reveals differences that cause the cranial apparatus of each to be subjected to different sorts of physical stress. Thus in *Capra*, but not in *Ovis*, impact produces transverse and dorso-ventral torques about the occiput which, if unopposed, would cause violent and injurious rotation of the skull. Such movement, it is believed, is prevented by voluntary contraction of the appropriate neck muscles which, as demonstrated by dissection and measurement of the relevant areas of insertion, are proportionately larger in goats than in sheep. Differences in horn shape and orientation also appear explicable in terms of behavioral differences between the two genera.

It is postulated that anatomically similar populations with differing patterns of intraspecific combat of low intensity gave rise to the divergent morphological types presently observed. Intensification of these "joustings," presumably favored by sexual selection, must have amplified the different stresses resultant from impact, thus rendering subsequent structural change adaptive. (Supported by NSF grant GE 6141.)

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C. RICHARD TERMAN, College of William and Mary.

An investigation of pheromone influence on reproductive maturation and function in populations of prairie deermice.

Ninety-five percent of the females born into and retained in freely growing laboratory populations of Prairie Deermice (*Peromyscus maniculatus bairdii*) fail to produce young. As a means of testing the influence on reproductive maturation of possible pheromones produced in the populations 20 bisexual pairs of mice were raised from weaning (21 days) until 100 days of age on bedding (a) soiled by asymptotic populations, (b) soiled by bisexual pairs from a breeding colony and (c) unsoiled by mice.

Analysis of the reproductive performances of the mice and weights of their ovaries, uteri and testes gave no indication of inhibition of reproductive development or function of animals maintained on bedding soiled by asymptotic populations. Rather the data indicated stimulation compared to the other two treatments. (Supported by grant MH-08289 from the U.S.P.H.S.).

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

Reproductive activity of individual females in three populations of house mice (*Mus musculus*).

Three populations of house mice (*Mus musculus*) were established by placing two sibling females with one sibling male into each of three separate 19 cubic-foot cages. Nesting materials, water and food were provided *ad libidum*, temperature was maintained at 25°C and light was automatically regulated. Individual mice were identified by clipping their toes. The populations were censused every two weeks and daily records of births and deaths were kept. The proportion of the total number of females that successfully reproduced was limited and decreased as the density of the populations increased. The mean percentage of the total number of suriving females that successfully produced litters in the three populations was 36.3%. However, 72.8% of the suriving females showed evidence of having been pregnant prior to sacrifice. At termination the mean age of mice that surived and successfully reproduced in the three populations was 221 days, while the mean age at termination of non-reproducing mice was 135 days Among the suriving females 33% had no corpora lutea at sacrifice As density increased the intervals between litters also increased. In all three populations factors implicated as affecting reproduction as density increased were fetal resorbtion, possible delayed implantation, inhibition of sexual maturation and inhibition of estrus. (Supported by Research Grants HD 00096 and GM 10530 from Public Health Service).

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ANDREW A. ARATA and MAURICE E. THOMAS, Tulane University.

Reproductive Patterns of Neotropical Bats.

Over 5000 bats were studied in Southwestern Colombia during the summers of 1964 and 1965 and monthly from June 1966 through August, 1967. Reproductive patterns within the different species are highly variable. As reported previously by others, certain forms (eg. *Artibeus lituratus*) appear to breed during all months at a rather constant rate. This generalization cannot, however, be extended to all Neotropical bat species. Some stenodermine phyllostomatids (eg. *Vampyrops dorsalis* and *Vampyressa nymphea*) breed intensely during certain periods of the year but at a lesser rate in other periods, suggesting cyclic activity.

Bats of numerous species have been found simultaneously pregnant and lactating, suggesting postpartum estrus. In some of these, ovarian sections reveal developed follicles making possible at least three consecutive pregnancies. Circumstantial evidence points to a gestation of no more than 90 days in certain stenodermines. Reproductive potential in such forms is estimated at 4 young/year, doubling the 1-2 young/year often attributed to Neotropical bats (Asdell, S. A. 1964. *Patterns of* Mammalian Reproduction. Cornell Press, Ithaca, 2nd edition: 1-670). Pregnant individuals with open phalangeal epiphyses have been taken demonstrating that female reproductive activity may start in the first year.

Collections made at the same time of the year in different areas show that individual populations of a species may not be synchronous, but experience different reproductive periodicity in different areas. Such periodicity may be directly or indirectly associated with the rainfall pattern of the specific zone. (Study supported by U.S.P.H.S., NIH TW00143 (I.C.M.R.T. Award) to Tulane University.)

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ALLAN J. STANLEY, L. G. GUMBRECK, J. E. ALLISON, and M. J. McBROOM, University of Oklahoma.

Hereditary vaginal agenesis in the rat.

This anomaly is well known in the rat, mouse and man, having been described for the rat in some detail by Plagge and Lamar in 1939 (Proc. Soc. Exp. Biol. & Med. 42:52, 1939) and for man by Bryan, Nigro and Counsellor in a review of 100 cases of *congenital absence of the vagina*. (Surg. Gynec. and Ob. 88:79, 1949).

Our material at present consists of 9 females of a closely inbred and isolated sub-strain of King-Holtzman hybrid rats now in the 18th generation. Externally the animals appear to be normal females except that there is no vaginal introitus. Laparotomy reveals that the posterior half of the vagina is represented by a solid cord of tissue without any opening into the anterior half. The uteri of the older animals are much distended and filled with fluid and cellular debris often to the extent of 100 ml or more which upon centrifugation consists of packed cells, 25%, and serous fluid, 75%. The cellular material consists of squamous epithelial nucleated and non-nucleated cells and leucocytes, predominantly lymphocytes. The fluid portion has the consistency of blood serum with electrolyte content essentially like that of blood serum in respect to the concentration of Sodium, Potassium, Calcium and Magnesium ions.

Examination of the ovaries showed corpora lutea to be present in all of 5 animals examined at one time. Normal estrous cycles are thus inferred.

Evidence from this limited kindred points to a double recessive gene as being responsible for the defect. (Supported by grants, NIH HD-01075-01 and Institutional Grant B1165605.)

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W. PATRICK LUCKETT, University of Wisconsin.

Evidence for the phylogenetic relationships of tree shrews (family Tupaiidae) based on the placenta and fetal membranes. (Introduced by H. W. Mossman).

Considerable disagreement exists concerning the phylogenetic affinities of tupaiids with insectivores and primates. Evidence for a close tupaiid-prosimian relationship may be due to convergence or parallelism. Many difficulties arise in assessing the relative primitive or specialized nature of characters used in determining ordinal affinities.

Fetal membranes have been demonstrated to be conservative characters of phylogenetic significance and are useful in determining relationships between mammalian orders and suborders (Mossman, 1937, 1953). The developmental stages of the conservative fetal membrane characters of tupaiids are most similar to those of soricoid insectivores and carni-

vores. An endotheliochorial placenta and retention of a large allantoic vesicle and yolk sac are characteristic of these three groups. These resemblances are thought to reflect the retention of primitive characters. There is no evidence for tupaiid-primate affinities suggested by the morphogenesis of their fetal membranes.

The primitive features of the tupaiid placentation support Romer's (1966) suggestion that tupaiids are the only living mammals closely related to the basal stock that gave rise to all of the eutherian orders. (Supported by NIH Fellowship 5-F1-GM-19, 136).

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HERBERT J. BERMAN and GEORGE R. SIGGINS, Boston University.

Direct measurement of the effect of endotoxin on the responsiveness of arterioles.

Thresholds for constriction were determined in 15 arterioles in the retrolingual membranes of nine frogs (*R. pipiens*). The vascular walls and associated nerves were stimulated with a microelectrode at intervals during 2 to 5 hours of exposure to *E. coli* endotoxin (.1 mg in .05 ml of Ringer's solution). Topical application of endotoxin first evoked arteriolar constriction for up to 1.5 hours, followed by progessive dilation to a mean of 138% of the original caliber at 4 hours. Throughout the periods of constriction and dilation electric thresholds increased progressively. After 2 hours the mean neurogenic and mural constriction thresholds were 3.8 and 3.5 times their mean pretreatment values (.26 and .12 ma), respectively. No period of hyperactivity to electric stimulation was found. In control experiments thresholds remained unaltered. At 5 hours, l-norepinephrine (NE) or l-epinephrine (E) was applied topically. Six of 11 endotoxin-treated arterioles did not respond to any concentration of either agent. In the five arterioles that responded, threshold concentrations for constriction were 10 to 100 times the control values of 5.9 X $10^{-7}$M for NE and 1.7 X $10^{-7}$M for E. The data indicate that in this *in vivo* preparation direct exposure of blood vessels to high concentrations of endotoxin reduced the reactivity of terminal arterioles to neural stimulation and E and NE. (Supported by Grant HE-902 and Contract DA-49-MD-2696.)

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FREDERICK G. DUERR, University of Saskatchewan, Regina.

Patterns of nitrogen excretion in marine prosobranch snails.

It has been generally considered that terrestrial, fresh-water, and marine snails excrete ammonia, urea, and uric acid. Several theories have been proposed to explain seeming correlations between environment and type of nitrogenous excreta in snails. Recent research indicates that many of these theories are in error and suggest that it would be of value to determine what compounds are excreted by marine prosobranch snails into their ambient medium.

Accordingly, seven species of prosobranch snails were collected near Juneau, Alaska during June and July of 1967. These snails were immediately placed in small quantities of artificial sea water (Instant Ocean) containing 1000 units of penicillin G per ml. From 24 to 48 hours later the snails were removed from this ambient fluid (held at 6°C.) and the fluid was analyzed for ammonia, urea, and total kjeldahl nitrogen.

No snails excreted urea. All snails excreted ammonia except *Acmaea digitalis*. There was no evidence to indicate that other nitrogenous compounds were added to the ambient fluid.

It has long been known that snails tend to store uric acid. Recently Duerr (Comp. Biochem. Physiol. *22*, 333, 1967) has suggested that snails tend to excrete ammonia and/or uric acid irregardless of their environment. The results of the research carried on this past summer at the Institute of Marine Science, Douglas, Alaska would tend to support this suggestion.

Evidence was also obtained which indicates that salinity influences the amount of nitrogenous excretion.

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WILLIAM B. STICKLE, JR., University of Saskatchewan, Regina.

Some physiological effects of starvation upon the foot and visceral mass of *Thais lamellosa* (Gmelin, 1792.) Introduced by Frederick Duerr)

Recent work indicates that pulmonates normally have a carbohydrate oriented metabolism (Emerson, Comp. Biochem. Physiol. *22*:1967; von Brand, Biol. Bull. *113*:1957). Emerson and Duerr (Comp Biochem. Physiol. *20*:1967) found the herbivorous prosobranch *Littorina planaxis* to utilize only lipid during seventy days starvation. This experiment was conducted to determine whether the carnivorous marine prosobranch, *Thais lamellosa*, also has a lipid oriented metabolism.

*T. lamellosa* were collected near Juneau, Alaska on June 24, 1967. A control group was frozen immediately, and seventy animals were maintained individually in 100% artificial sea water (Instant Ocean) at 6°C. Respiratory rates were taken periodically. Groups of animals were frozen at 9, 31, and 54 days. Frozen animals were flown to the University of Saskatchewan, Regina, where chemical analyses were made.

After the soft parts were removed from the shell, the snails were sexed, dissected into foot and visceral mass, and dried. At the time of sampling, the animals were in the late "gonadal ripening" stage of their reproductive cycle. During 54 days starvation they lost approximately 5% of their wet weight. The foot and visceral mass were analyzed separately for lipid, total nitrogen, protein nitrogen, and polysaccharide content.

It appears that both lipid and protein serve as major sources of energy during starvation. The visceral mass appears to contribute most of the lipid and the foot most of the protein to the energy requirements of the animals during starvation.

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ROBERT K. TCHOLAKIAN and SHERWOOD M. REICHARD, Medical College of Georgia.

A sea water system for experimental marine forms.

A proper environment for the survival of marine animals is essential for long term experiments. For

this purpose, a closed sea water system with the flexibility to accommodate a variety of marine animals was established. This was designed within an ordinary tile-floored, air-conditioned laboratory, and has the capacity of isolating at least 200 experimental adult and juvenile blue crabs, a fair number of stock crabs and a variety of other marine forms.

About 2,400 gallons of aerated, filtered sea water is constantly recirculated from overhead reservoirs through individual aquaria. The rate of flow is adjustable throughout the system, varying up to 25 gallons/hr. The filter design incorporates 3,000 pounds of selected gravels composed of dolomite, crushed oyster shells and marble flour. The filter inherently maintains the pH between 8.0 and 8.2, which is within the optimum range for most marine animals. Nitrogenous excretory products are minimal and have not shown any evidence of accumulation in the system. Temperature control is achieved by circulating refrigerated fresh water through polyethylene coils in the reservoir and maintains the temperature between 60-65°F.

Since the inception of the system, only distilled water has been added to compensate for evaporation. Success has been measured by the longitivity of the various forms of life. Marine animals have survived for 6 months to a year without mortality, immature blue crabs have undergone 3 to 4 molts successfully and crabs undergoing various surgical manipulations have survived with no difficulty. The water has remained crystal clear and odorless with no indication of purification.

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G. DI VIRGILIO, N. LAVENDA, F. INFANTE and P. PAUL, University of Geneva.

Viruses, polymorphonuclear leukocytes and neoplasia.

Evidence is presented for a new diagnostic procedure for cancer detection. Earlier reported studies involving over 600 cancer cases indicated viral association with human malignancy. In the female reproductive tract mutual antagonism exists between intracytoplasmic viruses and the normal bacterial flora. In chronic inflammatory states of the cervix of unknown etiology, often if not always preceding the onset of cervical carcinoma, there is an abundance of viruses and a paucity of bacteria. Although viruses abound in incipient cervical carcinoma, the vaginal fluid is well-nigh bacteriologically sterile. Viruses are invariably demonstrated within affected cells and polymorphonuclear leukocytes. In non-malignant viral infections polymorphonuclear leukocytes are consistently involved, the concentration of intracellular particles correlating with the severity of the infection. After recovery these particles are no longer observed. In these clinical viremias a hitherto unknown plasma phenomenon is reported. Factors in the plasma of patients with viremia interact with viral elements to form regular three-dimensional configurations whose development correlates with the disease state and fever curve. Although in early cancer the leukocytes respond in characteristic fashion, the aforementioned plasma phenomenon fails to occur. The consistent failure to demonstrate the geometric configuration in the presence of viruses in leukocytes suggests a reliable test for cancer diagnosis.

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HANS E. KAISER, The George Washington University and The National Aquarium, Washington, D. C.

Cytogenetic behavior of 3,4-benzopyrene and 1,2-benzopyrene in several species in the initial stage of multiphase carcinogenesis.

