

METABOLISM IN THE DOMESTIC FOWL  
(GALLUS DOMESTICUS). HEPATIC AND  
RENAL CONCENTRATIONS OF METABOLITES,  
AND MEASUREMENTS OF METABOLITE  
CONCENTRATIONS IN ABDOMINAL BLOOD VESSELS  
SUPPLYING AND DRAINING LEG, LIVER AND KIDNEY

CENTRE FOR NEWFOUNDLAND STUDIES

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METABOLISM IN THE DOMESTIC FOWL (Gallus domesticus).

HEPATIC AND RENAL CONCENTRATIONS OF METABOLITES, AND

MEASUREMENTS OF METABOLITE CONCENTRATIONS IN ABDOMINAL

BLOOD VESSELS SUPPLYING AND DRAINING LEG, LIVER AND KIDNEY.

BY

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#### ABSTRACT

Physiological levels of blood gases can be maintained in an anesthetized, abdominally opened fowl by means of a simple unidirectional ventilation technique. Hepatic ATP levels and ATP/ADP ratios in control birds measured using this ventilation technique have been found to be high (1.9 micromole/g and 1.4 respectively) and are comparable to those reported in rats. Hepatic/lactate levels and the lactate:pyruvate ratio were lower than is reported for non-ventilated chickens. Elevations of the tissue concentration of AMP, fructose-1,6-bisphosphate and inorganic phosphate and fall in ATP, in ischemic liver are consistent with an activation of glycogen phosphorylase, phosphofructokinase and pyruvate kinase - all indicative of the increased glycolytic flux in ischemic chicken liver.

A six-day fast in chickens results in a 55% drop in hepatic glucose, an increase in the measures of cellular energy level in the liver, and a shift to a more oxidized hepatic cytosolic  $NAD^+/NADH$  ratio. In contrast, glucose levels, cellular energy level indices and the redox state of the kidney cytosolic NADH system remain unchanged after a six-day fast. It is concluded that the relative availability of cytosolic reducing equivalents results in the process of gluconeogenesis from amino acids and pyruvate being more favored in the kidney of a fasted chicken than in its liver.

Measurements of afferent-efferent differences across liver in the fed and fasted states suggest a significant uptake of glucose and lactate and most amino acids. A negative afferent-efferent difference for urate across liver in the fasted, and not in the fed state, indicates that deamination of amino acids is more prevalent in the fasted liver.

Measurements of arteriovenous differences across muscle indicate that this tissue releases amino acids into the circulation in the fasted state and takes up no glucose. In the fed state no amino acids are released by muscle, although glucose is taken up.

In the fed state there is no significant afferent-efferent difference across kidney for glucose. Measurements of afferent-efferent differences across kidney suggest that in the fasted state there is a significant release of glucose and an uptake of amino acids, urate, and lactate. If the renal portal component of total renal blood flow is assumed to be greater than 30% then when the sum is calculated of the gluconeogenic amino acids for which there is a significant uptake across kidney, the value (in glucose equivalents), is equal to the figure for the release of glucose. It is concluded that the kidney is a major gluconeogenic organ in the fasted chicken.



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LIST OF ABBREVIATIONS .

2-OXOGL	2-Oxoglutarate
2PG	2-Phosphoglycerate
3PG	3-Phosphoglycerate
AMP	Adenosine-5'-monophosphate
ADP	Adenosine-5'-diphosphate
ATP	Adenosine-5'-triphosphate
cAMP	cyclic Adenosine-3',5'-monophosphate
F6P	Fructose-6-Phosphate
FBP	Fructose-1,6-Bisphosphate
GLUC	Glucose
GLYC	Glycerol
G6P	Glucose-6-Phosphate
GSH	Glutathione (reduced)
GSSG	Glutathione disulfide (oxidized)
LACT	Lactate
NAD	Nicotinamide adenine dinucleotide (oxidized)
NADH	Nicotinamide adenine dinucleotide. (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate
(oxidized)	
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NMR	Nuclear Magnetic Resonance
PEP	Phosphoenolpyruvate
PEPCK	Phosphoenolpyruvate carboxykinase
	GTP: oxaloacetate carboxy-lyase (transphosphorylating).
Pi	Phosphate (inorganic)
RNA	Ribonucleic acid
Triose-P	Dihydroxyacetonephosphate + glyceraldehyde-3-phosphate
URIC	Uric Acid

Abbreviations Used for Peptides and Amino Acids

3MH	3-methylhistidine	HPRO	Hydroxyproline
ALA	Alanine	ILE	Isoleucine
ANS	Anserine	LEU	Leucine
	(Beta-alanyl-1-methylhistidine)	LYS	Lysine
ARG	Arginine	MET	Methionine
ASN	Asparagine	ORN	Ornithine
BALA	Beta-alanine	PHE	Phenylalanine
CARN	Carnosine	PRO	Proline
	(Beta-alanylhystidine)	SER	Serine
CYS	Cysteine	TAUR	Taurine
GLN	Glutamine	THR	Threonine
GLU	Glutamate	TRP	Tryptophan
GLY	Glycine	TYR	Tyrosine
HIS	Histidine	VAL	Valine

CHAPTER 1

INTRODUCTION AND REVIEW OF THE LITERATURE



## 1.1 CARBOHYDRATE METABOLISM

Carbohydrate metabolism and its regulation in birds varies from that in mammals in several major ways. These differences are in the level of blood glucose, the response of blood glucose to fasting and the resistance to diabetes.

### 1.1.1 Level Of Blood Glucose

Weintraud (1894) appears to have been the first to notice the high level of blood glucose in birds when he reported blood sugar values for ducks (170 mg / 100ml) considerably higher than mammalian levels (90 mg / 100 ml). Many authors have verified since then that a blood sugar level, often twice as high as the mammalian level, is characteristic of avian species (See extensive review by Erlenbach, 1938). Early workers found that the chicken was no exception to this rule, having blood sugar levels ranging from 150 to over 200 mg / 100ml (Saito & Katsuyama, 1901; Gajja, 1912; Bierry & Fandard, 1912; Schunert & Pelchrzim, 1923; Hayden & Fish, 1928; Batt, 1939).

Bierry & Fandard (1912) postulated that the high blood sugar in birds was related to their high body temperature. Rodbard (1947) later demonstrated a positive correlation between body temperature and blood glucose in the chicken over a range of temperatures. The nature of the interaction, however, has not since been elucidated. There is, as yet, no explanation as to why birds maintain blood

glucose at such high levels.

#### 1.1.2 Blood Glucose Response To Fasting

In a chicken, the changes in the blood glucose concentration during a fast is unlike the normal mammalian pattern. In most mammals<sup>1</sup> the normal concentration of 5 mM falls to 4 mM after a short fast and to 3.5 mM during longer periods of fasting. [In the fasting neonatal human, blood glucose may fall below 1 mM (Bachmann et al., 1980)]. The blood glucose level (12 mM) of both juvenile and adult fowl on the other hand begins to decline within the first twelve hours of the beginning of a fast, but the fall is transient. Blood glucose returns to pre-fasting levels by the third or fourth day of a fast (Burrows et al., 1935; Henry et al., 1933; 1934; Nir et al., 1973; Opydyke, 1942). There is also a diurnal variation in the level of blood glucose in the fowl. Blood glucose falls from 12 mM during the day to 10 mM during the hours of darkness (Twiest & Smith, 1970).

This high level of glucose (12 mM) is maintained or increased throughout fasts of 6-8 days in the chicken.

---

1 By no means all mammals demonstrate this pattern. Weanling elephant seals (*Mirounga angustirostris*) maintain their blood glucose at levels exceeding 8 mM during natural fasts of 2 to 3 months (Ortiz et al., 1983).

(Hazelwood & Lorenz, 1959, Brady et al., 1978). It was reported by Houpt (1958) that while the blood glucose level of newly hatched chicks displays a similar response to fasting as the adult, young chicks of two to fourteen days of age appear less able to maintain blood glucose levels during a fast.

Fasts of up to 40 days without a decline in plasma glucose levels have been reported for geese (Vu Van Kha et al., 1979; Le Maho et al., 1981) and up to 120 days in the emperor penguin (Groscolas, 1978).

#### 1.1.3. Resistance To Diabetes

Another area in which birds differ from mammals is in their susceptibility to experimentally induced diabetes. In the classic experiments of Langendorff (1879), pancreatectomy (which causes diabetes in mammals) did not result in diabetes in a granivorous bird (pigeon) although in a carnivorous bird, the vulture, it did. Transient hyperglycemia was observed in pancreatectomized chickens (Gaja, 1912, Koppanyi et al., 1926) but a return to normal was seen within one week. On the other hand, Miahle (1968) has shown that in the duck, pancreatectomy does result in a permanent diabetes. More recent reports in chickens however, have shown removal of 100% of pancreatic tissue fails to cause permanent diabetes or to remove insulin from the circulation - leading to the suggestion of a

non-pancreatic source of insulin in this species (Colca & Hazelwood, 1976).

Chickens are also extremely resistant to diabetes induced chemically by the drugs alloxan or streptozotocin. Even near-lethal doses fail to induce diabetes or to abolish insulin from the circulation. (For reviews see Langslow & Hales, 1971; Hazelwood, 1976; and more recent work by Simon & Dubois, 1980; Danby et al., 1982).

It is possible that the high blood glucose and taurine levels of the chicken, act as agents which protect the bird from chemical diabetogens. High taurine levels have been shown to protect the islet cells of the mouse pancreas against streptozotocin toxicity (Tokunaga et al., 1979), and high glucose levels protect against alloxan toxicity in rat hepatocytes (Garman & Fischer, 1982).

Scott et al. (1981) have published a study which shows that in the rat, exogenously administered uric acid, which has structural similarities to the mammalian diabetogen alloxan, suppresses insulin levels and causes hyperglycemia. This has interesting implications in the fowl where there may be a relation between the naturally high plasma uric acid levels, the resistance to chemically-induced diabetes, and the uncertain role of insulin.

#### 1.1.4 Malic Enzyme And Hepatic Fat Synthesis

Lipogenesis is another area of metabolism where there is a pronounced difference between mammals and birds. Whereas lipid biosynthesis occurs in both liver and adipose tissue in mammals, in the fowl 90% occurs in the liver, adipose tissue serves primarily for storage (O'Hea & Leveille, 1969). Further it appears that reducing equivalents for lipogenesis in chicken liver arise from the oxidation of malate, as the oxidation of glucose via the phosphogluconate pathway and concomitant generation of NADPH is minimal in the chicken (O'Hea & Leveille, 1968; Goodridge, 1968; Madappally et al., 1971). The activity of another NADPH linked enzyme, isocitrate dehydrogenase is also low in chicken liver (Madappally et al., 1971).

## 1.2 HORMONAL EFFECTS ON CARBOHYDRATE METABOLISM

### 1.2.1 Insulin

Early workers found not only was the fowl resistant to diabetes (see Section 1.1.3), but also resistant to the extreme hypoglycemia and the convulsive or fatal effects of overdoses of mammalian insulin (Cassidy et al., 1926; Corkill, 1930; Golden & Long, 1942; Opdyke, 1942; Chen et al., 1945; Lepkovsky et al., 1967;). The resistance to insulin is a trait shared with several species of lizards (Miller & Wurster, 1956). Later studies showed this "resistance" in the fowl was due partly to a difference in the nature of mammalian and avian insulins. Fowl are between two-fold and ten-fold more sensitive to chicken insulin than to mammalian insulin (Hazelwood et al., 1968; Hazelwood & Barksdale, 1970; Simon et al., 1977). However, the density per unit surface area of insulin receptors in chicken liver is about one fifth that in rat liver, which may partially account for the resistance even to avian insulins exhibited by the fowl (Simon et al., 1977). The resistance of the fowl to insulin is also consistent with the recent findings of Cramb et al. (1982) that porcine insulin at physiological doses failed to prevent the glucagon-stimulated rise in cAMP in isolated chicken hepatocytes. Insulin is however, able to prevent this rise in cAMP in mammals (Exton & Park, 1972). Beef insulin is also unable to counteract in chickens the elevation of

plasma free fatty acids resulting from the injection of glucagon (Grande, 1969). It is also interesting to note that chicken insulin has a binding affinity for mammalian tissues (human lymphocytes and rat liver) twice as high as does porcine insulin (Simon et al., 1977).

The foregoing discussion notwithstanding, insulin is reported to induce hypoglycemia and elevated plasma fatty acids in the fowl (Heald et al., 1965; Lepkovsky et al., 1967; Hazelwood et al., 1968). This effect appears to be mediated by insulin's effects on the adipocyte where it causes an increase in glucose uptake, oxidation to carbon dioxide, and incorporation into triglycerides (Gomez-Capilla & Langslow, 1977). However in isolated hepatocytes physiological levels of insulin have no effect on glycogenolysis, glycogen synthesis, gluconeogenesis or lipogenesis (Cramb et al., 1982).

A seventy-two hour fast is without effect on the plasma insulin levels of chickens (Langslow, et al., 1970). It has also been reported that fasted chickens exhibit an impaired glucose tolerance even though insulin levels (measured with a mammalian insulin antibody and a chicken insulin standard) are unchanged or increased (Simon & Rosselin, 1978; Stellenwerf & Hazelwood, 1979). This has led these investigators to conclude that insulin in fowls is of secondary importance in the regulation of glucose metabolism. However the immediate increase of blood glucose

after the injection of anti-insulin serum in the duck (Mirsky et al., 1964) and the hypoglycemic effects of tolbutamide (which stimulates the release of preformed insulin from islet cells) demonstrates that insulin does play a role in the regulation of glucose levels, at least in the duck (Mirsky & Gitelson, 1957).

#### 1.2.2 Glucagon

The naturally occurring glucagon level in the fowl is ten-fold higher than the physiological level in the mammal (Fister et al., 1983). The metabolic response of the fowl to glucagon also differs from the mammalian response. In fed or fasted mammals an increase of blood glucose brought on by exogenously administered glucagon (which causes a prompt rise in blood glucose, and the stimulation of glycogenolysis, Picardo & Dickson, 1982), leads to a transient rise (5-10 minutes), followed by a fall in free fatty acids in the blood (Whitty et al., 1969). In the fowl however, glucagon administration results in a marked rise in plasma free fatty acids and the elevated levels are sustained for at least two hours (Heald et al., 1965). The same is true in the fowl for plasma glycerol levels elevated in response to exogenously administered glucagon (Carlson et al., 1964).



That glucagon is a more important pancreatic hormone than insulin in the fowl is suggested by the fact that total pancreatectomy results in hypoglycemia rather than hyperglycemia as in most mammals<sup>2</sup> (Mikami & Ono, 1962; Sitbon, 1967). Samols et al., (1968) have shown in ducks that both plasma glucose and plasma glucagon are reduced in the blood after pancreatectomy.