Cancer development is characterized by several stages of interaction of carcinogen and cell. An animal treated with 0.01% 3,4-benzopyrene will develop a neoplasm if a continuous application of a promoter follows. The application of the promoter is without any carcinogenic effect, without the initial application of 3,4-benzopyrene. A negative result will also be obtained if the non-carcinogenic 1,2-benzopyrene is used which differs only slightly from 3,4-benzopyrene in its molecular structure. The early stage of multiphase carcinogenesis is very important and hence our interest in short-interval studies of this topic, using a combined method and different plant and animal species. Results: 1. Differences in the distribution of 3,4-benzopyrene depend on body fluids and tissue lipoids. 2. Differences occur in closely related positive or negative reacting species and unrelated ones. 3. Cancers in mouse skin develop where a slow absorption and affection of mitochondria takes place (back), whereas fast absorption as in the tail does not lead to tumors and the involvement of mitochondria. 4. The first affection of mitochondria does not conform with the first application. 5. Differences occur in chemical combination in organelles. 6. Plants absorb the carcinogen more on a one-shot basis. The investigation was undertaken with the following methods (instruments): light microscopy (Zeiss fluorescence microscope and ultraphot II); electron microscopy (Zeiss EMA 9); gas chromatography (Perkin Elmer 881); spectrophotometry (Cary model 15) and other methods.

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SOPHIE JAKOWSKA and P. PAUL FAGUNDO, Autonomous University of Santo Domingo, Dominican Republic, and 27 West 96 Street, New York, N. Y.

Effects of intragastric cholesterol and/or corn oil in the newt, *Notophthalmus viridescens*.

Corn oil, less atherogenic for rabbits than medium chain triglyceride, tristearin or coconut oil (Kritchevsky, 1965), was used in a study lasting 23 weeks during which adult male newts (20) received 22 weekly doses of 0.1 ml of 5% cholesterol suspension. Ten newts received equivalent volumes of corn oil, and 10 others, of distilled water. The animals, collected in late fall, were kept unfed at 12-15°C, with a 12-hr artificial daylight cycle. Weight reduction occurred in all groups: cholesterol, 3.0-4.0 to 2.4-3.5; corn oil, 2.8-4.0 to 2.6-3.3; distilled water, 3.0-3.5 to 2.0-2.9 g. All water-treated newts survived and appeared normal. Three newts died after 15 doses of corn oil showing shedding edematous skin, pale spleen, and hyperemia of common duct and duodenum. Three other newts died shortly with similar gross changes. One newt died after 17 doses of cholesterol, showing thickened yellowish pericardium and aorta. Cholesterol-treated newts sacrificed after 22 doses or earlier had yellowish ventricles, thickening of the common duct

and, in one case, annular ridges on lung surface; the skin was normal. Large fat bodies and yellowish liver occurred in both groups receiving corn oil.

There was considerable fluctuation in liver weight in newts sacrificed at different periods, but the values expressed as per cent of body weight remained higher with corn oil alone than with cholesterol in corn oil, e.g., 5.3 vs 4.4% after 17 doses, and 5.1 vs 4.7% after 22 doses, with corresponding values lower in water controls. Preliminary histological evaluation indicated progressive fat accumulation in hepatocytes with corn oil administration. In one cholesterol-treated newt fat globules were observed in cardiac muscle fibers.

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GEORGE H. and ALICE F. BEATTY, The Pennsylvania State University.

The unusual oviposition behavior, habitat and the description of the larva of Palaemnema (Odonata: Zygoptera) of Mexico.

Inasmuch as the Odonata-Family Platystictidae (Zygoptera), with many ramifications in the Old World, is represented in the New World only by the rather large genus Palaemnema, it is of significance that practically no biological information is recorded on Palaemnema while the Old World Platystictids have been studied somewhat better due to the pioneer work of Fraser and Lieftinck.

Fieldwork in Mexico by the present writers in 1958, 1959, and 1963 has yielded considerable information on the biology of Palaemnema including: (1) Description of the habitat, habits, oviposition site and oviposition behavior of *Palaemnema desiderata*; (2) Description of the larval habitat and ecological requirements of the genus, and (3) Description of the hitherto unknown larva of the genus Palaemnema, the only New World representative of the family Platystictidae.

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EDITH A. MAYNARD, University of Michigan.

Microscopic localization of cholinesterase and acid phosphatase in brain and nerves of the spiny lobster following removal of antennule or eyestalk.

One antennule or one eyestalk was removed from each of 34 spiny lobsters (*Panulirus argus*). The brains and appropriate nerves were examined from 12 hours to 9 weeks post-operatively using the Koelle acetylthiocholine method for cholinesterase and the Barka-Anderson simultaneous coupling azo dye method for acid phosphatases in cryostat sections. Nine days following antennule removal there is a loss of cholinesterase normally present in the rim of the olfactory lobe on the operated side, and a concomitant increase in the number of small nuclei, presumably glial, in this area. No increased acid phosphatase reaction occurs at this site. The antennular nerve tract carrying the chemosensory fibers from their point of brain entry to the olfactory lobe, shows first an increase and later a decrease in cholinesterase content. By the third week cholinesterase reaction in this tract is limited to scattered glia which are no longer oriented in the parallel array seen in control material; nerve fibers are not visible. At this time also there are numerous small round cells rich in acid-phosphatase-positive granules, both in the antennular nerve tract and in the nerve itself just distal to the brain sheath. Five to 7 weeks post-operatively in nerve and tract there is a strikingly increased cholinesterase reaction possibly associated with new nerve fibers entering the brain from the newly forming antennule; slightly later, the cholinesterase reaction returns to the rim of the olfactory lobe. These results are compared with those in the eyestalk, where a heteromorph antennule regenerates. (Supported by grant NB-04179 from the U.S.P.H.S.)

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LANGLEY WOOD, Virginia Institute of Marine Science.

Acquisition of prey preference in a marine gastropod. (Motion picture)

*Urosalpinx cinerea* Say is a predatory marine gastropod native to the middle Atlantic coast of the United States. In its intertidal habitat, it preys chiefly upon barnacles, oysters, and mussels in direct proportion to these species' numerical ratio to one another, but circumstantial evidence is strong that detection and selection of prey individuals in nature is chiefly a chemoreceptory function.

Experimental evidence is presented to introduce the concept of *ingestive conditioning*, in which a predator's tendency to respond to effluents from a given invertebrate prey species is increased after ingestion of living tissue from that species. This process strongly resembles *imprinting*, in that juveniles are more easily conditioned than adults, and that responses after the initial conditioning are faster and more complete than are those following subsequent diet reversals.

Evolutionary aspects of this predator-prey relationship are discussed with particular reference to the adaptive value to the predator of ingestive conditioning. Restriction to a single prey species would have disoperative effects, so it is to the predator's advantage to be capable of feeding upon more than one species. However, different attack techniques are utilized for efficient penetration of various prey species, and these techniques are apparently acquired by individual *U. cinerea*. By concentrating upon a single species, *U. cinerea* probably increases its attack efficiency. The mechanism described here as ingestive conditioning provides such a concentrating influence without the irreversibility of genetically fixed prey-specificity.

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MICHAEL F. HALASZ, The University of Manitoba.

A behavioral evoked response: probing the stability of delayed conditioned approach (*albino rat*) with impulse-like changes of reinforcement schedule.

Delayed conditioned approach (DCA) to auditory CS (Skinner box) converts into a self-adjusting *behavior system*, responsive to experimenter incrementation of delay of water reinforcement availability relative to CS as servomechanisms adjust to standardized changes in demand (Amer. Zoolog. 6: 543, 1966; Activ. nerv. sup. 9:1, 1967). State of DCA system is specified by *relative error* ratio P = (Required DCA latency — Actual DCA latency)/(Change in required latency). We have defined a class of *impulse-like* perturbations of reinforcement

schedule that conform to the small-signal input employed by linear control theory to study systems in the neighborhood of an initial *operating level.* Upon application of impulses, time plot of (P — baseline P) gives the *behavioral evoked response* (BER). On assumption of no transition between operating levels (small preturbation), BER's may be superimposed (averaged) for truer picture of system impulsive response. Fitting of Lagrange polynomial to BER yields amplitude, rise time and decay criteria that, respectively, characterize gain, fidelity and stability of rat DCA. These quantities are predictors for system response to wider sets of disturbance in reinforcement conditions. Extent of prediction confirmation and limits of linear analysis will be discussed. (Supported by National Research Council of Canada Grant APA-189 and University of Manitoba Graduate School.)

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

Response consequences and free operant avoidance in goldfish.

Sixty goldfish, *Carassius auratus,* were given a single six hour session of free operant avoidance training with a constant shock to shock (S-S) interval of 5 seconds. The response required of S was that of swimming through a small tunnel from one compartment of an aquatic shuttlebox to another. S's behavior was monitored by a photocel circuit, and each response switched the program from the S-S cycle to the response to shock (R-S) cycle. One-sixth of the Ss were trained with R-S intervals of 2, 5, 10, 15, 20, and 40 seconds, respectively. Data were collected for each hour of the session, and included number of shocks received, number of responses, total time in R-S cycle, and number of responses made during R-S cycle.

The results suggest that the number of responses for the total 6 hours is roughly an inverted U-shaped function of R-S interval with the smallest number of responses being made by the 2 and 40 second R-S interval groups and the greatest number of responses being made by the 5 and 20 second groups. The 2 second group had a mean of 60.7 responses during the first hour and dropped with each hour until a mean of 4.2 responses was reached during the sixth hour. This might have been due to a punishment of responding. The 5 second group made the greatest number of responses during the third hour but had dropped considerably by the sixth hour. All other groups showed improvement over the 6 hours.

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MERLE E. MEYER, Western Washington State College.

Response of chicks with light onset or offset contingencies within a simple or complex environment.

Response contingencies of dim light onset and offset within a simple or complex environment were investigated using 160 24-36 hr. old cockerel chicks. Four treatment conditions with yoked controls were used to replicate reports by Sackett and by Eacker and Meyer. The general results substantiate the fact that light onset is an effective sensory reinforcer for chicks, and that visual complexity is one characteristic that contributes to this reinforcing property. The data, however, seriously question a stimulus change theory, in that the study does not verify onset as an effective reinforcer. (Support by grant from W.W.S.C.)

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HANS ZEIER and MICHEL CUENOD, University of Zurich, Switzerland.

Interhemispheric transfer of visual discriminations in commissurotomized pigeons. (Introduced by H. J. Karten)

Various brain commissures were cut in pigeons (*Columba livia*) with a stereotactically inserted knife. The operated animals were placed in an operant conditioning situation (Zeier, 1966, Z. Tierpsychol. 23:161-189) and required to peck a key for reinforcement (food). When they performed sufficiently well, monocularly, with either eye, they were trained on a two-choice color discrimination task (e.g., blue positive and green negative) with one eye. Then the untrained eye was tested for transfer of this discrimination. After that, a second discrimination task (e.g., yellow positive and red negative) was presented to the second eye and transfer was tested in the reverse direction.

Both experimental and normal control animals acquired the second discrimination faster than the first. Intact animals invariably showed a complete interhemispheric transfer. However, animals in which the tectal and posterior commissures had both been cut, showed a deficit in transfer in both directions. The degree of impairment was proportional to the numbers of fibers cut, but even maximal lesions failed to produce complete loss of transfer. Some deficits resulted from combined transsections of anterior and pallial commissures; but greater deficits from those confined to posterior and tectal commissures. Still, combined sections of all these four interhemispheric connections left some residual capacity for transfer. (Supported by Grant 4395 from the Swiss National Foundation of Scientific Research.)

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JOHN H. HOLLIS, University of Kansas.

Direct measurement of the effects of drugs and alternative activity on stereotyped behavior.

Infrahuman primates and children reared under restricted conditions frequently display stereotyped behavior patterns. These environments not only reduce physical stimulation, but are also void of the contingencies and consequences that maintain adaptive behavior. This investigation was designed to use operant conditioning techniques in the analysis of the effects of manipulandum responding and drugs on stereotyped rocking behavior in severely developmentally retarded children. First, drug dose-effect curves were obtained for Chlorpromazine, Chlordiazepoxide, and Amphetamine. The response was pulling a ball-manipulandum, which was maintained by fixed-ratio reinforcement schedules. Second, the effective drug dosages were tested for their effect on stereotyped rocking behavior. The results were as follows: (1) high dose levels of Chlorpromazine (3 mg/Kg) and low dose levels of Chlodiazepoxide (0.2 mg/Kg) significantly reduced

ball-manipulandum responding: (2) rocking behavior was reduced or eliminated depending on the drug dose level; (3) data obtained from Amphetamine was equivocal; and (4) manipulandum responding under a fixed-ratio schedule eliminated rocking. (Supported by Grant No. 00870-04 from U.S.P.H.S.)

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RONALD R. KEIPER, University of Massachusetts.

Stereotypies in caged canaries.

Caged canaries exhibit two general repetitive stereotypies which have been termed route-tracing and spot-picking. Several experiments were conducted to determin what factors might alter the frequency of these stereotypies. Increasing cage size, social interaction, or environmental complexity all significantly reduced route-tracing, but only increased cage size significantly reduced spot-picking. The frequencies of stereotyping shown by several species of birds caged for different lengths of time were compared. Route-tracing was generally higher in wild caught species while spot-picking was either not observed or occurred at very low levels in these birds. The evidence suggests that route-tracing may be a cage-related stereotypy, while spot-picking is less directly related to this factor. (Supported by grant NSG (T) 137 from NASA.)

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JOHN BUCK, ELISABETH BUCK and HOWARD BRUBACH, National Institutes of Health.

Mechanism of synchronization in finger tapping.

In an investigation of phase-shifting mechanisms by which animals attain and maintain synchrony with an external rhythm, subjects were tested for ability to maintain rhythmic tapping of a telegraph key at self-chosen frequencies and to synchronize with rhythmic acoustic signals in the 1/sec to 5/sec range. Though spontaneous tap frequencies are most commonly in the 2/sec to 3/sec range, coefficients of variation are proportional to cycle length and are of the same magnitude whether the signal being followed is endogenous or external. Steady-state synchrony is maintained by alternation of too long and too short tapping cycles, with a strong tendency toward bimodal distributions of the cycle lengths attained in cycle shortening and also of those attained in lengthening. Since taps often anticipate the signal, and since the mean T-S (or S-T) interval is much shorter than the minimal ear-finger latency, the synchronization mechanism depends on cues in the preceding, not current, episode. Most subjects show a marked sequential bias, usually leading the signal. In resynchronizing after a sudden change in S frequency, T cycle length is immediately adjusted, by major shortening or lengthening, to approximate the new S cycle length. This adjustment also makes T later than S, regardless of whether the T cycle has had to be shortened or lengthened. The final step in adjustment is then a gradual resumption of the early bias position by the cumulative effect of a succession of tap cycles that are slightly shorter than the S cycle.

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HARRY R. KISSILEFF, The Rockefeller University.

Loss of saline preference in rats drinking prandially.

Rats recovered from aphagia and adipsia after lateral hypothalamic lesions and neurologically normal rats with ligated salivary ducts drink water in small draughts, alternately eating small morsels, when dry food is available ad libitum (prandial drinking, Teitelbaum & Epstein, Psychol. Rev. 69: 74-90, 1962; Kissileff & Epstein, Fed. Proc. 24: #2, 1965). Both operations abolish preferences for saline solutions in a 2-bottle 24-hour test (Kissileff & Epstein, Am. Zool. 2:533, 1962; Vance, Psychol. Monog. 79: #5, 1965) although Wolf (Am. J. Physiol. 212:113, 1967) finds no decrement in recovered lateral rats.