It is also possible in the fowl to produce a selective glucagon deficiency syndrome. Treatment with Synthalin A, an agent believed to destroy glucagon synthesizing cells, causes intense hypoglycemia and convulsions in the chicken, followed by death (Beekman, 1956; Hazelwood, 1971).

Despite these differences, the mechanism of glucagon action appears to be the same in chickens as in mammals. Glucagon at physiological concentrations is able to induce in chicken hepatocytes, a rise in cellular cAMP (Langslow & Siddle, 1979; Cramb et al., 1982). This suggests glucagon's mode of action is via an activation of adenylate cyclase and the stimulation of glycogenolysis through the cAMP-sensitive protein kinase cascade.

---

2 It is interesting to note that the lizard (Eumeces obsoletus), another uricotelic species, becomes hypoglycemic after pancreatectomy (Miller & Wurster, 1958).

### 1.2.3 Adrenaline

In the fowl, adrenaline has an effect similar to glucagon in increasing blood sugar through hepatic glycogenolysis and gluconeogenesis (Cramb et al., 1982). The hyperglycemic effects of adrenaline were known more than seventy-five years ago when it was noted adrenaline administration caused glycosuria (and by implication hyperglycemia) (Paton, 1905). To continue the catalogue of differences between birds and mammals, in the adult fowl, unlike mammals, adrenaline does not effect the release of free fatty acids from adipose tissue (Carlson et al., 1964; Langslow & Hales, 1969), though apparently glucagon does (Sect. 1.2.2).

### 1.2.4 Glucocorticoids

The major steroid synthesized by adult fowl has been shown to be corticosterone (Phillips & Chester Jones, 1957; deRoos, 1960; Flack & Freeman, 1983). The fowl demonstrates the classic glucocorticoid response (hyperglycemia and increased liver glycogen & gluconeogenesis) when cortisol or corticosterone is administered, but unlike the rat (White et al., 1978) fails to respond to cortisone (Stamler et al. 1954; Greenman & Zarrow, 1961; Snedecor et al., 1963; Dulin, 1956). Kochi et al. (1980) have reported that administration of glucocorticoids to the fowl induces in liver, the synthesis

of a cytosolic form of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (see Sect. 1.4.3).

Egana et al. (1981) have shown that in addition to the effects on carbohydrate metabolism at the protein synthesis step, in chickens, in vivo, glucocorticoids cause a rapid glycogenolysis which Egana et al. (1981) postulate may involve calcium<sup>2+</sup> ion effects on membranes.

Plasma levels of corticosterone in the fowl are known to vary diurnally and to be dependent on photoperiod, which implies that there are diurnal and photoperiod dependent effects on glucose metabolism (Johnson & van Tienhoven, 1981; Wilson & Cunningham, 1981). In this regard, both Twiest & Smith, (1970) and Davison (1975) have shown a diurnal rhythm for plasma glucose and hepatic glycogen in the chicken.

#### 1.2.5 Stress

The effects of short-term "stress" on glucose metabolism have been examined by several investigators. Early workers noted a profound rise of the plasma glucose in "excited" birds (Honeywell, 1921). This initial hyperglycemia may be followed by hypoglycemia 30 minutes after handling (Freeman & Manning, 1976). In addition to these effects on blood glucose, short-term stress (handling) is also known to cause hyperlipidemia and an increase in blood glucocorticoids (Freeman & Manning, 1976, 1980;

Freeman, 1971; Beuving & Vonder, 1978). Food withdrawal is reported to cause hyperlipidemia in the fowl within one hour of that stress and after two hours, a hypoglycemia (Freeman et al., 1980). A recent review on stress in birds has been published by Siegel (1980).

As many of the effects of stress on birds are very similar to the effects of adrenaline, glucagon and the glucocorticoids, it is likely that many of the effects of stress in birds are mediated through these hormones. In this regard, Freeman & Manning (1976) have shown that glucagon concentration increases in fowl in response to handling.

### 1.3 RELATION BETWEEN URICOGENESIS AND GLUCONEOGENESIS

#### 1.3.1 Urate Excretion

It has been known since the classic experiments of Fourcroy & Vauquelin (1811) and Minkowski (1886), and from later work by Davis (1927) that uric acid is the principal compound for the excretion of nitrogenous waste in the urine of the fowl. The first reliable determination of the concentration of uric acid in blood however, had to wait until the introduction of a new method by Folin & Denis (1913) who reported levels in chicken blood more than tenfold those in mammals. Benedict (1915) and Pupilli (1923) later showed uric acid was contained only in the plasma compartment of the fowl, whereas in the ox, the uric acid was solely in the corpuscles (Benedict, 1915).

Austic & Cole (1972) reported plasma urate in fed chickens varied directly with the protein content of the diet. In the early stages of a fast, plasma levels of urate in the adult fowl remain constant but begin to rise after three to four days of starvation, presumably due to an increase in protein catabolism (Henry et al., 1934; Okumura & Tasaki, 1969; Homma & Sato, 1960). One early study however, (Bell et al., 1959) reported a decrease in the plasma level of urate upon a short period of fasting (36 hours). In another study, there was no significant difference between the plasma urate levels of 1.2 kg broiler

chicks fasted for 1, 4 or 8 days, although the comparison to the fed level of urate was not made (Brady et al., 1978). There was also no significant effect of fasting on plasma urate levels of White Mountain broiler chicks fasted between 1 and 5 days. Some of the differences reported above may result from a difference in the metabolic responses of broiler and layer breeds of chickens.

Urate excretion by the kidneys occurs primarily as a result of tubular secretion. This accounts for approximately 90% of the urate excreted (Shannon, 1938). Renal excretion of urate depends upon both the concentration of urate in plasma (Sykes, 1971) and on renal plasma flow (Martindale, 1969), which in turn is dependent on the action of the renal portal system in shunting blood through, or in bypassing the kidney (See Akester, 1971 and Sect 1.5.2.1).

Urate can comprise more than 80% and ammonia, 7% of the nitrogen excreted in the urine of fed chickens, though in the fasted state urate composition drops to 60% and ammonia rises to 23% of the nitrogenous waste in the urine (Sykes, 1971). In the well-fed fowl the bulk (80%) of the urate produced is synthesized in the liver, with the remainder being made by the kidney (Chin & Quebbeman, 1978). Chou (1972) has suggested, based on concentrations of urate precursors, that the kidneys of the fowl produce from 60% to 96% of the amount of urate produced by the liver of fed cockerels or 1.75 to 2.7 times as much urate per gram of

tissue as does liver. Urate synthesis is reduced (Chin & Quebbeman, 1978) or absent (Martindale, 1976) in the renal tissue of the starved fowl. As urate excretion in a fasted chicken is increased<sup>3</sup>, this implies that the amount of urate synthesized in the liver is increased.

Uricogenesis is an energy intensive process in the cell; six to nine high energy ATP phosphate bonds must be hydrolysed for each urate produced (Barratt et al. 1974; Mapes & Krebs, 1978). In fact initiation of uricogenesis in perfused chick liver or in chicken hepatocytes may inhibit gluconeogenesis up to 100%, which Deaciuc et al. (1982) postulate could be due to competition for common substrates and possibly for ATP, though they discount the latter possibility. Mapes & Krebs (1978) have reported that ammonia detoxification takes precedence over glucose synthesis in chicken liver. It is the view of these latter authors that uricogenesis and gluconeogenesis may be antagonistic processes in chicken liver. However Coolbear et al., (1981) have suggested that uricogenesis may supply cytosolic reducing equivalents for gluconeogenesis from

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3 Surmised from the increase in hepatic xanthine dehydrogenase activity after a fast (Wiggins et al., 1982; Coolbear et al., 1981), and the findings of Chin & Quebbeman (1978) of urate excretions of 616 and 752 micrograms/min/kg in fed and 18-hour fasted adult hens, respectively.

amino acids.