To elucidate the mechanism of this loss, 2-bottle 24-hour sodium chloride preference aversion curves were first obtained during free feeding on dry Purina lab chow from desalivate rats and from recovered lateral rats. Ingestion patterns were obtained using a box equipped for continuous recording of feeding and drinking. All desalivate rats showed loss of salt preference, but only recovered lateral rats with consistent prandial drinking patterns showed the loss. Draughts were larger from saline than from water.

In desalivate rats the two bottle saline preference test was repeated in absence of food for 16 hours daily (0.1 to 3.0%) and the preference reappeared, showing that feeding while drinking (prandial drinking) interfered with expression of the preference. When the 2-choice situation was replaced by a 1-bottle test, (food continuously available) preference also reappeared.

In conclusion, lateral hypothalamic lesions and interruption of salivary flow abolish saline preference during 2-bottle tests by disrupting the normal distribution of draughts, not by altering taste sensitivity. (Supported by U.S.P.H.S. grants NB-03496, 5-T-1-GM-281, 1 F2 AM 34,509-01, and NSF GB-4198.)

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ALAN N. EPSTEIN and GERARD P. SMITH, University of Pennsylvania.

Feeding in response to decreased glucose utilization in rats.

Two-deoxy-D-glucose, a glucose analogue that decreases intracellular glucose utilization, has recently been shown to increase food intake in monkeys (G. P. Smith. The Physiologist *10*:308, 1967). We report here the same effect in rats. Feeding was markedly stimulated in 9 rats given 750 mg/kg (100 mg/ml solution, ip) of 2DG just before a 6 hour test beginning at 11 AM. They ate earlier and they ate more. Baseline (non-deprived) intake (Purina pellets) averaged 2.9 g (Range: 0.0-6.2) but reached 5.6 g (Range: 0.8-8.6) after 2DG. Seven of the 9 rats ate more. Average latency to the first bout of eating decreased from 171 min (Range: 23-360) to only 39 min (Range: 9-198) after 2DG. All 9 rats ate sooner, 8 did so within 30 minutes of the injection. Only one had done so on baseline day. Latency of 2DG feeding was also much shorter than that (124 min, Range: 24-360) observed with a dose of insulin (Iletin, 6U, ip) that yielded comparable total food intakes. Lower doses of 2DG (200-500 mg/kg) produced small and inconsistent effects, higher doses (1000 mg/kg) depression and

ataxia followed by eating and, in one rat, retching. In a separate experiment in which feeding was permitted, a severe and sustained hyperglycemia occurred after 750 mg/kg 2DG (resting: 96.7 mg%, 1 hr: 296.3 mg%, 6 hr: 178.5 mg%, glucose oxidase analysis) thus confirming earlier reports that the competitive inhibition of glucose utilization by 2DG produces a hyperglycemia. This work together with the earlier findings in monkeys, is strong evidence that decreased glucose utilization 1) drives mammals to eat and 2) is an important short-term factor in the normal control of food intake. (U.S.P.H.S. NB 03469 and 06073.)

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CHARLES T. SNOWDON, University of Pennsylvania.

Meal parameters and motivational aspects of oral and intragastric feeding in rats. (Introduced by Alan N. Epstein)

Rats are remarkably constant in daily food intakes, adjusting rapidly and precisely to dietary dilution. This work examines the effects of dietary adulterations on meal parameters (meal size and meal frequency) and the role of oral sensations in maintaining constancy of food intake. Rats fed themselves a liquid diet by depressing a bar in their home cages. Printing timers recorded the size and frequency of all spontaneous meals. Chronically implanted intragastric tubes permitted feeding without oropharyngeal sensations (Epstein and Teitelbaum, J. Comp. Physiol. Psychol. *55*:753, 1962).

All 27 rats feeding orally and 17 of 19 feeding intragastrically responded to dietary dilution by increasing meal size, usually by the fifth meal of dilution. Total nutritive intake remained constant whether the diluent were distilled water (nutritive + osmotic dilution) or isosmotic NaCl (nutritive dilution only). Thus nutritive rather than osmotic properties of the diet control daily regulation. Rapidity and precision of regulation were similar with oral and intragastric feeding.

The tansition from oral to intragastric feeding required special training to prevent extinction of bar depression. Intragastric daily intakes were 5-10 ml less than oral. Intragastric meals were shorter, consisting of bursts of brief bar depression. Oral meals consisted of a few sustained bar depresions. Unlike rats feeding orally rats feeding intragastrically did not gain weight during the experiments (21-63 days).

Oral sensations act as motivators, maintaining feeding at optimal levels, but they are not essential for the rapidity and precision of regulation. (Supported by USPHS NB 03469 and MH 32066.)

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DAVID W. JACOBS, The American Museum of Natural History and Fairleigh Dickinson University.

Light vs. sound cues in avoidance behavior in the pinfish *(Lagodon rhomboides).*

Previous investigators have been concerned with the role of "emotional" conditioning as a variable in avoidance conditioning. The effect of differences across modalities stimulated by the conditioned stimulus was not considered. In this study, the effects of light vs. sound cues on rates of acquisition, extinction, re-acquisition and re-extinction, and the effects of retention interval upon these rates were investigated in the pinfish.

The avoidance conditioning procedure followed closely that employed by Jacobs and Tavolga (An. Beh., 1967, 15, 324-335) in work on auditory thresholds. Significant differences in the rate of initial acquisition and extinction occurred as a function of the modality stimulated. Animals trained to a light cue showed faster acquisition and slower extinction rates than did those trained with sound stimulation. These differences were reduced for re-acquisition and all animals showed increased resistance to re-extinction. No effect of a one week retention period was noted. (Supported by Grant GB-4364 from the N.S.F. and Contract 552 (06) with the O.N.R.)

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PHYLLIS H. CAHN, J. WODINSKY and W. SILER, Yeshiva and Long Island University, Brandeis University, State University of New York's Downstate Medical School.

A signal detection study on the acoustico-lateralis system of fishes.

Fish appear to have a dual acoustic detection system, with separate but probably coupled pressure and particle velocity or displacement sensors (ear and lateral line, respectively; Cahn, in press, Lateral Line Detectors, Indiana University Press). A well calibrated test chamber with both far-field and near-field approximations of sound was used to obtain pressure and velocity/displacement thresholds. Avoidance conditioning methods (Tavolga and Wodinsky, 1963, Auditory Capacities in Fishes, Bull. Amer. Mus. Nat. Hist. 126:179) were applied to young grunts (*Haemulon sciurus* and *parrai*, approximate total lengths 5.1 cm), collected from Biscayne Bay, Florida.

Sound was transmitted from two Chesapeake Instrument Company J9 underwater speakers, mounted with their driving faces at the ends of a transparent, plexiglas, water-filled tube (12 inches diameter, 54 inches long, ¼ inch wall thickness). A plywood, water filled box (18 × 18 × 24 inches) was sealed to each end of the tube, and housed the J9's. The tops of the boxes were kept open to insert monitoring hydrophones (pressure sensitive Chesapeake Instrument Company SB 154B, and velocity sensitive Geo-Space Corporation HS-1) and fish shuttle box (transparent, plexiglas, with inside dimensions of 2¾ × 2¾ × 5½ inches).

Electrically, the speakers could be driven "in phase," resulting in a pressure peak and velocity node, or "out of phase," resulting in a velocity peak and pressure node. The magnitude of the parameter peaked (velocity or pressure) was uniform within 1 db in the shuttle box where the fish were located.

The thresholds will be discussed in terms of the signal-to-noise ratios of the pressure and velocity/displacement parameters. (Supported in part by NSF GB4791, and by NIH Institutional Grant Allocation to Brandeis University, FR-07044-02.)

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J. H. TODD and J. E. BARDACH, University of Michigan.

Chemical communication in the social behavior of the Yellow Bullhead *(Ictalurus natalis)*.

Blinded yellow bullheads in large aquaria exhibit complex social behavior, establishing hierarchies and territories through agonistic displays and/or fighting. Although they have a well developed sense of smell, bullheads locate their food primarily by means of their external taste sense (Bardach et al., Science, 155:1276, 1967). Our work indicates that chemical communication is important for this species and that the nares act as receptors of pheromones.

Bullheads were able to discriminate between the odors of two donor fish after reward-punishment training. This ability was lost after deprivation of their sense of smell. The main source of the intraspecific chemical stimuli involved in discrimination was the mucus. The fish remembered the learned discrimination, without retraining, for at least three weeks.

Individual recognition of one fish by another is a dominant factor in social behavior; individual identity and changes in social status after fighting are chemically communicated to other bullheads. (Supported by Public Health Service Grant NB-04687.)

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ARTHUR N. POPPER, The City College of The City University of New York.
WILLIAM N. TAVOLGA, Department of Animal Behavior, The American Museum of Natural History and Department of Biology, The City College of The City University of New York.

Hearing thresholds in the Mexican blind cavefish.

The Mexican blind cavefish, *Astyanax mexicanus (Anoptichthys jordani)*, may have increased auditory sensitivity compensating for lack of eyes. Auditory threshold were determined for five specimens utilizing avoidance conditioning. The animals were tested with pure tones from 50-6400 Hz. The minimum threshold was —48.2 dB (re 1 ub) at 1000 Hz. The threshold at 50 Hz was —19.7 dB; at 200 Hz, —42.1 dB; at 2000 Hz, —21.8 dB; and at 4000 Hz, —5.76 dB. At 6000 Hz the threshold was +8.6 dB.

At 50-2000 Hz, the audiogram for the cavefish closely parallels that reported for goldfish *(Carassius auratus)* by Jacobs and Tavolga (An. Beh., 15, 324-335, 1967). In contrast to the goldfish and many other species, the cavefish responded readily to sounds above 3000 Hz. (Supported by Grant GB-4364 from the N.S.F. and Contract 552 (06) with the O.N.R.)

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LESTER KRAMES and W. J. CARR, Temple University.

The effect of previous visual experience upon the response to depth in domestic chicks.

Emlenn (Behaviour, 22:1-15, 1963) reported that gull chicks reared in cliff nests are less likely to descend on a visual cliff than birds reared in plateau nests. However, Tallarico & Farrell (J. Comp. Physiol. Psychol. 57:94-96, 1964) found that domestic chicks reared in nests which approximate the deep side are more likely to descend on the deep side of a visual cliff than are shallow-reared birds. The present experiment was performed to resolve this apparent conflict.

Eighty-four chicks (DeKalb #131) were reared for 35 hrs. post-hatch in one of three environments and then were observed on a modified visual cliff. One group was reared in nests with opaque walls and floors (shallow-reared). A second group was reared in nests with tansparent floors and opaque walls (deep-reared), and a third group in nests with opaque floors and transparent walls (cliff-reared). The transparent surfaces allowed *Ss* to view a large textured surface which was 12 in. below the floors.

During testing, *Ss* were placed individually on a platform situated 12 in. above a textured surface. A greater proportion ($p<.05$) of the deep-reared *Ss* descended than did the shallow- or cliff-reared *Ss*. The latter two groups did not differ. These results suggest that the location of textured surfaces exterior to the rearing environment influences the chick's reaction to depth.

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HELMUT C. MUELLER, University of North Carolina.

Prey selection by a sparrow hawk.

An adult female sparrow hawk (*Falco sparverius*) was subjected to a series of prey-selection experiments in the laboratory. The experiments were designed to test two hypotheses: (1) that conspicuous prey is selected and (2) that "odd" prey (which differs in appearance from the majority of conspecifics) is selected. White laboratory mice were used as prey. Some of the mice were dyed gray with food coloring. Backgrounds were either white to match white mice or gray to match the dyed mice. There was no evidence for the selection of odd prey. The bird selected significantly more white mice on a gray background. Random selection of gray or white mice occurred on a white background. Thus selection appears to occur for (1) conspicuous mice and (2) white mice. Further experiments will be performed to determine the ontogeny of the preference for white mice.

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THEODORE D. SARGENT, University of Massachusetts.

Background selections of cryptic moths.

A variety of cryptic moths selected appropriate backgrounds in an experimental apparatus allowing a choice between black and white paper backgrounds. In an attempt to define the mechanism behind these selections, the circumocular scales of some moths were painted with quick-drying "Flo-Paque" paints (circumocular here includes the head, the collar on the thorax, and the base of the forewings). *Catocala antinympha*, a coal-black noctuid, invariably rested on black backgrounds. Individuals of this species which were painted white also selected black backgrounds, as did control moths which were painted black. Similar results were obtained with small numbers of a pale noctuid, *Apatela innotata*; here, untreated and black-painted individuals selected white backgrounds. These results support the view that appropriate background selections of cryptic moths are genetically fixed, and are not the result of a matching

mechanism which enables the moth to compare its own reflectance with that of its background. (Supported by a Faculty Research Grant from the University of Massachusetts.)

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H. B. GRAVES and P. B. SIEGEL, Virginia Polytechnic Institute.

Behavioral heterosis and the approach response of chicks.

Naive chicks from 8 crossbred and 7 purebred mating types were exposed to a distant audio-visual imprinting apparatus at 24 ± 2 hours posthatching. Measurements included length of incubation period, 24-hour body weight, time to respond, time to approach, and time spent near the apparatus during a testing period of 5 minutes duration. Results showed a significant heterotic effect for the tendency of young chicks to approach a parent surrogate. Incubation period was also shorter for crossbreds than for purebreds. The low, but significant, heritability of .18 ± .03 for time to respond (Graves and Siegel, 1966. Bul. Ecol. Soc. Amer. 47:144) and the heterotic inheritance of the approach tendency indicates selection for these traits during the evolution of this neonatal species.

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GILBERT GOTTLIEB and JOHN G. VANDENBERGH, Dorothea Dix Hospital, Raleigh, North Carolina.

Technique for de-vocalization of duck embryos.

Duck embryos (*Anas platyrhynchos*) are capable of vocalizing 3-4 days before hatching and domestic chicks (*Gallus gallus*) can vocalize 2 days before hatching. Since both species are also capable of hearing at this time, studies of supposedly "naive" hatchlings are not actually free of the influence of auditory and vocal experience which occurs *in embryo*. To help rectify this difficulty, we have recently developed a technique for muting duck embryos 3 days before hatching. The special advantages of the present technique are that it can be used with embryos and it is reversible.

Prior to devising the de-vocalization technique, the internal and external tympaniform membranes were identified as the indispensable site of sound production in ducklings. These membranes are located immediately below the syrinx at the head end of the bronchi. (Each bronchus has one set of tympaniform membranes.) These membranes collapse and vibrate when vocalizations are produced. De-vocalization of the embryo is achieved by surgically exposing the syrinx and applying "Collodion Merck" (24% alcohol—245 grains ether per fl oz) to the tympaniform membranes. Collodion is nontoxic and it rapidly forms a glue-like sheath over the membranes. When the tympaniform membranes are thus rigidified, the embryo can not vocalize. Thus far 82 embryos have been treated and 81% were completely mute when tested after hatching. Vocal ability is re-established by exposing the syrinx and removing the Collodion sheath with forceps. (Supported by the Division of Research, North Carolina Department of Mental Health, and U.S.P.H.S. Research Grant HD-00878.)