It has been found that insulin must be included in the medium in order to stimulate uricogenesis from amino acids in both perfused chick liver (Barratt et al., 1974) and in isolated chicken hepatocyte preparations (Badenoch-Jones & Buttery, 1975). As mentioned in Section 1.2.1, however, insulin has no effect on glycogenolysis, glycogen synthesis, or gluconeogenesis in isolated chicken hepatocytes (Cramb et al., 1982).

### 1.3.2 Xanthine Dehydrogenase And Cytosolic Reducing Equivalents

The ultimate enzyme in the synthetic pathway for uric acid in the fowl is xanthine dehydrogenase, an NAD<sup>+</sup>-linked dehydrogenase (Richert & Westerfeld, 1951). The chicken liver enzyme was first characterized by Remy et al. (1955). The purification and properties of the enzyme were further described by Rajagopalan & Handler (1967). Fasting was found to cause an increase in hepatic levels of the enzyme up to fourfold (Della Corte & Stirpe, 1967; Stirpe & Della Corte, 1965; Scholz & Featherston, 1968; Coolbear et al., 1981; Wiggins et al., 1982). Feeding a high protein diet can cause increases in hepatic activity up to thirteenfold (Westerfeld et al., 1962; Itoh & Tsushima, 1974; Scholz & Featherston, 1969; Wiggins et al., 1982). The influence of strain was examined as well: fasting had no effect on



xanthine dehydrogenase levels in the liver of a strain of white leghorn layers, while in a broiler strain, a significant reduction in xanthine dehydrogenase was seen (Scholz, 1970). This latter observation may explain in part the difference between broiler and layer strains in the effects of fasting on plasma urate (Sect 1.3.1)

In chicks fed high protein diets, a correlation was noted between xanthine dehydrogenase activity and urate synthesis in the liver (Featherston & Scholz, 1968; Hevia & Clifford, 1978; Wiggins et al., 1982). The adaptation of this enzyme in response to fasting and feeding high protein diets has led some investigators to suggest that xanthine dehydrogenase, a cytosolic enzyme in chicken liver, may provide cytosolic reducing equivalents for gluconeogenesis in the liver. "In both circumstances gluconeogenesis is presumed to be active in the fowl's liver. Some of the NADH necessary for the cytosolic reduction of 1,3-diphosphoglycerate in the synthesis of glucose may arise from xanthine and hypoxanthine oxidation (Coolbear et al., 1981).

Xanthine dehydrogenase is found in both liver and kidney in the fowl, although the pigeon lacks xanthine dehydrogenase activity in the liver (Morgan, 1926). This latter fortuitous circumstance allowed Edson et al. (1936) to identify hypoxanthine as an intermediate in the synthetic pathway for uric acid. In the pigeon only the steps up to

hypoxanthine synthesis occur in the liver, while the final oxidations to uric acid take place in the kidney. This would imply that in the pigeon reducing equivalents for gluconeogenesis could not be produced by the oxidation of hypoxanthine to urate in the liver cytoplasm.

#### 1.4 REGULATORY ENZYMES IN GLUCOSE METABOLISM

##### 1.4.1 Hexokinase And Glucose-6-phosphatase

Four isoenzymes of hexokinase have been characterized in rat liver which catalyse the phosphorylation of glucose. Three of the isozymes have a low  $K_m$  for glucose and a fairly broad specificity for a variety of hexose sugars. These are hexokinases. The fourth isozyme is designated glucokinase and is specific for glucose with a high  $K_m$  for that sugar (Gonzalez et al., 1967; Parry & Walker, 1966).

In many species glucokinase is regulated by diet, starvation or hormonal changes (see review by Weinhouse, 1976). In most avian livers however, glucokinase has not been found (Sols et al., 1964; Ureta et al., 1972, 1973). Reports of a 'glucokinase-like' activity in chicken liver have been published (Wallace & Newsholme, 1967; Pearce, 1970) but more recent studies by O'Niell & Langslow (1976, 1978) reported no glucokinase activity in chicken liver; only hexokinase activity, distributed evenly between

particulate and soluble fractions of the cell.<sup>4</sup> Another recent paper by Wals\* & Katz (1981) has reported a glucokinase activity associated with the cell membrane but this observation could not be confirmed (G.R. Herzberg, unpublished observations).

O'Niell & Langslow (1978) report a small but significant drop in hepatic hexokinase in the chicken and a doubling of glucose-6-phosphatase caused by fasting. These authors conclude glucose flux into and out of chicken liver cells is regulated by the activity of glucose-6-phosphatase and the provision of glucose-6-phosphate. The amount of glucose-6-phosphatase present in chicken liver is highest at hatching but declines rapidly during the first two months of life until at 8 weeks of age, liver activity is only a third of the activity present per gram of liver in the chick (Raheja et al., 1971).

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4 It appears that in rats hexokinase is an ambiguous enzyme. The enzyme may be present in either the soluble fraction of the cell or bound to the mitochondrial membrane, the latter form possessing the most catalytic activity. The amount of hexokinase bound to the membrane is increased by high polyamine or Magnesium ion ( $2^+$ ) concentrations, and decreased by high glucose-6-phosphate concentrations (Kurokawa et al., 1983).

#### 1.4.2 Phosphofructokinase And Fructose-1,6-bisphosphatase

Phosphofructokinase, which catalyses the phosphorylation of fructose-6-phosphate using ATP as the phosphoryl donor, is another enzyme considered important in the regulation of gluconeogenesis. As with many other enzymes, phosphofructokinase is known to exist as a number of different isozymes, at least two and perhaps four in some animals. An excellent review of this topic has recently been published (Dunaway, 1983). In the chicken, the two major isozymes are those present in the liver and muscle (Kono et al., 1973). The activity of chicken liver phosphofructokinase is very low and that of fructose-1,6-bisphosphatase which catalyses the hydrolysis of fructose-1,6-bisphosphate, fairly high (Wallace & Newsholme, 1967). Chicken phosphofructokinase is inactivated by glucagon and epinephrine, while insulin alleviates the inhibition by glucagon (Fister et al., 1982, 1983). Chicken fructose-1,6-bisphosphatase is also inhibited by low molecular weight metabolites including AMP and fructose-1,6-bisphosphate, its substrate (Wallace & Newsholme, 1967).

Fister et al. (1983) have recently demonstrated a decrease in phosphofructokinase activity in extracts of hepatocytes exposed to glucagon. They have demonstrated this decrease was independent of fructose-2,6-bisphosphate, a potent regulator of phosphofructokinase in mammals (Pilkis

et al., 1981; Van Schaftingen et al., 1980; Furuya & Uyeda, 1980). In contrast Chaekal et al., 1983, reported an inhibition of fructose-1,6-bisphosphatase and stimulation of phosphofructokinase by fructose-2,6-bisphosphate in chickens. They showed that compared to hepatocytes from fasted rats, in hepatocytes from fasted chickens a lower concentration of exogenous glucose was required to produce a rise in fructose-2,6-bisphosphate. It also appears from the work of Chaekal et al. (1983) that the chicken phosphofructokinase and fructose-2,6-bisphosphatase enzymes are sensitive to lower concentrations of fructose-2,6-bisphosphate than are the rat enzymes.

#### 1.4.3 Pyruvate Kinase, Phosphoenolpyruvate Carboxykinase And Pyruvate Carboxylase

As with hexokinase, pyruvate kinase exists as a number of isoenzymes. In the mammal three isozymes are distinguished by kinetic, electrophoretic and immunological techniques: Type L, the major hepatic form; Type K (or M2), the major foetal form; and type M, the predominant form in muscle and brain (Seubert & Schoner, 1971). The activity of the L type, but not the K or M type isozyme can be altered by the diet in the rat (Sandoval & Carbonell, 1973). Compared to a mammal, levels of pyruvate kinase activity in the liver of a fowl are low (Wallace & Newsholme, 1967) and are not subject to dietary modification (Pearce, 1971; 1980). Several authors have reported finding only the K type and not the L type isozyme in chicken liver (Strandholm et al., 1975; Schloen et al., 1974; Cardenas et al., 1975). More recent reports however, have demonstrated very convincingly, the presence of both type L and type K in the liver of chickens in the ratio 1:4 (Eigenbradt & Schoner, 1977).

Phosphoenolpyruvate carboxykinase (PEPCK) is another key enzyme in the regulation of gluconeogenesis (Soling et al., 1976). Its intracellular distribution varies considerably among different species of animals. In the livers of rats and mice, PEPCK is located primarily in the extramitochondrial fraction (Nordlie & Lardy, 1963). In the

livers of guinea pig (Nordlie & Lardy, 1963), rabbit (Johnson et al., 1970), sheep (Taylor et al., 1971), cow (Heitzman et al., 1972) and human (Diesterhaft et al. 1971), the enzyme is found in both the mitochondrial and cytosolic compartments. In avian species there are conflicting reports as to the subcellular distribution of the hepatic enzyme. In the adult chicken, the enzyme is reported to be entirely mitochondrial (Chiao, 1976; Deaciuc et al., 1982; Bannister & O'Neill, 1981) and apparently unchanged by a fast (Gervers, 1967; Watford et al., 1981; Bannister & Cleland, 1978; Brady et al., 1978; Soling et al., 1973). Further, Hód et al. (1982, 1983) report that no messenger-RNA coding for the chicken kidney-cytosolic PEPCK can be detected in chicken liver. Other investigators however, report significant amounts of a cytosolic enzyme: up to 50% of the total (Jo et al., 1974a, 1974b; Kochi et al., 1980; Shen & Mistry, 1979; Felicioli et al. 1967; Migliorini et al., 1973). In addition there are reports of changes in the amount and subcellular distribution of PEPCK during development (Felicioli et al., 1967; Peng et al., 1973; ), as a result of fasting (Veiga et al., 1978) as a result of glucocorticoid treatment (Kochi et al., 1980; Jo et al., 1974a) or as a response to the season of the year (Jo et al., 1974b). (None of the investigators who reported significant amounts of cytosolic PEPCK in the chicken carried out investigations on subcellular distribution employing subcellular markers to control for contamination

by other cellular fractions.)

A possible explanation to reconcile the conflicting findings of these investigators is in the report of Tinker et al. (1983a) who found that the cytosolic activity of PEPCK decreased with age from the time of hatching, until in the adult, no soluble activity was present. Tinker et al. (1983a) further reported that cytosolic levels of PEPCK activity in juvenile chickens were sensitive to the photoperiod. Both the total and cytosolic activities were higher in birds raised under 24 hour daylengths than under 12 hour daylengths.

The knowledge of whether PEPCK is cytosolic or mitochondrial is important in considering factors affecting the regulation of metabolism, especially whether it is the reduced malate or the oxidized phosphoenolpyruvate which is translocated from the mitochondria for cytoplasmic gluconeogenesis (See discussion in Section 4.1).

PEPCK has been reported in the nuclear fraction of species other than chickens (Garthoff et al., 1972; Swiatek et al., 1970; Nordlie and Lardy, 1963; Ballard and Hanson, 1967; Nagano et al., 1973). It was not shown, however, that the nuclear activity was not due to contamination by mitochondria or cytosol. Using marker enzymes to control for contamination by other fractions, Tinker et al. (1983a, 1983b) have reported that chicken liver PEPCK is distributed



in both the mitochondrial and in the nuclear fraction.

Although pyruvate carboxylase, which is located in the mitochondria, is often considered to be important in the regulation of gluconeogenesis, it does not seem to be a limiting enzyme in glucose synthesis in the fowl except during biotin deficiency (Arinze & Mistry, 1970) and in birds afflicted with the fatty liver and kidney syndrome (Bannister, 1976).

In the rat liver pyruvate carboxylase is known to be sensitive to several low molecular weight intermediates such as acetyl CoA, ATP, glutamate, pyruvate and divalent calcium ions (See references in Chisholm et al., 1983). The chicken liver enzyme is also activated by acetyl CoA (Utter & Fung, 1971), though unlike the rat and human liver, the chicken liver enzyme is entirely without activity in the absence of acetyl CoA. As in rats, pyruvate carboxylase of the chicken is also inhibited by ADP (Keech & Utter, 1963). Additionally, while fasting increases the activity of the enzyme in the liver of several species, the increase in the avian liver (pigeon) is less pronounced than in rats (Soling & Kleineke, 1976).

## 1.5 ANATOMICAL DIFFERENCES BETWEEN BIRDS AND MAMMALS RELEVANT TO GLUCOSE METABOLISM

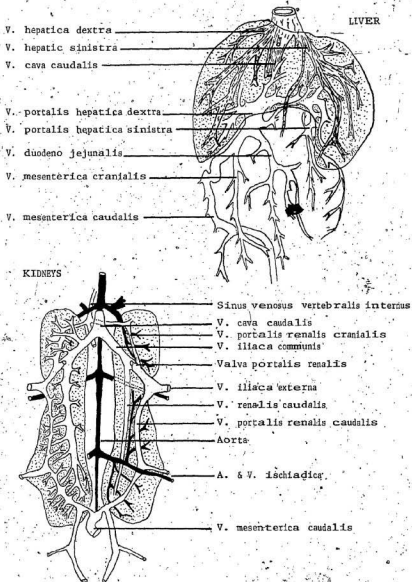
### 1.5.1 Respiration

One of the major differences between a bird and a mammal is the way the blood is oxygenated (See review by King & Molony, 1971). Avian species lack a diaphragm, so inhalation and exhalation forces are provided by rib cage motions. Opening the abdominal cavity of any bird therefore renders it unable to breathe because it cannot raise or lower intra-abdominal pressures. Additionally, in order to exchange blood gases, the bird uses a complex system of airsacs to circulate air unidirectionally through parabronchi whereas mammals use muscular diaphragm movements to inflate and deflate alveoli.

### 1.5.2 Circulation

The arrangement of the circulatory system is also quite different from the mammalian model (See review by Akester, 1971). The major points of difference are described in the following sections. Figure 1 illustrates the major abdominal vessels in a domestic fowl.