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ROBERT G. WOLK, Nassau County Museum of Natural History.

Comparative morphology of some avian retinas.

The Black Skimmer, *Rynchops nigra*, which preys upon small fish by "plowing" the water surface with its greatly compressed mandible only immersed, is largely crepuscular but is active during both night and day also. This unique species utilizes a largely scotopic retina which is protected during periods of intense illumination by a vertically-slit pupil. Rods outnumber cones at a ratio of 8:1. This and the relative thicknesses of the outer and inner nuclear layers and the thin ganglion cell layer indicate a degree of scotopic vision and limited visual acuity which does not, however, preclude some photopic ability.

The morphology of the retinas of closely-related species of diurnal Laridae (*Larus* and *Sterna*) as well as those of unrelated species whose daily activity patterns are also largely crepuscular (e.g., *Chordeiles*) is compared to that of *Rynchops*. (Supported by Grant GE-6267 from the N.S.F.)

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R. J. CABRAL and J. I. JOHNSON, Michigan State University.

Somatic sensory projections in thalamus of sheep.

To study the relationship between behavioral specializations and brain organization, somatic sensory projections in the thalamic ventrobasal complex of sheep were mapped electrophysiologically. Tungsten microelectrodes were used to systematically sample the activity of thalamic neural units in response to mechanical stimulation of body surfaces. As in other mammals, projections from caudal body regions were found laterally, those of rostral regions medially, in the thalamus. In carnivores, rodents and primates, these projections are almost entirely from contralateral body surfaces; in sheep thalamus, however, about 80% of the nuclear volume contains projections from ipsilateral receptive fields, all located in and around the mouth and nose. One fourth of this volume (20% of the total nuclear volume) responded to stimulation of tissues inside the mouth: tongue, palate, and teeth. Half the projections from contralateral surfaces were from the head; half were from the rest of the body. The trigeminal component of this thalamic region appears overwhelmingly predominant, with its input almost entirely uncrossed. This pattern is consistent with that found in cerebral cortex of sheep (Adrian, Brain 66:7, 1943; Woolsey and Fairman, Surgery 19:684, 1946; Hatton and Rubel, Anat. Rec. 157:256, 1967), and with anatomical studies indicating a prominent uncrossed trigeminal tract in ungulates (Rose and Mountcastle, Handbook of Physiology 1:396, 1959). This extensive trigeminal representation appears to be related to the importance of grazing in the behavior repertoire of these animals. (Supported by NIH grant NB 05982, NASA Contract NSG 475, and NIH grant 10890.)

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ETHEL TOBACH, YVES ROUGER and THEODORE C. SCHNEIRLA, The American Museum of Natural History.

Development of olfactory function in the rat pup.

Olfactory bulbs were removed from Wistar (DAB) strain rat pups aged 1 to 14 days. Pups bulbectomized before day 10 did not survive. Beginning with day 10, survival rate improved and by day 13 almost all pups survived. Pups aged 1 through 14 days were observed in the following six situations: home cage pan with home cage sawdust, with fresh sawdust and with no sawdust; fresh pan with home cage sawdust, with fresh sawdust and with no sawdust. Activity was greatest on the bare home cage pan, and decreased in the situations in the order in which they are listed: fresh bare pan, home cage pan with home cage sawdust, fresh pan with home cage sawdust, home cage pan with fresh sawdust and fresh pan with fresh sawdust. Pups deprived of olfactory bulbs were not differentially active on the six types of sawdust. (Supported by grant MH-13294-01.)

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BARRY R. KOMISARUK and JAMES OLDS, Rutgers University and University of Michigan.

Behavioral correlates of single-neuron activity in freely-moving rats.

Single neurons in the limbic system-midbrain circuit of awake, freely-moving female rats showed firing patterns which were temporally correlated with feeding, lordosis, or exploratory behavior. Their firing patterns were clearly related to behavioral changes, but there was no precise moment-to-moment correlation of firing with visible muscular movements or with the application of sensory stimuli. Since these neurons apparently serve neitheir primary sensory nor motor functions, they are probably involved in higher integrative functions. Action potentials were completely absent for minutes at a time in some neurons, appearing almost exclusively during the performance of a particular behavior pattern, such as exploration. While the majority of neurons were more active in wakefulness than during sleep, changes in their activity were related more closely to particular behavior patterns than to degree of arousal. (Supported by grant MH 13279 and others from U.S.P.H.S.)

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DAVID R. HERTZLER and WILLIAM N. HAYES, State U. of N. Y. at Buffalo.

Role of the midbrain in the optokinetic response of turtles.

Red-eared turtles were subjected to midbrain transections, and their pre- and post-operative performance in an optokinetic drum was compared. Transections brought about severe reductions in binocular performance only when the direction of movement of the striped drum was suddenly reversed following a period of habituation. The operated turtles then responded as if they were monocular. (Supported by grant NB-05-001 from the U.S.P.H.S.)

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MARTHA McMULLEN and BURTON M. SLOTNICK.

Fighting behavior in mice following septal forebrain lesions.

Numerous reports indicate that rats and mice bearing septal lesions appear hyperemotional or hyperreactive to normally innocuous stimuli. Effects of septal lesions on aggressive behavior in pairs of CF-1 strain male mice matched for age, weight and exploratory activity were evaluated by observing fighting behavior in staged encounters. The same pairs were maintained throughout all tests.

The fighting arena was a neutral cage similar to each animal's home cage. Each staged encounter was terminated when, following a minimum of three fights, no further fighting occurred for 1.5 minutes.

Twelve pairs of animals were used. Six pairs had no pre-operative encounter; septal lesions were placed in one member of the pair and the second received a sham operation. The other six pairs had one pre-operative encounter. The winner of this encounter received a septal lesion and the other member was sham-operated. One to two weeks post-operatively three encounters were staged on alternate days; a fourth encounter was staged two weeks later.

In no instance did an animal with a septal lesion win a fight in any of the four post-operative encounters. A control group demonstrated that the animal which wins one encounter prior to sham surgery will be the winner in subsequent re-tests.

While normal animals either counter-attack or show submissive postures when attacked, septal animals generally show extreme escape reactions at the approach of their opponent. Brief defensive fighting was shown by experimental animals with pre-operative winning experience but not by the naive experimental mice.

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WALTER B. ESSMAN and GEORGE E. SMITH, Queens College of the City University of New York.

Behavioral and neurochemical differences between differentially housed mice.

Male CF-1 mice were housed from weaning to adulthood under either isolated (1/cage) or aggregated (5/cage) conditions. Isolated animals tended to show somewhat greater levels of locomotor activity (15%), and when free exploratory activity through a tube was tested, there were no significant differences in the number of tube entries and exits as a function of differential housing. Under a 23 hr. schedule of food deprivation aggregated mice showed significantly more tube exploration than isolates ($\chi^2 = 6.56$; $p < .02$) and differences between exploratory activity under free- and food-deprived conditions were greater for aggregated mice ($\chi^2 = 19.93$; $p < .001$). Brain norepinephrine was greater in aggregated (0.277 $\mu$g/g) than in isolated (0.256 $\mu$g/g) mice ($t = 3.39$; $p < .01$) and was inversely related to activity level in isolated animals ($\delta = -0.753$; $p < .01$). The concentration of brain serotonin and 5-hydroxyindoleacetic acid did not significantly differ as a result of differential housing, although the metabolism of serotonin, estimated by steady state kinetics, differed appreciably between isolated and aggregated mice; serotonin turnover rate was higher in isolated animals (0.423 $\mu$g/g/hr) than in aggregated mice (0.176 $\mu$g/g/hr), and the serotonin turnover time was significantly lower in the isolated animals ($\chi^2 = 4.16$; $p < .001$). The

data support the hypothesis that several behavioral differences which emerge with differential housing, such as locomotor activity and motivated exploratory behavior, are related to changes in brain amine metabolism among differentially housed mice. (Supported in part by Grant MH 13191-01 from the U.S.P.H.S.)

371

CHARLES H. SOUTHWICK, Johns Hopkins University.

Effect of maternal environment on aggressive behavior of inbred mice.

The aggressive behavior of young adult male mice of a non-aggressive strain (A/J) was significantly increased by fostering at birth to females of an aggressive strain (CFW). The chase-attack-fight score of cross-fostered A/Js increased 85% over non-fostered controls, and 52% over in-fostered controls. The attack score alone of cross-fostered A/J mice (11.6 ± 3.8/hr.) was more than twice as great as that of control (4.6 ± 1.8/hr.) and in-fostered (5.5 ± 1.9/hr.) A/Js. Attack latency of cross-fostered A/Js decreased from 35 to 13 minutes, and percent wounding increased from 3.7% to 17.5%. The aggressive score and attack latency of the CFW males were not altered by fostering to females of the A/J strain. The mechanism by which the maternal environment influences the ontogeny of aggressive behavior is not known. (Supported by U.S.P.H.S. Grant HD 00365-03.)

372

A. STANLEY WELTMAN, ARTHUR M. SACKLER and RALPH SCHWARTZ, Brooklyn College of Pharmacy, Brooklyn, N. Y.

Isolation-induced aggressiveness and behavioral abnormalities in female mice.

In man, solitary confinement has been found to cause paranoid psychoses. The development of viciousness, aggressive behavior and hyperadrenocorticalism has been noted in rats and mice isolated for 13-16 weeks. This study presents sequential changes and effects of more prolonged periods of isolation stress on aggressiveness, head-twitch frequencies, locomotor activity, w.b.c. counts and mating behavior of female albino mice isolated for 26 weeks. Thirty test mice were housed singly; equal numbers of control mice were paired 2 per cage. Measurements of body weights and neck-twitch responses were recorded weekly. Locomotor activity, w.b.c. counts, aggressiveness and mating behavior were noted at random periods. Analyses of the data revealed no significant changes in the body weights of isolated females. Behaviorally, isolation produced heightened irritability and nervousness. After the 1st week, 87% of the isolated mice showed rapid, head-twitch responses. Marked and/or significant increases were observed in locomotor activity after the 6th week. Isolation caused significant increases in the number of aggressive mice ranging up to 69%. Significant increases were also noted in the test females revealing aggressive-defensive reactions to breeding males. Significantly fewer isolated females permitted the males to mount. Total w.b.c. counts were significantly decreased at the 22nd and 24th weeks. When mated at the 26th week, no significant differences were noted in the fertility and fecundity of isolated vs. control females. The data confirmed the continued induction of aggressiveness, behavioral abnormalities and hyperadrenocorticalism by isolation stress which persisted during a 6½ month period.

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ROBERT P. SCHWARTZ, A. H. ANTON and S. KRAMER, University of Florida.

Behavioral and neurochemical correlates of isolated and grouped mice.

This study concerns itself with the interaction of behavior, environment, and sex on the catecholamine level (norepinephrine, epinephrine) in mice. Three weeks old DUB/ICR mice were housed singly and in groups of six, according to sex. After 4 weeks of isolation or grouping, individual mice were tested as follows: isolated *versus* isolated, isolated *versus* group and group *versus* group mice within each sex. Catecholamine determinations of adrenal, heart and brain followed 24 hours later.

Male mice raised in isolation were the most aggressive and fought in 78% of their encounters, whereas group-raised mice fought in only 18% of the tests. Aggressiveness appeared sex-linked since none of the females fought, and castration or estrogen treatment decreased the incidence of fighting threefold in the isolation-raised males. Testosterone treated females, however, were not more aggressive. Isolation-raised male mice, when exposed to a female, showed only sexual aggressive behavior.

The change in behavior of the isolation-raised males could not be related to any change in catecholamine levels in brain, heart, or adrenals. Male mice (grouped and isolated) had more adrenal catecholamines, of which a higher percentage was norepinephrine, than did the females even though the female adrenals weighed more. No differences in adrenal catecholamines were found between isolated and grouped males, however, even though the greatest behavioral change occurred in the male mice raised in isolation. (Supported by Grant HE-05251 from U.S.P.H.S.)

374

ANNEMARIE S. WELCH, University of Tennessee, Knoxville.

Some effects of fighting on central and peripheral catechol amines and serotonin.

Mice of many strains can be made aggressive by prolonged isolation in individual caging units. Such mice become hypersensitive to all forms of stimuli, and when placed in the presence of another mouse they will normally attack the stranger within 1-2 minutes. The fighting that ensues may continue sporatically and viciously for several hours, especially if the animals are evenly matched. The initial encounter often causes a measurable reduction in brain norepinephrine, and sometimes dopamine and serotonin, within the first 5-10 minutes; subsequently there is a gradual accumulation of all three of these brain amines, so that after 1-2 hours they may be elevated 30-50% above normal. Fighting enhances the accumulation of these brain monoamines induced by the monoamine oxidase inhibitor Pargyline, but it also enhances the amine-lowering effects of d-amphetamine. Fighting retards the lowering of brain catecholamines normally

induced by the tyrosine hydroxylase inhibitor alpha-methyltyrosine. These dynamic changes have been measured in the pons and medulla, the midbrain-diencephalon, and the telencephalon at several time periods with and without drugs. Concomitant with the apparent increase in turnover of brain amines there is a marked release of adrenaline—but not noradrenaline—from the adrenal gland.

375

BRUCE L. WELCH, University of Tennessee, Knoxville.

The brain as integrator of population stress and programmer of feed-back controls.

Population "stress" is mediated through the brain. When population density increases, central nervous activity is increased both as a result of the general increase in stimuli and as a result of increased emotional conflict. This is reflected in increased rates of production, release and breakdown of neurotransmitters; decreased responsivity of postsynaptic neurochemical receptors: increased levels of activity and of tonic inhibition of activating systems of the brain: and decreased behavioral responsivity and emotionality. The brain controls the level of activity of the adrenal glands and the reproductive organs; and changes in their function dictated by changes in population density follow only secondarily upon changes in the brain. Negative feedback regulators are operative at several levels of organization to slow the growth of populations in a density-dependent manner.

376

BENSON E. GINSBURG and JOANNE ELLSWORTH JUMONVILLE, The University of Chicago.

Genetic variability in responses to early stimulation viewed as an adaptive mechanism in population ecology.

Laboratory analyses have recently focused on a variety of mechanisms by means of which crowding, diminished food supply and other factors inimical to population growth limit reproductive behavior and interfere with successful gestation and raising of young, thereby providing feedback mechanisms which adjust population size to prevailing conditions. The present researches are concerned with genotypic variability in behavioral responses to early post-natal stresses in laboratory mice under controlled experimental conditions. They demonstrate that the period at which such stresses are most effective in altering adult behavior is a function of genotype, and that the direction and magnitude of the effects are also genotype dependent. A population may thus be viewed as inherently variable in these respects, and this is seen as adaptive since some segments of the population will respond with increased aggressiveness, heightened emotionality, and increased activity when subjected to adverse conditions during various intervals within the preweaning period, while others will respond in the opposite fashion, and still others will remain unaffected, thus insuring a variety of responses to fluctuations in environmental conditions, some of which are almost certain to be adaptive. Selection pressure must, on this view, favor continued variability in this aspect of genotype. (Supported by grant MH-03361 from the U.S.P.H.S.)

377

LOUIS LEVINE and LESTER WOLK, City College of New York.