FIGURE 1. Anatomy of Abdominal Blood Vessels in the Fowl



#### 1.5.2.1 Renal Flow

It was recognized in the nineteenth century and confirmed by later investigations of Spanner (1924) and Sperber (1948) that the chicken has a renal portal system and a renal portal valve in each common iliac vein (V. iliaca communis). Renal portal blood flow through the kidneys can be complicated because of this arrangement. In effect, blood entering the renal portal system from the external iliac vein (V. iliaca externa) has four potential routes: 1) perfusion of the renal tissue via the renal portal system; 2) a bypass of the renal tissue by flowing through the renal portal valve into the caudal vena cava (V. cava caudalis); 3) a bypass of the renal tissue by flowing through the caudal renal portal vein (V. portalis renalis caudalis) to the coccygeomesenteric vein (V. mesenterica caudalis) and through this vein to the right hepatic portal vein (V. portalis hepatica dextra) and liver; 4) a bypass of the kidney via the cranial renal portal vein (V. portalis renalis cranialis) to the vertebral venous sinus (Sinus venosus vertebralis internus) and thence to the jugular vein (V. jugularis). (See Figure 1). These four alternatives may be a function of the state of the renal portal valves (open/closed). The function of the valves is under nervous control, but the regulation is not yet understood (Burrows et al., 1983). It is not certain how

the renal portal valve affects blood flow, as blood enters the renal portal system even if the valve is open (Akester, 1971; Sturkie et al., 1978)

#### 1.5.2.2: Hepatic Flow

Blood flow through the liver is also complex. The liver is fed by two portal veins and two hepatic arteries. The right portal vein is the larger vessel and receives blood from the large intestinal veins before entering the liver. The regulation of hepatic portal blood flow is not well understood. Blood in the right hepatic portal vein, flowing into the liver from the gut, may be augmented by blood leaving the kidney or hindquarters via the coccygeomesenteric vein (Akester, 1971, Sturkie & Abati, 1975, Sturkie et al., 1977)

#### 1.5.3 Red Blood Cells

Erythrocytes of the chicken, as of nearly all non-mammalian vertebrates are nucleated. The intact red blood cells of chickens contain no glucose, and they do not consume glucose (Shields et al., 1964) even though the cells possess all of the glycolytic enzymes (Rosa et al., 1983). Apparently red cells derive their metabolic energy from fatty acids via Beta-oxidation and the tricarboxylic acid cycle (Bell, 1971). Simons (1983) has demonstrated the presence of a sugar transport system for the uptake of glucose, in pigeon erythrocytes, but noted it functions at

only one one-thousandth of the rate of the human red cell sugar transporter.

The red cells contain no 2,3-diphosphoglycerate and instead inositol pentaphosphate fulfills 2,3-diphosphoglycerate's role as the molecule regulating oxygen binding to hemoglobin (Benesch & Benesch, 1967).

It has also been noted, that compared to mammals, chickens have a higher concentration of leucocytes, a lower concentration of erythrocytes in their blood and the erythrocytes of chickens are very large. The avian hematocrit is approximately 30% in females (and in chicks) and ranges from 38% to 45% in adult cocks. (Hayden & Fish, 1928; Newell & Shaffner, 1950; Bond & Gilbert, 1958; Wels et al., 1967).

## 1.6 METHODS USED TO STUDY GLUCONEOGENESIS IN THE FOWL

### 1.6.1 Studies In Vivo

#### 1.6.1.1 Fasting

Early workers attempting to understand glucose metabolism in chickens did so through in vivo studies. Glucose was measured in the urine of depancreatized birds (Langendorff, 1879), of birds dosed with adrenaline (Paton, 1905), and in blood (Bierry & Fandard, 1912; Giaja, 1912). Investigators became interested early in this century in the nature of the glucose precursors. In 1911, Mostowski published a study showing a decrease in hepatic glycogen of fowl after a four day fast and provided evidence that dihydroxyacetone (force-fed) was a precursor of hepatic glycogen, as indicated by a rise in hepatic glycogen after the treatment.

In addition to the early work concerned with glucose and glycogen, investigators have since examined the effects of fasting on blood amino acids and other metabolites as well as glucose in an effort to come to a more complete understanding of glucose metabolism in the domestic fowl. In general, investigators who have examined blood have reported little or no change in the plasma glucose concentration, a drop in the plasma concentration of most glucogenic and essential amino acids, and a rise in plasma threonine (See Zimmerman & Scott, 1967; Swenson, 1970;

Boomgaardt & McDonald, 1969; Hill & Olsen, 1963; Murray & Rosenberg, 1953; Belo et al., 1976a; Brady et al., 1978).

The effects of fasting on metabolite levels in tissues were examined in the early 1930's and 1940's but major experimental drawbacks make it advisable to be careful in drawing conclusions from these studies. Early investigators (Corkill, 1930; Silvette & Britton, 1934; Henry et al., 1934; Emslie & Henry, 1933; Golden & Long, 1942; Murray & Rosenberg, 1953) determined hepatic glycogen simply by sacrificing the animal and excising liver tissue for analysis. Hepatic glycogen levels in such birds in the fed state were found to vary from 0.6 to 6%, while in fasted birds the values ranged from 0 to 0.4%. While these levels are not too different from measurements made with current techniques, doubt should be cast on any reported measurements of lactate, or the phospho-sugars because the levels of intermediary metabolites change very rapidly after the onset of ischemia in liver (Hems & Brosnan, 1970). Introduction of "freeze-clamping" (Wollenberger et al., 1960) to cool tissues to liquid nitrogen temperatures rapidly and stop metabolism, when applied to the domestic fowl, resulted in data more representative of the situation in vivo and the changes induced by fasting (Allred, 1969; Nakatani & Gotoh, 1961; Simon & Blum, 1972; Rinaudo et al., 1976; Brady et al., 1978; Wittman & Weiss, 1981; Bannister & Cleland, 1977; data have also been obtained



from quail, Didier et al., 1981, 1983; and pigeon, Kaminsky et al., 1982).

#### 1.6.1.2 Precursors For Glucose Synthesis

Emslie and Henry (1933) expanded the force-feeding experiments of Mostowski (1911) and determined hepatic glycogen formation from a variety of substrates force-fed to fasted chickens. They found glucose and dihydroxyacetone were the best precursors with less glycogen formation from alanine, glycerol and lactate. Pyruvate was ineffective in elevating hepatic glycogen.

Two more recent experiments involving intraperitoneal injections of substrates into fasted chickens were carried out by Sarkar (1971) and by Davison & Langslow (1975) who found similar results - glycerol, malate or lactate were effective in elevating blood glucose, while pyruvate and a variety of amino acids were less so.

Considerable research has also been carried out on the effect of feeding non-carbohydrate diets, especially high-fat diets, on blood metabolites and growth. It has been found that fat plus protein can completely replace carbohydrate in the diet of chickens without any ill effects or decrease in plasma glucose. However when protein plus fatty acids are used instead of protein and triglycerides, birds display effects similar to those exhibited by fasting animals (poor growth, elevated levels of blood lysine &

threonine, and hypoglycemia) (See Zimmerman & Scott, 1967; Brambila & Hill, 1966, 1967; Allred, 1969; Hill & Olsen, 1963; Evans & Scholz, 1971; Renner & Elcombe, 1964, 1967). These findings can be interpreted to mean the chicken can produce all the glucose it needs by a high level of gluconeogenesis from a combination of the glycerol in fat and the amino acids in protein, although gluconeogenesis from amino acids in the absence of glycerol is impaired. The stoichiometry of gluconeogenesis from glycerol is such that an NADH is produced for each glycerol molecule converted to glucose. This NADH could be used in the production of glucose from pyruvate and therefore from the several amino acids whose transformation into glucose is via pyruvate or PEP.