Behavioral characteristics of ST/bJ and CBA/J male mice. (Motion picture)

Three aspects of the behavior of ST/bJ (albino) and CBA/J (black agouti) male mice were recorded: (1) their exploratory behavior, (2) their mating behavior, and (3) their fighting behavior. When placed in a strange environment, the albino males exhibit a greater amount of exploratory behavior than the black agouti males. When placed with an estrus induced albino female (ST/bJ), each type of male exhibits a distinct mount-and-thrust pattern. The albino males mount more frequently and thrust fewer times per mount than the black agouti males, (Levine *et al.*, 1966, Amin. Behav., 14:1). With regard to fighting behavior, both types of males can be trained for victory. There does not appear to be any difference in the pattern of attack or submission in the two types of males (Levine *et al.*, 1965, Anim. Behav. 13:52). (Supported by grant GB-3695 from the N.S.F.)

378

J. P. SCOTT, CHING-TSE LEE, and JOHN HO, Bowling Green State University.

Effect of agonistic behavior on rectal temperatures of male BALB/C and C57BL/6 mice.

Eight males of each strain were trained to fight by the dangling method. Rectal temperatures were taken with a thermister probe before and at intervals up to 1 hour after a standard training session. These were compared with temperatures taken 1 day sooner or later in a situation identical except that no training was given. Maximum effects were observed immediately after training. 14 out of 16 animals showed a greater rise in temperature after training than under corresponding contol conditions ($p < .01$). Training produced more fighting in the C57 animals, and temperature changes were greater in this strain. Mean changes of two trials in each strain after training and non-training sessions were: C57BL/6, 3.09°F., 1.24°F.; BALB/C, 1.90°F., .73°F. Relationships between these findings and the crowding effect on amphetamine toxicity are discussed. (Supported by grant MH-13431-01 from the U.S.P.H.S.)

379

HERBERT FRIEDMAN, PETER L. DERKS, and ROBERT ZEMORE, College of William and Mary.

Stimulus factors in the disruption of maternal behavior in crowded C57BL mouse populations.

Two populations maintained with 8 male and 16 female mice were each confined in a box with a free floor space of 147 cm × 20 cm. The long walls of the box contained two types of satchels (each with an area of 190 $cm^2$) which faced the inside of the box. A total of 126 visibly pregnant females from the populations were placed in "open" (½" wire mesh front panel) or "closed" (solid plywood front panel) satchels in either the individual's own

or the other population. The satchels confined the female and prevented members of the population from directly intruding on the nest. The "open" satchel permitted visual and some tactile stimulation from the population. Olfactory and auditory stimuli were the same for the two types of satchels. Litters born in the population were not tended to and had 100% mortality within 5 days. Based on number of young to survive 15 days in the satchel, larger mean litter size was found with the closed than the open satchels in the same population (4.54 vs. 2.85, $p < .05$) and in the different population (3.90 vs. 1.82, $p < .01$). Therefore direct interference by members of the population with the nest or the pups is not a necessary factor in the poor survival of pups in a crowded situation. A sufficient factor is the disruption of maternal behavior itself by limited stimulation from a population. (Supported by NASA Grant NSG 567-G.)

380

ERNST S. REESE, University of Hawaii.

Shell selection behavior of hermit crabs. (Motion picture)

In recent years there has been considerable interest in hermit crab biology. Basic to most of these studies is the shell in which the crab lives. The shell can be considered as a microhabitat. It is undoubtedly the most important stimulus object in the hermit crab's world, and not surprisingly hermit crabs have evolved highly adaptive patterns of behavior toward shells.

The film deals with the behavior shown by *Pagurus samuelis* to three species of shells, and is based on work reported by Reese (Anim. Beh. 10: 347-360, 1962; Behaviour 21:78-126, 1963). The crab's preference for *Tegula* shells and the experimental manipulation of shells to determine releasing stimuli are illustrated. Initial orientation to shells is visual; the crab approaches objects which contrast with the background. Then tactile stimuli take over; the crab touches objects with its first walking legs and small cheliped. If the shell is moved by this contact, the crab usually begins to explore. Shape and texture are important. External exploration of the shell leads the crab to the aperture which is probed with the chelipeds and occasionally the first walking legs. The crab then positions itself above the aperture and slides the abdomen into the shell. A rapid withdrawal movement is followed by the characteristic righting movement accomplished by placing the second walking legs beneath and behind the shell and bringing them quickly forward.

381

RICHARD A. BOOLOOTIAN and MANERT KENNEDY, Biological Sciences Curriculum Study, University of Colorado.

The use of behavioral films for teaching science as a Process of Inquiry. (Motion picture)

The BSCS Single Topic Films on "Social Behavior in Chickens" and "Mimicry" are designed to encourage an attitude of inquiry. They pose questions, raise problems, and present experimental data that promote student participation. The design of each film is such that students and teacher are engaged in active discussion during its presentation. The teacher's role is to guide student discussion and interaction and not to dominate the presentation.

Problems and questions are presented by the teacher and the film. The students are expected to supply hypotheses, interpret data, or suggest implications of experimental results.

The film on "Social Behavior in Chickens" is based on experiments by Professor A. M. Guhl and the late Professor W. C. Allee. Chickens are brought together for the first time and paired off in ten possible combinations. The chickens fight for dominance and eventually establish a social order or rank within the group.

In the film students are asked to observe the details of behavior among chickens. Observation of activity in animals requires very careful attention on the part of the observer since the slightest movement, change in position, or response might be of importance.

The second film is an abbreviated version of Drs. Lincoln P. and Jane V. Z. Brower's film on "Mimicry" which shows the results of several experiments on the effect of mimicry on predation of insects by toads. Through a series of experiments, students are presented the opportunity of examining the applicability of mimicry as an explanation to the similarity between bumblebees and a species of robber fly.

The BSCS Single Topic Films are designed to present science as inquiry. They represent the basic philosophy found in BSCS materials.

382

A. O. D. WILLOWS, University of Oregon.

The function of single nerve cells in the control of behavior. (Motion picture)

The nudibranch *Tritonia gilberti*, has over a hundred large, brightly pigmented nerve cells distributed on the surfaces of its cerebral-pleural-pedal ganglion complex. Cytoplasmic pigments give the individual cell bodies contrasting outlines and characteristic colors. The functional roles of fifty identifiable cells were explored in experiments in which the intact animal was suspended by an array of small hooks from the sides of a small aquarium. The brain, exposed by a short incision, was immobilized on a rigid circular arrangement of hooks so that prolonged intracellular recording from single cells was feasible. Intacellular recording from, and stimulation in the recognizable cells permitted a number of observations. (i) Long term spontaneous spiking, often seen in the cells of the isolated ganglia of Tritonia, does not occur in the intact animal. (ii) Certain cells, whitish in appearance, produce no obvious motor responses when stimulated. (iii) Contractions in regions of the body wall musculature, sometimes producing recognizable motor acts such as branchial tuft retraction and left or right turning, are elicited by stimulation of other cells. (iv) Generalized withdrawal and stiffening of the entire animal are produced by stimulation of any of another group of cells. Occasionally, a single spike in one of these cells triggers a prolonged escape response, comprised of over thirty seconds of sequentially coordinated activities. (Supported by N.S.F. GB3160 to G. Hoyle and GB3386 to Friday Harbor Laboratories.)

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EDWIN M. BANKS, University of Illinois.

Reproduction in the collared lemming, *Dicrostonyx groenlandicus* Traill. (Motion picture)

This film illustrates the salient features of reproduction in the collared lemming. Sequences include courtship, copulation parturition and maternal behavior. (A detailed analysis of sexual behavior will appear in a 1968 number of Animal Behaviour.) (Supported by N.S.F. GB 4996.)

384

BENJAMIN DANE, Tufts University.

Social behavior of the mountain goat. (Motion picture)

A long term study of mountain goat social behavior has been undertaken in the Coast Range of central British Columbia. This study focuses on mountain goat's general social behavior, and the possible changes in their behavior as man moves into the study area. Mountain goats have been chosen for the study because they are highly social animals that are easy to observe.

The study's initial phase has concentrated on communication, play, group size, areas of habitation, and reactions to man. In contrast to other studies of mountain goats, the animals in this study area stay in one group numbering between 28 and 47 individuals. Adult males frequently remain with the group during the summer months. During the first year, young mountain goats were never seen playing. Some play was observed during the second year. The goats have shown little fear of man, and when frightened usually flee very short distances. (Supported by grant 5TI-GM-365-04 from the N.I.H.)

385

MELBOURNE R. CARRIKER, Marine Biological Laboratory, Woods Hole.

Boring gastropods (16 mm silent color motion picture.)

The two major families of shell boring snails known, the Muricidae and Naticidae, penetrate shell of live prey by alternately pressing the accessory boring organ (abo) against the boring site, and rasping and swallowing the shell weakened by the abo secretion. The secretion, an unidentified viscid acid demineralizing substance, etches shell on contact.

The film illustrates: representative phases of the boring behavior of *Urosalpinx cinerea follyensis* in the shell of a glass-shell oyster model; magnified views of the radula; radula rasp marks and imprint of the abo at the bottom of incomplete bore holes; apparatus for determination of pH of abo secretion by means of a Charlton micro-glass electrode, and strip chart recording of pH of the active abo in bore hole (minimum recorded was pH 3.8); and feeding by the proboscis in the cavity of the oyster model.

The next scenes show live *Eupleura caudata etterae* boring *Crassostrea,* and resulting bore hole; *Thais lapillus* boring *Mytilus,* and bore hole; *Lunatia triseriata,* and bore hole in *Macoma;* and *Polinices cuplicatus,* and bore hole in *Mya.*

In conclusion there is demonstrated a ground section of the shell of the large boring rock snail *Murex fulvescens* thickly penetrated by a variety of boring clams, barnacles, worms, and sponges. (Supported by NIDR Research Grant DE 01870 from the U.S.P.H.S.)

386

E. G. F. SAUER, University of Florida.

Mother-infant relationship in Galagos and the oral child-transport among primates. (Motion picture)

The maternal child-transport in the lorisiform *Galago senegalensis* and *G. crassicaudatus* and early behavior patterns of the infants are described and compared. The carrying of the infant in the teeth of its mother, or of another female acting as "midwife," is the specific mode of child-transportation in both species. However, this behavior and its function are influenced in the two species by their different ontogenetic patterns, as defined in terms of the developmental stages of the neonates and their rates of development. The neonate of the smaller *G. senegalensis* is less developed and shows a more prolonged dependency on this special maternal care than the larger *G. crassicadatus.* The infant of the former species resists and escapes maternal transport in its fourth week, the infant of the latter species on its eighth day is already able to avoid the oral grasp of the female.

The typical primate mode of transportation by unassisted clinging of the infant to the mother's body, as it is characteristic for most nonhuman primates, occurs only under very exceptional circumstances. Oral transport is found in several prosimian species of the Lemuriformes, Lorisiformes, and Tarsiiformes, as well as in one simian form among the Cercopithecoidea. The mouth-transport as established in galagos is discussed as a phylogenetically old behavior pattern which these species share with nonprimate mammals. At present no phylogenetic lineage can be established for this behavior. It quickly disappears with the phylogenetic advancement of the primate ontogenetic pattern to the "dependent nidifugous" condition of the neonate.

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GILBERT GOTTLIEB, ZING-YANG KUO, and ANNE W. SMITH, Dorothea Dix Hospital, Raleigh, North Carolina.

Development of behavior in the duck embryo. (Motion picture)

The film shows the main features of the behavior of the Peking duck embryo (*Anas platyrhynchos*) over the course of its 27-day incubation period. The embryo's heart begins to beat as early as 42 hours and by Day 3 the heartbeat becomes regular and rhythmic. Beginning around Day 3 beating of the heart induces passive nodding of the embryo's head. Active head-lifting begins around Day 4 and lateral turning of the head begins on Day 5. When the embryo is normally positioned (i.e., with its left side in contact with the yolk sac), it turns its head more frequently to the (embryo's) right than to the left. Amnion contractions passively rock the embryo from Day 7 to Day 12. Oral activity begins around Day 8, and by Day 12 bill-clapping and tongue protrusion occur quite frequently. Excretory substances appear in the egg about Day 12, indi-

cating that digestive and urinary functions begin before incubation is half completed. The embryo's bill penetrates the air-space at the large end of the egg on Day 24 or 25, at which time it begins to vocalize fairly regularly.

The present observations indicate that the embryo is exposed to various kinds of sensory stimulation during the normal course of incubation. From other evidence it is known that all of the embryo's sensory system become functional before hatching. The role which embryonic sensory stimulation may play in the postnasal behavior of the duckling is an important question for future research.

(This narrated color film is available for rental through the Psychological Cinema Register, Pennsylvania State University, College Park, Penna. Work on film was supported by N.S.F. Grant B-14676 and U.S.P.H.S. Grant HD-00878.)

388

HOWARD E. WINN, University of Rhode Island.

Pilot whale drive, Newfoundland. (Motion picture)

Fishermen in the various large bays of Newfoundland drive with small boats schools of pilot whales, *Globicephala melaena* (Traill), into narrow arms of the bay until they eventually panic and beach themselves. Data was obtained on a drive completed on August 13, 1967. There were about 150 animals in the group. The animals after beaching are killed, flensed, and the meat processed for use as mink and pet food. Several interesting features of the animals' behavior will be seen and sound recordings of various stages in the drive were obtained. (Supported by Office of Naval Research Contract Nonr 396 (08) NR 083-165.)

389

CHARLES W. BROWN, University of California, Berkeley.

A motion picture study of *Ensatina subspecies.* (Introduced by R. A. Boolootian)

*Ensatina eschscholtzi* is a species of salamander whose populations are distributed throughout the mountains of California, Oregon, and Washington. In California, this species consists of a series of populations which show gradual clinal variations in color patterns along the coastal mountains (generally unblotched in coloration) and down the interior mountains (blotched). In northern California, there is a smooth, broad band of intergradation between the coastal unblotched subspecies *oregonensis* and the interior blotched *platensis*. In central California unblotched *xanthoptica* populations meet those of *platensis* in the Sierra Nevada, forming fairly narrow zones of hybridization. In southern California near San Diego, the zone of hybridization is extremely narrow, and some symparty without evidence of introgression between *eschscholtzi* and *klauberi* has been observed. The above data suggests a north to south gradient in genetic divergence and the evolution of partial reproductive isolating mechanisms.

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DENZEL E. FERGUSON, Mississippi State University.

Sun-compass orientation in amphibians. (Introduced by R. A. Boolootian) (Motion picture)

Seven families of frogs, toads, and salamanders use celestial cues to orient to familiar segments of shorelines and to locate breeding sites. The test procedure involves transporting animals in light-tight containers and releasing them in large circular arenas on land or in water. Orientation persists in daytime and nighttime tests but fails under complete cloud cover and after prolonged exposure to darkness. The method is easily adapted to training situations involving an artificial "sun" or an artificial shoreline.

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C. S. LIN, Huston-Tillotson College.

Nesting behavior of *Trypargilum spinosum* Cameron (Hymenoptera:Sphecidae).

*Trypargilum spinosum* Cameron is a Neotropical wasp but also occurs in Texas. The nesting activities were observed in May and August, 1966 on the front porch of the author's residence in Austin where a dozen bamboo stems were used as trap nests for *Isodontia mexicana* (Saussure). Both sexes of *T. spinosum* were seen flying about the porch and inspecting the bamboo stems. They mated and began to occupy the one stem which was not used by *I. mexicana*. No hunting and provisioning activities during first two days presumably the preliminary work on cleaning the nest and erecting the inner partitions of mud discs has been performed by the female. The male stands on guard at the entrance while the female is away. They also stay inside the nest until it is fully provisioned and sealed off. The 15 cm long bamboo stem is neatly partitioned into six brood cells, each is separated by a mud disc. A fully stocked cell consists of 16 small spider prey belonging to the families of Anyphaenidae, Argiopidae, Oxyopidae, Salticidae, and Thomicidae. The egg is glued to the dorsum of last spider's abdomen. There are three generations in the summer.

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JOHN D. SPOONER, Augusta College, Augusta, Georgia.

Bush Katydid oviposition behavior.

Bush Katydids (Insecta, Orthoptera, Tettigoniidae) exhibit stereotyped sequences of behavior patterns during oviposition resulting in eggs laid in the soil on the edges of leaves, or inside leaves between the epidermi, the oviposition site being species-typical. All species investigated sample oviposition sites with the palpi to determine where to lay. Once a spot is chosen, the ovipositor is brought forward ventrally, grabbed between the mandibles. and held between the mandibles until inserted into the oviposition medium. One egg is layed at a time. After each egg is layed, the female proceeds to groom the entire ovipositor. (Supported by NSF Grant GY-2218.)

394

JEROME S. ROVNER, Ohio University.

Territoriality in the spider *Linyphia triangularis.*

The sheet-web weaving spider *Linyphia triangularis* occurs in dense populations. The adult male

does not construct a web. He visits the webs of female conspecifics for variable lengths of time. While in the web, the male is dominant over the female. The female's web is used by both sexes for prey capture and is the site of courtship and copulation.

If a second male enters the female's web, semi-ritualized fighting between the males occurs. The enlarged chelicerae are used as weapons during such territorial defense.

Prior to copulation the male usually performs web reduction. Chemical or vibratory signalling by the receptive female may provide the stimulus releasing this behavior. In web reduction the male uses his chelicerae to tear the peripheral fibers of the female's sheet-web and thereby decrease the web's area. Web reduction apparently serves to lessen the likelihood of interference during the lengthy copulation. (Supported by an N.S.F. post-doctoral fellowship.)

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LLEWELLYN T. EVANS, Research Laboratory, Troy, N. H.

How are age and size related to mating in the Wood Turtle, *Clemmys insculpta*?

Wild adults, collected and studied in New Hampshire, varied in growth ring count from 12 to 25 (years?) and in size (mean of six carapace-plastron dimensions) from 13.4 to 17 cm.

Comparing, for example, the time-interval-data of the mounted phases: 1) rolling, 2) pumping, 3) thumping, in older mates (mean of 21 g.r.c.) with that of younger mates (mean of 14.5 g.r.c.), the results averaged 9.5, 6.0 and 16 minutes, respectively, for the former and 3.5, 3.5 and 5 minutes, respectively, for the latter.

However, the number of rhythmic pumps and thumps per-minute elicited by older mates were only 66 and 5 respectively, compared with highs of 108 and 12 respectively for the younger mates.

When males (21+ g.r.c.) were mated with females (14-15 g.r.c.), the data for both time-intervals and rhythms-per-minute were intermediate to the data given above. This was true also when the young male (14 g.r.c.) was mated with older females (20-21 g.r.c.). Evidently the length or duration of time-intervals of phases 1, 2 and 3 was directly proportional to the mean g.r.c. of the partners in courtship; whereas the number of rhythmic pumps and thumps per-minute was inversely proportional to the mean g.r.c. of the partners.

Results show that size is also a factor. For example, an older male mated with a larger than average young female, and a larger young male with an older female both yielded data somewhat higher than comparable data from average size-age mates.

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SALVATORE F. BONGIORNO, Louisiana State University.

Substrate and nest location in *Larus atricilla* (Laughing Gull).

On a salt marsh in Cape May, N. J., for the past three summers, within a permanent grid, the locations of Laughing Gull nests have been recorded. The topography of the gullery, as well as a total mapping of the vegetation mosaic indicate that substrate exerts a major role in the actual configuration of nests within a colony. Field experiments altering the marsh grasses and debris changed the nesting pattern: birds nested in spatial configurations dictated by placement of debris; where grasses were cut, they did not nest although they had nested previously in control (uncut) years. The prevalent view of the function of nest spacing in ground nesting gulls is that it has evolved as a consequence of predator selection pressure. Instead, this study suggests that spacing of nests is dependent upon a bird's "double" response to the habitat and to its neighbors.

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LARRY C. HOLCOMB, Creighton University.

Egg turning behavior of color-marked eggs in birds.

Eggs were numbered with fingernail polish in 28 species. Eggs were manipulated so that all the numbers faced up one day and faced down the next in 12 species. The frequency of those found up or down on the following day was recorded. Nine of the 12 species where manipulations occurred did not turn the eggs at random. Instead, Chi-square values show that the numbered side of the egg was turned down. Furthermore, Chi-square values showed that the same nine species turned their eggs numbers down when left up more often than they turned them up when left down.

Species that are frequently parasitized by cowbirds reacted to the colored eggs in the same manner as infrequently parasitized species. The colored numbers apparently acted as a "disturbing" factor to the incubating parent but they did not differ enough from the original color to cause ejection or desertion.

398

CAROL A. DIAKOW and LESTER R. ARONSON, American Museum of Natural History.

The role of genital sensation in the maintenance of sexual behavior in the female cat.

Penile sensation is important for the integrity of mating behavior and for long-term maintenance of sexual arousal in male cats (Aronson and Cooper, 1966), but previous experiments on female cats (Bard, 1935) and on other mammals indicated that estrous behavior is retained after genital desensitization. The object of this experiment is to determine quantitative and long-term effects of genital desensitization on the mating pattern and on sexual receptivity in the female cat.

The vulva and clitoris were desensitized in 4 sexually experienced females by bilateral section of the pudendal nerve. In 30 weekly postoperative tests no differences in the mating patterns between the nerve-cut and two sham-operated females were seen. Post-copulatory rubbing and rolling decreased significantly after nerve section, and other post-operative changes in behavior were noted, but similar changes occurred in the control group. Behavior associated with receptivity did not change in either group. Desensitization was confirmed by histological and neurophysiological methods.

The vagina and distal portion of the uterus are innervated by branches of the pelvic plexus. Bilateral section of these branches in four sexually experienced females changed their copulatory re-

sponses. The loud "copulation cry" normally given at intromission was heard less frequently, on many more occasions the male was not dislodged after copulation, and the rubbing and rolling component of the "after-reaction" was less vigorous. Thus, specific genital sensations are as necessary for the maintenance of normal mating behavior in the female as they are in the male. (Supported by Grant HD-00348, U.S.P.H.S. and U.S.P.H.S. Fellowship No. 1-F1-MH 20, 805.)

399

LORRAINE L. ROTH, Princeton University.

Effects of young and of social isolation on maternal behavior in the virgin rat.

Virgin rats initially unresponsive to young become maternal after 5 days of living with them (Weisner, B. P. & Sheard, N. M., 1933, *Maternal Behaviour in the Rat.* Oliver & Boyd, Edinburgh; Rosenblatt, J. S., *Science, 156,* 1512-1513). This observation is important because (1) it shows that, contrary to previous opinion, maternal behavior is not directly hormone-dependent, and (2) it raises further questions about the effects on maternal behavior of the presence and absence of young.

In the present study, it was shown that both the appearance *and* maintenance of induced maternal behavior is dependent upon a gradient of stimulation coming from young. Sprague-Dawley virgins which were initially unresponsive became maternal either by living in direct contact with young, or by indirect contact, when young lived "next-door" to the female in wire baskets placed on the side of her cage. Under condition one, direct tactile cues from the young were present as were long-range ones (odors, sights, sounds), whereas under condition two, only long-range cues were available. Under condition one, maternal behavior appeared in about 5 days and remained high and constant for the duration of testing (12 days). Under condition two, the latency was 9 days, and the behavior varied. Control females simply tested with young showed no behavior.

It was shown further that maternal behavior is decreased markedly by a reduction in environmental stimulation. 60% of the Sprague-Dawley rats tested showed an initial interest in young. After 2 weeks of living alone in sound-proof boxes, however, only about 35% of them showed a response. This suggests that housing conditions can alter behavior. (Supported by Post-doctoral Grant 2- F2-HD 19, 140- 03 from U.S.P.H.S.)

400

RONALD J. BARFIELD, Rutgers University.

Activation of sexual and aggressive behavior by androgen implants in the brain of the male Ring Dove.

The site of hormonal control of sexual and aggressive behavior in the Ring Dove *(Streptopelia risoria)* was investigated by implanting minute amounts of testosterone propionate (TP) into the brains of castrate males. Crystalline TP was compressed into 27 gauge stainless-steel tubing so that the hormonal surface was equal to the cross-sectional area of the lumen of the tube (0.03 $mm^2$). The tubes were implanted bilaterally in the brains of 33 doves 4 to 6 weeks post-castration. Upon testing, 14 birds exhibited bow-cooing behavior. All of these birds bore implants in the anterior hypothalamic-preoptic region. Of the 14 responding birds, 6 showed wing-flipping behavior also. These birds all had implants in the preoptic area. Six responding birds showed copulation behavior, but their implants were distributed throughout the anterior hypothalamic-preoptic region. The 19 doves not responding bore implants throughout the diencephalon and in the basal telencephalon.

Androgen appears to act within the anterior hypothalamic-preoptic region to activate copulatory, courtship and aggressive behavior in the male Ring Dove. This finding contrasts with results obtained in the male fowl where androgen implants in the preoptic area activated copulatory behavior, but not courtship or aggressive behavior (Barfield, Amer. Zool., 4:301, 1964). Thus a basic neural organizational difference appears to exist between these two species. This difference may be related to differences in social organization between the two species or to the fact that the fowl is sexually dimorphic while the dove is not. (Supported by Grant GM-1135 from the USPH to Dr. D. S. Lehrman.)

401

DONALD J. KIMELDORF, Oregon State University, Corvallis.

Prompt reactions of organisms to ionizing radiations.

A variety of animals can detect promptly the presence of ionizing radiations. In mammals, detection has been identified experimentally through immediate behavioral reactions such as prompt arousal from a sleep state and the immediate suppression of on-going activity when radiation is used as a warning cue for electric shock. These reactions are accompanied by transient charges in electrical activity of the brain and brief acceleration in heart rate. Potential mechanisms for detection appear to include visual and olfactory pathways and one or more visceral pathways. It is presumed that ionizing radiation constitutes an effective stimulus for receptors of the identifiable pathways, although non-sensory mechanisms have not been entirely eliminated. Reflex-like behavioral reactions to radiation exposure have been reported for insects and other invertebrates. The invertebrate reactions may often be produced by radiations in the visible energy spectrum, suggesting photochemical reactions for ionizing radiations as well. Information gained from detection has been utilized to promote aversive behavior with respect to exposure field or cues associated with exposure. The evidence supporting the thesis of biodetection of radiation exposure to less than one Roentgen will be presented.

402

JAMES C. SMITH, Florida State University.

Radiation as an aversive stimulus.

Experiments were conducted conditioning aversions to distinctive taste substances by irradiating rats in the presence of the taste solution. The role of the quality and concentration of the taste substance was studied. It was shown that saccharin solution (.1%) yields better conditioning than sucrose (4%) when studying the interval between

presentation of the CS (sweetened water) and US (100 r $Co^{60}$ irradiation). In addition, higher concentrations of sucrose yield better conditioning than lower concentrations.

Also demonstrated was the fact that all preferences could be reversed by one pairing of the Gamma rays with the preferred solution. This included conditioning rats to prefer lower concentrations of sucrose over higher concentrations, to prefer lower concentrations of saccharin over higher concentrations, and to prefer saccharin solution over sucrose. The results of the final experiment indicated that if the rat was irradiated in the presence of a mixture of glucose and saccharin, the subsequent aversion was to the saccharin solution only. Implications of these aversions for the study of basic taste discrimination studies were discussed. (Supported by Contracts AT-(40-1)-2903 and AT-(40-1)-2690 with the Division of Biology and Medicine, U. S. Atomic Energy Commission.

403

GARY S. SHABER, ROBERT L. BRENT, JAMES A. RUMSEY, and GAIL NEWINGHAM, Jefferson Medical College.

The effects of low doses of ionizing radiation on taste thresholds.

The dependence of taste upon continuous mitotic activity in the taste buds has been previously well documented. Since radiation is a mitotic inhibitor, alteration of taste thresholds should occur following irradiation of the taste buds. Therefore, taste thresholds in the rat for saccharin were determined using a condition suppression behavioral technique with hypothalamic stimulation for reward. This method of determining taste thresholds was used so that a prolonged training period would not be required, rapid and repeatable determinations of the threshold could be obtained during one experimental trial, and so that the animals' motivation to discriminate between test solutions would remain high throughout the entire trial. Following determination of the taste threshold (three to four weeks), eight animals received 50-100 R of X-irradiation to the tongue, using a 250 KVP X-ray generator, 2 mm Cu filtration, and their thresholds remeasured beginning three hours after radiation exposure and daily thereafter. At least a two-fold increase in taste thresholds was observed following irradiation. In conjunction with the changes observed in taste threshold, a morphological study of the taste buds was conducted to document any anatomical change, to obtain a correlation between structure and behavior. (Supported by Grant 3359 from the U. S. A. E. C.)

404

JOHN R. TESTER, D. B. SINIFF and ORRIN J. RONGSTAD, James Ford Bell Museum of Natural History, University of Minnesota.

Effects of radiation on behavior of unconfined raccoons and snowshoe hares determined by telemetry.

Behavioral effects of sub-lethal doses of ionizing radiation on unconfined animals are being studied by telemetry at the Cedar Creek Natural History Area in east-central Minnesota. One adult male and one yearling female raccoon *(Procyon lotor)* and one adult and one young female snowshoe hare *(Lepus americanus)* were exposed to 300 r gamma irradiation from a cesium-137 source. Comparative data have been obtained from the pre- and post-irradiation periods and from paired control animals.

Movements and activity of each animal are monitored by an automatic radio tracking system which can obtain data on each animal every 45 seconds.

Analyses of the behavior of each irradiated animal will be discussed with emphasis on effects of radiation on circadian activity patterns and on size of home range. For example, data on irradiated raccoon 625 show no marked change in circadian activity pattern, but suggest a slight increase in the amount of time spent resting in the post-irradiation period. A reduction in size of the home range occurred during 2 consecutive 15-day periods following irradiation. The control raccoon increased the size of its home range during this period. (Supported by U. S. Atomic Energy Commission Contract AT(11-1)-1332, Document COO-1332-39.)

405

ERNEST FURCHTGOTT, University of Tennessee.

Behavioral effects produced by irradiation during the developmental period.