#### 1.6.1.3 Glucose Turnover

A high level of gluconeogenesis in chickens has been shown by studies of glucose turnover in fed or fasted chickens. Glucose turnover is higher in chickens (15-20 mg/min/kg) than in mammals (7-12 mg/min/kg). There is also a higher rate of Cori cycle (Cori & Cori, 1929) recycling of glucose (Chickens - 30-60%, Belo et al., 1976a; Brady et al., 1978; Riesenfeld et al., 1981; Veiga et al., 1982; Rats - 14%, Katz et al., 1974; Ponies - 15%, Anwer et al., 1976; Dogs - 14%, Belo et al., 1976b). Glucose turnover in the chicken is decreased by a fast (Anniçon et al., 1966; Riesenfeld et al., 1981; Brady et al., 1977), to approximately the same extent as in mammals (Katz et al.,

1974; Anwer et al., 1976).

### 1.6.2 Studies In Vitro

During the 1930's, Dyer & Rde (1933) and Heller & Pursell (1937) published reports on the constituents in the blood of the fowl; and Krebs & Henseleit (1932) published their classic paper on the preparation of physiological media. This provided investigators with the information needed to prepare artificial incubation media and laid the groundwork for another phase of study, namely in vitro experiments.

The levels at which metabolism may be studied are, in descending order of complexity: 1. the whole animal; 2. a perfused tissue; 3. organ slices; 4. isolated cells; 5. a tissue homogenate or fraction; and 6. isolated enzymes in solution. The first level as it pertains to the study of glucose metabolism in chickens was reviewed in Section 1.6.1; and the sixth level, for the enzymes considered regulatory in gluconeogenesis, was discussed in Section 1.4. In this section, (1.6.2), the remaining subjects will be discussed.

#### 1.6.2.1 Perfused Tissues

The first level of organization beneath the intact organism useful for metabolic studies is often considered to be the perfused tissue. Experimenters using the perfused avian liver to study gluconeogenesis report results similar to the in vivo studies, i.e. low rates of gluconeogenesis from pyruvate and amino acids while lactate, glycerol and fructose are more effective as glucose precursors (Deaciuc & Ilonca, 1981; Sugano et al., 1982; Deaciuc et al., 1982; Soling, 1974). Perfused chicken liver has also been used in the study of uricogenesis (Deaciuc et al., 1982; Barratt et al., 1974.)

Perfused chicken breast and wing muscles have also been successfully employed in the study of protein degradation using 3-methylhistidine release as an indicator (Hillgartner et al., 1981)

#### 1.6.2.2 Organ Slices

The use of organ slices to study metabolism was pioneered by Warburg in a series of papers in the 1920's (See, for example, Warburg, 1923)

Both liver and kidney slices have been used to examine metabolism in birds. The liver slice has not proved a useful tool for studying gluconeogenesis as rates of glucose synthesis are lower than in vivo (Krebs, 1964), presumably

as a result of extensive tissue damage. Nevertheless, liver slices have been used by investigators to examine gluconeogenesis in birds, especially in comparative work (Veiga et al., 1978; Migliorini et al., 1973; Bannister & Cleland, 1977). Liver slices have been used for investigations into hepatic urea synthesis (which is very low in birds, Lemonde, 1959) and chicken fatty liver and kidney syndrome (Bannister & Cleland, 1977).

Slices of kidney cortex, which apparently suffer less tissue damage than liver slices during preparation, have been used with greater success in the study of gluconeogenesis. Various investigators have found kidney cortex slices to have a very active gluconeogenesis from most amino acids, pyruvate and lactate (Krebs & Yoshida, 1963). Isolated kidney tubules, prepared by digesting kidney slices with collagenase, have proved a useful research tool, and have been used effectively in studies on gluconeogenesis with results similar to the kidney slice findings (Wittman & Weiss, 1981; Watford et al., 1981).

### 1.6.2.3 Isolated Cells

In studying avian hepatic glucose metabolism for short-term experiments, the isolated hepatocyte has been used most often in the last decade or so. This means of studying metabolism has allowed investigators to obtain much more data in a shorter time than in vivo or in perfused organ studies due to the fairly great number of hepatocytes that can be obtained from one animal. The method was refined for rats by Berry & Friend (1969). Modifications of the original method have been developed for the chicken (Mapes & Krebs, 1978), and methods to determine the viability of cells have also been established (Anderson et al., 1976).

Hepatocytes have been used by a number of authors to study gluconeogenesis in chickens (Deaciuc et al., 1982; Langslow, 1978; Ogata et al., 1982; Ochs & Harris, 1978, 1980; Bannister & O'Niell, 1981; Dickson & Langslow, 1977, 1978; Brady et al., 1979; Dickson et al., 1978; Mapes & Krebs, 1978; Dickson, 1983). The results obtained are similar to those found for the perfused liver. (See more complete discussion in Sect 2.4.3)

#### 1.6.2.4 Tissue Homogenates

The homogenate technique has been used to study chemical reactions in disrupted cells. Some of the cellular organelles (nuclei, mitochondria) are still intact in a properly prepared homogenate, although the endoplasmic reticulum is disrupted (Potter, 1972).

Krebs et al., (1964) used a liver homogenate to examine gluconeogenesis from lactate in pigeons. While significant rates of glucose were formed in the pigeon liver homogenate, in a chicken liver homogenate, glucose synthesis from lactate was insignificant. The technique has not found wide use in avian metabolic studies.



## 1.7. PROBLEM OF INVESTIGATION

Much is known about glucose metabolism in the chicken and much is not. Although general textbooks of biochemistry often make sweeping statements about glucose metabolism, and its regulation, most of the conclusions have been based on research in mammals, especially Rattus rattus and Homo sapiens. Such generalizations are not true in all cases for all mammals. The number of exceptions to these generalizations increases when other classes of vertebrates are examined.

One of the general rules concerns gluconeogenesis from amino acids in a fasting animal. The belief is that protein breakdown and metabolic processes in muscle result in the release of more alanine and glutamine into the circulation than any other amino acids. The alanine is taken up by liver where the carbon skeleton is used for glucose synthesis and the nitrogen for urea production. The glutamine is taken up by kidney, where the carbon skeleton enters the gluconeogenic pathway and the nitrogen is released as ammonia. (For a review see Felig (1973) and papers by Chang & Goldberg, (1978), Aikawa et al (1973), Ruderman & Berger (1974) and Odessey et al., (1974).

Glucose needs of the mammal are reduced on starvation. While glucose released by liver and kidney is taken up and oxidized by the brain, the muscle ceases to oxidize glucose. Blood glucose levels fall and glucose turnover is decreased. The majority of the glucose needs of the mammal are met through hepatic gluconeogenesis from alanine and a lesser contribution of renal gluconeogenesis from glutamine. Lactate produced by glycolysis in the red blood cells is converted to glucose in the liver.

Several investigations have indicated that this general pattern is not adhered to in the chicken. Glucose levels are not lowered by fasting in the chicken (Section 1.1.1 and Section 1.6.1). Red blood cells of the chicken are unable to utilize glucose and hence form no lactate (Section 1.5.3). Hepatic urea production from alanine does not occur, as the chicken is a uricotelic species, and urate is the compound by which nitrogenous wastes are excreted. In addition, there are major enzyme differences between birds and mammals (especially the lack of hepatic glucokinase and hepatic cytosolic PEPCK, Section 1.4). In vitro investigations have demonstrated that the fowl's liver and liver cells make glucose far less readily from alanine than from lactate, which is not the case for mammals nor for chicken kidney (Section 1.6.2). And lastly, the hormonal control of glucose metabolism varies significantly from the mammalian pattern (Section 1.2).