A number of different behavioral changes have been observed in animals exposed to ionizing radiation during the developmental period. In most of the studies the observations were conducted relatively early in life. In a few instances data are available on older animals, but only cross-sectional comparisons can be made. So far there have been few attempts to interpret the diverse findings.

In the present series of studies different behavioral measures were obtained on prenatally exposed rats. Most of the data are based on animals which had received 200 R (250 kVp X-rays) on day 16 of gestation. Both cross-sectional as well as longitudinal comparisons were made of age changes in the irradiated animals. Data were obtained on: open field activity, eating in a novel place, swimming speed, activity wheel performance, runway performance, electric shock escape and avoidance conditionings, stove-pipe emergence, inclined plane performance and heart rate reactivity to white noise.

Using cross-sectional comparisons no age changes were apparent. However, in the longitudinal studies, early observed differences between irradiated and control animals tended to diminish with repeated testing.

It is hypothesized that the irradiated animals show behavioral hyperreactivity to novel stimulation. This is associated with increased heart rate reactivity. However, once adaptation to the testing situation has occurred, the behavior of the irradiated animal is similar to that of a control. Many diverse findings would seem to fit this hypothesis. (Supported by Grant RH0065, U.S.P.H.S.)

406

SYLVAN J. KAPLAN, Armed Forces Radiobiology Research Institute, Bethesda.

Some psychophysiological effects of a single dose of gamma-neutron radiation upon monkey.

Twenty-four monkeys, divided into four groups of

six subjects each, were exposed to a single whole body dose of 5000 rads of mixed gamma-neutron radiation. Time of delivery of the dose was on the order of 20 milliseconds. One group was studied for physiological functions only (EEG, ECG, and peripheral temperature). The remaining three groups were examined primarily for their performance capabilities following irradiation with specific attention focused upon retention of three different behavioral tasks learned prior to exposure. Performances required for these tasks included: (1) capacity to discriminate between visual and auditory cues in a short time frame, (2) memory for six pairs of discrete forms and colors, (3) arm-hand motor requirements for pressing a button and a lever, and (4) running, which exercised the subject's gross musculature. Responses were measured in terms of latency and accuracy. All animals were examined physiologically and psychologically at regular intervals from exposure to death.

Results indicate that, while marked individual differences are seen, postirradiation performance falls into three phases capable of objective description: (1) transient phase—characterized by complete work cessation for some of the subjects, begins during the first five minutes postirradiation and varies in duration among subjects; (2) recovery phase—subject appears to have regained its preexposure performance behavior; (3) total and complete behavioral incapacitation phase—precedes death, the duration varying from subject to subject. Characteristic EEG, ECG, and temperature changes are observed during the above mentioned phases. These physiological changes are considered to be related significantly to some of the behaviors noted.

407

STEPHEN H. VESSEY, Laboratory of Perinatal Physiology, San Juan.

Interactions between free-ranging groups of rhesus monkeys.

Interactions of three groups of rhesus monkeys were studied for 15 months on a small island off the coast of Puerto Rico. A stable dominance hierarchy positively correlated with group size persisted throughout the study. The overall interaction rate was 0.36 per hour; significant differences among groups were found. Peripheral males were most often involved in interactions; in descending order of frequency other categories of animals involved were central males, adult females, immature males and immature females. Peripheral males were also responsible for most of the aggressive behavior which accompanied about one-half of the interactions. Intergroup play was seen among immature males, but grooming was never seen between members of different groups. Because a few members of a dominant group often displaced an entire subordinate group, recognition of individuals from other groups and conditioning from previous encounters are assumed to occur.

408

DONALD G. LINDBURG, University of California, Davis

Patterns of reproduction in wild rhesus monkeys.

Reproductive behavior of rhesus monkeys was observed during a 21-month field study in India. Previous reports of a spring birth season are confirmed. Virtually all mating activity occurred between October and January. About 30% of the adult males in the study area changed groups during the mating season. A similar phenomenon occurs annually in the Cayo Santiago colony. In India most cases of change involved activities related to reproduction. Change was not restricted to males of a particular age or social class, nor to those deprived of the opportunity to mate in their group of origin. These patterns are of theoretical importance, since sexual attraction in primates has usually been considered an important cohesive force. The evidence for this species shows that it can be disruptive. Male interchange also provides for distribution of genetic material among the groups of an area. (Supported by National Center for Primate Biology, Davis, California.)

409

STUART A. ALTMANN, Yerkes Regional Primate Research Center.

Testing Mason's hypothesis of sex differences in the affective behavior of rhesus monkeys.

According to Mason, Green and Posepanko (1960), adult female rhesus monkeys are more "anxious" than adult males, and tend to over-react to stressful situations. They tend to show a higher incidence of affective reactions than do adult males, including threat behavior and particularly the milder agonistic responses. This hypothesis was tested against data that were gathered on free-living rhesus monkeys on Cayo Santiago. These data gave no indication that females showed a higher incidence of affective responses as a whole, nor that they were more inclined to exhibit the milder forms of agonistic behavior. There was an indication that the affective social behavior of adult males was somewhat more likely to be aggressive; that of females, more submissive. Adult females were more likely to ignore their social partner and were less likely to lip-smack in agonistic situations. While the available data do not confirm the hypothesis of Mason *et al,* they do support an alternative hypothesis, namely, that in agonistic situations, adult females tend toward a strategy that can be crudely characterized as "hit-and-run," whereas males tend to a strategy of "come-closer-or-scream." (Supported by grants GB-2879 and GB-4415 from N.S.F. and MH-07336-01 from N.I.H.)

410

JOHN R. OPPENHEIMER, University of Illinois.

Vocal communication in the white-faced monkey, *Cebus capucinus.*

During the period from March 1966 to August 1967 several groups of wild and caged *Cebus capucinus* were studied on Barro Colorado Island in the Canal Zone. Recordings were made in the jungle to help in identifying the vocalizations and for their later analysis. Those vocalizations that were given during situations of close individual contact, and which were usually given in low volume were recorded from caged individuals. Approximately 16 calls were distinguished, plus three noises—tooth grinding, sneezing, and coughing. The vocalizations can possibly be divided into 3 or 4 groups within which they are closely related in terms of their

function and physical formations. Each call is given usually in specific circumstances in the wild, such as when an individual is lost or when a large bird flies overhead. Some comparisons are made with the vocalizations of other New World primates. (This work was supported by a Predoctoral Internship from the Smithsonian Institution.)

411

IRWIN S. BERNSTEIN, Emory University.

Territoriality and the lutong, *(Presbytis cristatus)*: a field study.

During an 18 month field study in Malaya, interactions among five adjacent lutong monkey troops were recorded. The nature of the interactions suggests territoriality.

A single adult male in each troop actively defended a specific geographic area against intrusion by the males of adjacent troops. The territorial overlap between troops consisted of only a few trees and disputes were most common in these areas. Although males defended their troops against other males, they would leave the troop in response to a territorial infringement by another male anywhere within their territory.

Conflicts were expressed in: vigorous maneuvering, loud vocalizations, chases and rare aggressive contacts. Chases and counterchases were observed as the males crossed territorial boundaries. Fighting consisted primarily of grappling with little biting. Injuries noted resulted from falls; most of which occurred during territorial disputes.

In the absence of male conflicts, females and immature animals of adjacent troops came together peacefully. No exchanges of membership were observed. (Supported by N.S.F. Grant GB 3008 and N.I.H. Grant FR-00165.)

412

GORDON R. STEPHENSON, University of Wisconsin.

Cultural aspects of communication among nonhuman primates. (Motion picture)

Culture among nonhuman primates appears as a constellation of behavior patterns characteristic of a social group, transgenerational, and socially learned. Many such patterns have been described by Hall (1963, Brit. J. Psych. 54-3), Kawai (1965, Primates 6-1) and others. Incidence of some communicatory behavior patterns appears similarly coincident with group membership.

Culturally derived signals can be distinguished from fixed action patterns by detailed comparison of signal form in terms of the spatial integration of behavioral units over time. A kinesic analysis and a scaling procedure can independently test for similarities and differences in the form of a given signal as performed by different individuals. Digital signals are more readily compared than are analogic ones. Analogic signals can be compared by ranking them for similarity in a scaling procedure adapted from Clifton and Odom (1966, Psych. Monogr. 80-5). This procedure was applied to randomized sets of film clips of individual macaque monkeys performing particular behavior patterns. Social distribution of such differentiated signal performances serves as a criterion for discerning the derivation of a given signal. Applied across natural groups of a primate species, the degree of openness (cf. Sebeok, 1965, Science 147, p. 1009) of communication for this species could be ascertained.

The cultural derivation of some communicatory behavior patterns cautions against the indiscriminate use of the manifestation or incidence of the various patterns to monitor the ontogeny of sociality among laboratory-reared nonhuman primates. (Supported by NSF through the University Research Committee, NSF Grant GB-4193, and the Wisconsin Alumni Research Foundation.)

413

ROBERT J. PALUCK, JONATHAN D. LIEFF, and ARISTIDE H. ESSER, Rockland State Hospital.

Artificial group formation with juvenile *Hylobates lar*

To explore group formation and development in *Hylobates lar* three juvenile gibbons without previous social contact were placed together and observed in a naturalistic environment. Preliminary analysis of 1200 observations in 96 hours indicates that the gibbons formed a group which remained together in most activities, e.g. foraging, playing, sleeping. Foraging categories accounted for about 76% of total daytime activity; social interaction, 18%; resting, 6%. These figures essentially parallel findings of Carpenter (1940) and Ellefson (1967) with gibbon groups in the wild. Observations of mechanisms of group formation and maintenance, and computer-analyzed data on spatial organization and periodicity of individual behavior will be presented.

Sixteen juvenile gibbons, two to four years old, have been maintained with minimum human contact for two years on Laulanui Island in Hawaii. Of these one male and two females were selected for the study from three separate cages and released onto a specially prepared one acre lobe, isolated by electric fences and viewed from two unobtrusive observation posts. Data were obtained from all-day observations on twelve separate days during a three week period; they include discrete and continuous individual records of location, posture, and activity as well as social interactions, e.g., peer and sex play, dominance, and group movement.

At the end of the study period, the remaining 13 gibbons were released onto the lobe. After a period of territorial hooing, 3 were integrated into the group; the others were rejected. This observation parallels information on maximum size of wild gibbon groups. (Supported by General Research Support Grant FR 05561-04, New Jersey Foundation for Mental Hygiene, Inc., and The Zaret Foundation, and grant from Harvard Medical School.)

414

CARL S. HELLMANN, Trident Laboratories, Inc., Washington, D.C.

Vocal simulation in *Lemur*.

Experimental simulation of *Lemur* vocalizations has been attempted and the resulting behavior and apparent understanding or at least recognition, by the experimental animals has been examined to determine the effects of frequency, phase, amplitude,

and variable attack and roll-off portions of the impressed signals. LLLTV, x-ray, sonographic and oscillographic approaches have been used to study the effects of varying the temporal content of several basic *Lemur* vocalizations (grunts, howls), and the resulting behavioral sequences. Specificity of the calls was examined to determine the most meaningful, or responsive, portions of the vocal signals, and a first attempt was made to determine the thresholds applicable to several frequencies in the presence of noise typical in the *Lemur* vocal signals. While the experiments are not yet complete, it appears that *Lemur* has highly sensitive hearing with sophisticated cortical processing techniques similar to those of most higher primates, but with different frequency, threshold, and modulation parameter ranges.

415

L. L. ADLER and H. E. ADLER, The American Museum of Natural History and Yeshiva University, New York, N. Y.

Age as a factor of observational learning in puppies.

Learning by observing the behavior of another member of the same species (imitation) has been relegated to relatively minor importance in past discussions of the development of canine behavior. Using a split-litter technique, observational learning was tested in a duplicate cage apparatus on four litters of miniature dachshund puppies, 21, 28, 38 and 60 days of age at the beginning of the experiment. Although the total N was small, clear-cut differences emerged. No observational learning could be demonstrated for the youngest litter, but significant differences between learning times of demonstrators and observers were found for the older puppies. The limiting factor appeared to be the development of adequate visual functioning after the opening of the eyes at age 2-3 weeks.

416

JUNE F. HARRIGAN, University of Hawaii.

Preliminary observations on rhythms in locomotion and feeding in *Metapograpsus messor*. (Introduced by E. S. Reese) (Motion picture)

*Metapograpsus messor* is a semi-terrestrial crab common in Hawaii along rocky shores with light to moderate wave action. Hourly crab counts were taken through a Questar telescope; temperature, tide height, and number of crabs feeding were also recorded. Multiple regression analysis showed a significant regression (P = less than 0.01) only for number of crabs present against time and tide height, and not for number of crabs feeding. There were more crabs out in the early morning, especially at low tide, when the rocks were damp and the temperature lower than were visible in the late afternoon when the rocks were drier and warmer.

The number of crabs feeding was 10% higher after dark, indicating a tendency toward nocturnal feeding.

Estimated population density ranged from one crab per 750 $cm^2$ at low tide to one crab per 594 $cm^2$ at high tide. A laboratory tank held one crab per 363 $cm^2$; that this was above optimum density was indicated by the abnormally high frequency of aggressive encounters.

In the laboratory tank the crabs distributed with respect to each other rather than with respect to each of several water levels. There were more crabs above water in the afternoon than in the morning at all water levels. This afternoon upward movement was not observed in the field, where higher temperatures and drier surfaces may have restricted the crabs to crevices or below water level.

417

TALBOT H. WATERMAN and DONALD A. BERRY, Yale Univesity.

Evidence for diurnal vertical plankton migration below the photic zone.

To determine whether diurnal vertical migrations extend below the photic zone (about 1000 m max.) 57 horizontal two-meter closing-net hauls were made at 2300-0100, 0500-0700, 1100-1300 and 1700-1900 hours at depths grouped in adjacent layers centered at 600, 1000, 1400 and 1700 m at a station 5120 m deep between Funchal and Lisbon. The resulting $4 \times 4$ catch matrix for time of day vs. depth has been analyzed for 207 species (17 medusae, 14 chaetognaths, 5 mysids, 40 amphipods, 23 euphausiids, 36 decapods and 72 teleosts).

Differences between matrix categories ($j$, $k$) have been evaluated as

$$\delta_{jk} = \sum_{i=1}^{n} [\ln(X_{ij} + C) - \ln(X_{ik} + C)]^2$$

where $X_{ij}$ is the number of species $i$ caught in category $j$, n the number of species and $C$ a constant various values of which were tested. When the 16 resulting points were fitted by a multidimensional procedure onto one or two dimensions, they are clustered and ordered so that depth in the water column is a major vector, time of day a secondary one. Dispersion for time of day is marked at 600 m, much smaller at the three deeper levels.

Twenty-five individual species gave evidence of diurnal vertical movements partly or wholly within the depths sampled. Two behavior patterns were most interesting. Species of *Eukronia, Eucopia, Acanthephyra* and *Gennadas* apparently shifted their depth of maximum abundance from 1000 m at night to 1400 m during the day. Also two species of chaetognath peaking around 1700 m at midnight appeared to descend below this depth in the daytime. (Supported by NSF G-8638, G-9690, the British National Institution of Oceanography and USONR contract 60723.)

418

HUGH DINGLE, University of Iowa.

Environmental and hereditary influences in the migration of the milkweed bug *Oncopeltus*.

Most migration in *Oncopeltus* takes place early in adult life following maturation of the cuticle. In bugs reared on a 16 hours light, 8 hours dark 23°C daily regimen, approximately 20% of males and 30% of females were migrants: raising or lowering the rearing temperature, to 27°C and 19°C respectively, reduced the proportion migrating. Altering the daily light regimen to 12 hours light, 12 hours dark or temporarily depriving the bugs of food extended the period during which migration occurred; both these treatments delay or depress reproduction. The proportion of migrants of both sexes was increased to between 60% and 70% by

selective breeding thus indicating a genetic basis. The data are consistent with the hypothesis that migration is promoted or suppressed in genotypical migrants by those environmental factors which influence reproductive (and corpus allatum) development. (Supported by NSF grants GB-2949 and GB-6444.)

419

ROY L. CALDWELL and MARY ANN RANKIN, University of Iowa.

Temporal segregation of various cyclic behaviors in the milkweed bug, *Oncopeltus fasciatus*. (Introduced by Hugh Dingle)

Various cyclic activities occur in the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae) including flight, feeding, mating and oviposition. Mating and feeding reach maxima at sunset and flight and oviposition in the afternoon. Some of these activities are mutually exclusive; for example, flight or oviposition can not occur during mating and feeding. Analysis shows, however, that the expression of each activity is virtually independent of other activities. In addition, mating and feeding cycles of ovariectomized females are unaltered even though oviposition and flight do not occur. The occurrence of mating and feeding in the evening allows maximum opportunity for flight and oviposition during the afternoon when conditions may favor these activities. Evening occurrence of mating and feeding may serve to concentrate bugs at feeding sites, increasing the probability of contact between potential mating partners. This segregation of behavioral events may have particular adaptive value for a migrant colonizer such as *Oncopeltus*. (Supported by NSF grants GB-2949 and GB-6444 to H.D. and a NSF Pre-doctoral Fellowship to R.L.C. and NSF Traineeship to M.A.R.)

420

WALTER LENER, Nassau Community College, Garden City, New York.

Pheromone produced by *Oncopeltus fasciatus*, the large milkweed bug.

Pheromones (ectohormones, sociohormones) affect the behavior of other individuals of the same species. Adult male milkweed bugs reared in isolation or with all male container-mates produce a pleasant fruity aroma in their containers. Humans can readily detect this scent. Female milkweed bugs do not produce this fragrance. It was hypothesized that the male aroma served as a pheromone, and a study was conducted to evaluate this hypothesis. The results are discussed in detail.

Investigations of *Oncopeltus fasciatus* are particularly pertinent at this time because the *ad hoc* Committee of the Phenology Program of the United States, as part of our country's contribution to the International Biological Program, has listed this as one of seven species of insects suggested for extensive study.

421

ROBERT M. MURPHEY and CHERYL F. HALL, University of California, Davis.

Some correlates of *Drosophila* negative geotaxis.

Hirsch and his associates (e.g., Erlenmeyer-Kimling, Hirsch & Weiss, J. Comp. Physiol. Psychol. 1962, 55:722-731) have reported extensive analyses of genetic selection for geotaxis in *Drosophila melanogaster*. When male descendents of their negatively geotactic strain were tested in a different kind of maze, they showed a statistical tendency toward repeating responses at sequential choice points rather than alternating them (Murphey, J. Comp. Physiol. Psychol., 1965, 60:196-199). The geometry of the geotaxis apparatus, which Hirsch *et al.* used for selection, requires that subjects repeat responses at choice points in order to receive high geotaxis scores. Hence, it is possible that their animals were selected for response perseveration in addition to gravitational responses.

The hypothesis was supported when negatively geotactic and unselected "wild type" males were tested for response stereotypy, with the result that the negatively geotactic flies had a statistical tendency toward repeating responses, whereas the wild type animals did not. Also, a greater proportion of the geotactic subjects were able to complete the test during the time limit imposed. This suggested that selection for negative geotaxis may have included selection for an ability to survive a dry, plastic environment and/or selection for locomotor activity as well as response perseveration. In testing these hypotheses, geotactic flies did outlive wild types in a dry environment, but wild type flies were the more active under a variety of conditions. Further experimentation demonstrated that activity in a dry environment is associated with mortality.

422

JERRY HIRSCH, University of Illinois.

Tropisms *are* forced movements. (Motion picture)

For Jacques Loeb tropisms were oriented movements of both plants and animals which were forced "by outside sources of energy." Fraenkel and Gunn (1960, p. 307) have objected that " . . . neither Loeb nor anyone else has ever demonstrated that any animal response is forced. . . . " Kellog (1958) has described "Galvanotropism as an avoidance response." Kellog reported that fish first escape and then avoid the current flow, they are not attracted (forced) to either the cathode or the anode.

Rabaud's (1922) elegant experiment demonstrated the forced nature of phototropism. Viaud (1948 and 1959), Médioni (1961) and Hirsch and Boudreau (1958) have reported observations consistent with such an interpretation. Viaud et Dreyfus (1956 and 1957) and Viaud (1959) have reported on the forced nature of galvanotropism, and Murray and Hirsch (1965) have reported observations which, though focussed on conditioning, both assumed and corroborated Viaud's interpretation.

Part of the history of the tropisms question will be discussed and our recent observations (on goldfish in a galvanic field) demonstrating the nature of their forced responses will be presented.

423

LOUIS C. GRAUE, Bowling Green State University.

Distance and initial orientation in pigeon homing.

A relation between distance of release and the accuracy of initial orientation for pigeons homing to a loft in Durham, N. C. was obtained by Schmidt-Koenig (1966, Z. Vergleicherde physiologie

milk production and milking time were recorded once weekly for one year.

Expt. 1—Cows (87) were separated into loose or free-stall housing and milked as 5 different groups. Four groups had access to one side while group 5 had access to both doors in the milking area. Cows developed stable entrance orders. Group 5 developed two entrance orders—one for each side. Positive correlations between entrance rank and milk production approached significance. Significant negative correlations occurred between entrance rank and: age, percent chased in and days in milk.

Expt. 2—Primiparous animals (16) in late lactation, grouped together and pasture-fed, exhibited constant entrance orders. Significant negative correlations existed between entrance rank and milk yield.

Expt. 3—Three groups (48) were milked together, housed separately and fed complete rations away from milking area. Significant positive correlations were computed between entrance rank and milk yield, days in milk, previous rank index and breed differences (with Holsteins preeminent to Red Danish cows for average entrance order, total milk and milking rate).

Highly significant correlations were found between previous training and present entrance order. A Chi-square test of previous preference of Expt. 3 cows with previous records in Expt. 1 showed that animals trained to enter one door developed significant preference for that side.

Cows developed significantly consistent entrance orders which were maintained over time, transfer from one group to another or starting new lactations. Higher-yielding cows entered the milking area earlier than low-yielding cows during early lactation. (This research was financed in part from a trust agreement between Purdue University and Normandy Farm, New Augusta, Indiana.)

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CARLETON RAY, Johns Hopkins University.

Natural history of the Weddell Seal in Antarctica. (Motion picture)

Coordinated on-ice and underwater studies revealed aspects of the social behavior of the Weddell Seal, *Leptonychotes weddelli*. Observations were made during the courtship seasons from 1963 through 1966 in McMurdo Sound, Antarctica. Correlations were made of seal numbers with season, time of day, and weather. Movements and growth of the first twenty-five pups born were observed in detail, as was mother/infant behavior. Underwater techniques involved scuba-diving, underwater tape recording, and a sub-ice observation chamber (Ray, Bioscience 1965, Vol. 15, No. 4; 274-277).

It was shown that males spend much of their time in the water and that their trill (Schevill and Watkins, Zoologica, 1965, Vol. 50, No. 1; 45-46) is ascribable to them in loose territorial "display". Though more than one male may occupy a "territory", there is clearly a dominance system in each. Females show dominance behavior and preference for certain locations, but not to the extent of "territory". They actively lead pups to water when the latter are about 14 days old and take a role in acquainting the pup with the sub-ice environment. Females use sound principally in threat to other seals and in identification with pups. Mating was not observed to occur while females and pups remained together, that is until late November. (Supported by grants GA-57,126 and 234 from the N.S.F.)

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RONALD A. BRANDON, Department of Zoology, Southern Illinois University.

*Typhlotriton spelaeus*, the grotto salamander; influence of food on seasonal abundance, reproductive activity, and relative growth rate.

Over a five-year period some 200 specimens of the troglobitic "grotto salamander" were collected, during every month except February, from a cave in Shannon County, Missouri. The peak of salamander abundance in the cave occurs in May, and the fewest have been found in December and January. Periods of salamander abundance are positively correlated with rainfall, food in stomachs, and reproductive activity as judged by the amount of body fat, presence of enlarged and yolked ovarian eggs, hypertrophy of the oviducts, and prominence of the mental hedonic gland.

The Shannon County cave is small with only a small summer colony of bats and no extensive guano deposits. The salamanders in this cave transform and mature at a strikingly smaller body size than in several larger Pulaski and Laclede County caves which contain larger colonies of bats and bigger guano deposits and/or a rich invertebrate fauna.

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STEWART B. PECK, Harvard University.

The life cycle of the troglobitic catopid beetle *Ptomaphagus loedingi*.

The troglobitic catopid beetle *Ptomaphagus loedingi* can be cultured at 15°C and saturated humidity. Doing so has shown that: a female can lay up to one egg per 24 hours, beginning one month after her emergence from the pupal chamber; eggs hatch in 13 to 17 days; the larvae pass through three instars in 14 to 24 days; the larvae construct mud pupal chambers in which they reside for 9 to 12 days before pupating; the pupal stage lasts 18 to 29 days; the newly emerged adult stays within the chamber for 1 to 7 days; the adult beetle can live for at least 6 months.

In caves the beetles exhibit clumped distribution. The clumps are usually within a few hundred feet of the cave entrance and are in the vicinity of animal dung or decaying debris. The microflora of these substances is the probable food of *Ptomaphagus*. Competition for food may come from collembola, millipedes, and phorid flies.

Trapping in an Alabama cave during the summer of 1965 indicated a low adult population in July. Numerous adults in early August suggest the control of an emergence cycle from hiding places or pupal chambers. Numerous larvae appeared two weeks after the adults. In 1967 a mark-recapture census in the same cave indicated 126 beetles in early July, 28 beetles in late July, and .... beetles in middle August.

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THOMAS C. JEGLA, Kenyon College.

Annual cycles in cave organisms, especially crayfish.

Annual periods of reproduction have been reported in cave amphipods, crayfish, and fish but not in terrestrial beetles. In a population of cave crayfish annual periods of molting also occur. Although reproduction in the amphipods and molting in the crayfish occurs throughout the year, there are definite "seasonal" peaks. This suggests that the cycles of some of the individuals are out of phase with respect to the majority of the population. The majority may be synchronized by seasonal changes other than light or temperature. Individual beetles may have a seasonal cycle but they are not synchronized by a seasonal cycle.

Individual cave crayfish show molting and reproductive cycles of about a year in a "cueless" laboratory environment of darkness and constant temperature. Some lived for two years in this environment. Individuals had molting and/or reproductive periods ranging from about 330 to over 400 days in length.

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DAVID C. CULVER, Yale University and West Virginia Association for Cave Studies, Inc.

Structure of some West Virginia cave stream communities.

Surber samples from several cave streams in Greenbriar County, West Virginia were taken at various times during the year. The data are considered both in terms of presence or absence of species and in terms of abundance of the more common species (amphipods). Correlations of the data that showed significant departure from a Poisson distribution with environmental parameters are attempted. Predictions concerning niche breadth were possible because of the simplicity of the cave environment. These predictions were tested in the laboratory by simple experiments such as substrate preference and tendency to aggregate. Certain aspects of game theory proved to be a useful conceptual framework. The problems involved in using game theory in a more rigorous sense are discussed. Both the theoretical models and the laboratory experiments are applied to the field results in an attempt to explain some of the important parameters in the structure of the cave community.

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ROBERT W. MITCHELL, Texas Technological College.

Diversity and niche structure in temperate and tropical caves: Texas and Mexico.

Striking differences exist between temperate and tropical cave communities. More troglobites (obligate cave-dwellers) are found in temperate caves, but the most drastic difference lies in the paucity of terrestrial troglobites in the tropics. Most tropical troglobites are aquatics. Three hypotheses are offered to explain these disparities. First, there are fewer troglobites in tropical caves since the higher energy input into these caves lessens the positive selection pressures for the "regressed" phenotype and slows evolutionary rates. Second, the scarcity of terrestrial troglobites in tropical caves is further promoted by the tropical climate which presents few barriers to gene flow between surface and cave populations of the same species. Third, most tropical troglobites are aquatic since the aquatic species are readily isolated geographically from the surface fauna by being trapped in subterranean waters.

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THOMAS L. POULSON and DAVID C. CULVER, Yale University and The Cave Research Foundation.

Diversity in aquatic and terrestrial cave communities of Flint Ridge, Mammoth Cave National Park.

Within-habitat diversities were calculated using species-abundance criteria. Stream data are from complete censuses of fish and crayfish and samples of plankton, isopods, and flatworms. In terrestrial habitats both timed search per area and trapping contributed to calculations of diversity.

Diversities were rank-correlated with productivity, spatial heterogeneity, stability and predictability of primary production, predation, competition, and rigor of microclimate.

Several hypotheses might explain diversity differences within each habitat. Climate is stable within caves and, due to local geology, ecological and evolutionary time were constant throughout the cave system. Predictability of primary productivity and rigor of microclimate showed positive correlations with diversity. Spatial heterogeneity, predation, competition, productivity, and stability of primary productivity showed generally poor correlations with diversity although spatial and temporal distribution of microhabitat and food provide a possible explanation of differences in patterns of diversity between the terrestrial and aquatic communities. (Supported by U.S.P.H.S. Grant GM-12231.)

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K. V. KHIRUVATHUKAL, Canisius College.

Comparative histology of the adrenal glands of some chelonians.

The purpose of this investigation is to describe the comparative histology of the adrenal glands of *Pseudemys scripta, Clemmys insculpa, Trionyx spinifer, Emydoidea blandingi, Chelydra serpentina, Malaclemys terrapin* and *Chrysemys picta.*

The structure of the adrenals was studied with routine histological stains. The presence of sudanophilic lipids and cholesterol was demonstrated by Sudan Black B and the Shultz test, respectively.

In the above turtles, the adrenals are paired bodies, irregular in outline and yellowish in color, extending along the ventrolateral surface of the kidneys in a retroperitoneal position. The glands are completely capsulated, but there is an intermixture of renal and adrenal tissue on the ventral surface of the glands. The ventral surface of the gland is covered by pleuroperitoneum, which at the ventrolateral edge gives rise to the mesentery supporting the gonads.

The stroma of the adrenal glands consists of a connective tissue capsule, composed of collagenous and reticular fibers. The capsule invaginates into the body of the gland, dividing the gland into small masses of tissue. The gland consists of an intermixture of cortical and medullary tissue. The cells of the cortex range from irregular polyhedral to columnar cells. These cells show positive reactions with Sudan Black B and Shultz tests. They show a uniform distribution of lipid.