Thus when this project was conceived it was expected that inter-organ relationships of amino acids would be different in the chicken than those described for the rat by Aikawa et al. (1973) and Chang & Goldberg, (1978). The objective of this investigation was to answer the questions (1) In what organ or organs is glucose produced in the fasted domestic fowl, (2) Which amino acid(s) is(are) the primary substrate(s) for glucose synthesis, and (3) From which tissue(s) are the amino acid substrates released?

The search for the answers to these three questions was divided into two lines of investigation. The first of these was the determination of the levels of the metabolites of glycolysis (gluconeogenesis) in liver and kidney. These two organs were suspected of being the primary gluconeogenic tissues because of their role in glucose synthesis in the man and the rat (Harris & Crabb, 1982). The results of this line of investigation are reported in Chapter Two of this thesis. The second line of investigation was the measurement of amino acids and metabolites in abdominal vessels of fed and fasted chickens in order to understand inter-organ relationships of amino acids and glucose. The results of this line of investigation are reported in Chapter Three of this thesis.

Because of the body of evidence that the ability of the chicken's liver in vitro to synthesize glucose from amino acids is impaired (Section 1.6.2), while that of the kidney in vitro is not (Watford et al., 1981; Ogata et al., 1982), it was hypothesized that the kidney of the fowl would be a more important gluconeogenic organ in vivo than liver.

CHAPTER 2

ARTIFICIAL RESPIRATION AND METABOLITE LEVELS IN LIVER AND KIDNEY

## 2.1 INTRODUCTION

### 2.1.1 Hypoxia And Metabolite Content Of Tissue

There is, in addition to the metabolic differences between birds and mammals, an anatomical difference making avian investigations difficult; that is, unlike mammals, birds lack a muscular diaphragm. Thus, opening a bird's abdominal cavity is analogous to the opening of the thoracic cavity of a mammal. The bird is unable to breathe, and anoxia rapidly ensues with the resulting shift of metabolites from their in vivo concentrations. In freeze-clamped rat liver the ATP concentration and ATP/ADP ratio is high ([ATP]= 2.74 (micromoles/g), ATP/ADP= 2.04) (Hems & Brosnan, 1970). Previous investigators have found the concentration of ATP or the ATP:ADP ratio, or both, in freeze-clamped avian liver to be lower as is seen in the list below.

Bannister & Cleland, 1977,	[ATP]= 0.99, ATP/ADP= 0.61
Deaciuc & Ilonca, 1981,	[ATP]= 1.44, ATP/ADP= 0.61
Dickson et al., 1978,	[ATP]= 2.46, ATP/ADP= 0.24
Barratt et al., 1974,	[ATP]= 0.58, ATP/ADP= 0.34
Locke et al., 1972,	- ATP/ADP= 0.25
Rinaudo et al., 1976,	[ATP]= 0.47, ATP/ADP= 0.42

These reports contrast with the findings of an ATP level of 2.24 micromoles per gram and an ATP/ADP ratio of 2.6 in perfused chicken liver (Ogata et al., 1982) and ATP/ADP ratios in chicken hepatocytes of 2.4, 1.9 and 2.5, reported by Dickson et al., (1978), Dickson & Langslow,

(1978) and Cramb et al., (1982) respectively. Additionally, in a note added in proof, Soling et al., (1973) report without any details, that in vivo measurements of adenine nucleotide levels in livers from pigeons under artificial respiration showed an ATP/ADP ratio of 2.99.

In freeze-clamped rat liver the lactate concentration is low (0.45 micromoles per gram) (Hems & Brosnan, 1970). The in vivo lactate level and lactate/pyruvate reported ratio for avian liver is higher than the rat's. Values found by several investigators are given below. (For lactate, units are in micromoles per gram of liver)

Brady et al., 1978	Lactate = 1.57	Lact/Pyruv = 14.1
Barratt et al., 1974	Lactate = 11.8	Lact/Pyruv = 14.8
Kaminsky et al., 1982	Lactate = 1.14	Lact/Pyruv = 27
Didier et al., 1983	Lactate = 1.79	Lact/Pyruv = 22.4

These figures are high in comparison to the lactate level of 0.097 and lactate:pyruvate ratio of 2.8 reported in perfused chicken liver (Ogata et al., 1982).

I postulate in this thesis that many of the differences previously reported between the metabolite concentrations of rat tissues and chicken tissues are attributed in part to the hypoxia in the chicken resulting from surgery. An older method therefore, (Burger & Lorenz, 1960), was adapted to maintain blood oxygen, carbon dioxide and pH at physiological levels for extended periods in an anesthetized

chicken whose abdominal cavity has been opened. This technique allows the taking of tissue samples under conditions which eliminate the hypoxia previously encountered with tissue sampling procedures in birds.

### 2.1.2 Application Of Artificial Respiration To The Study Of Gluconeogenesis

Most research to date suggests the presence of active gluconeogenesis in fasting chickens, yet hepatic glucose synthesis from alanine, a major source of glucose carbon in the fasting rat, appears to be low in avian species both in vivo and in vitro (Golden et al., 1982; Soling et al., 1970; Langslow, 1978).

Sugano et al. (1982) have reported that in perfused chicken liver, regeneration of NADH in cytosol is limiting and therefore gluconeogenesis in vitro is regulated in part by alterations in redox state. I postulate that cytosolic NADH availability in the liver may be limiting for gluconeogenesis from pyruvate and amino acids in vivo as well. Evidence will be presented in this chapter to support this hypothesis.



In this chapter are presented details of a method of artificial respiration for birds, and the levels of blood gases measured while employing the technique. The levels of hepatic and renal metabolites in artificially ventilated fed and fasted birds and levels of metabolites in ischemic liver of fed birds are also reported. Preliminary reports have appeared in abstract form (Tinker et al., 1981; 1982) and two papers have been published on this work (Tinker et al., 1984a, 1984b).

A ventilation technique similar to the one described in this chapter was used by Hillgartner et al. (1981) as part of a muscle perfusion procedure. However, they employed 95% oxygen plus 5% carbon dioxide as the ventilatory gas. It is expected that there would have been a deviation from physiological values of pH,  $pO_2$  and  $pCO_2$  of the blood. Boelkins et al. (1973) have also employed artificial respiration in chickens. They were able to maintain arterial blood gases within the normal range (Chiodi & Terman, 1965) using a respirator but a slight alkalosis was observed.

## 2.2 METHODS AND MATERIALS

### 2.2.1 Experimental Animals

Fertilized eggs were obtained from a flock of white leghorn layers (Gallus domesticus) maintained at Memorial University's Animal Care Facilities. Newly hatched chicks were reared at 25 degrees Celsius with room lighting on 24 hours daily. A 24 hour daylength was chosen as it has been shown that chicks raised under this photoperiod have a higher hepatic PEPCK content than chicks raised in 12 hour daylengths (Tinker et al., 1983). It was expected that higher rates of gluconeogenesis would be found in fasted chickens raised under such conditions, and differences from the fed state more easily seen. Water and commercial chick starter feed (Supersweet Feeds, St. John's, NFLD; 20% protein; 2% fat, 6% fibre) were available ad libitum. Experimental animals were eight-week old males which weighed approximately 800 g. The animals were processed immediately or fasted six days before use. Fasted chickens lost on average, 18% of their initial body weight. A six-day fast was chosen somewhat arbitrarily, in that preliminary work on 3-day fasted chickens indicated that blood 3-methylhistidine levels, an indicator of muscle proteolysis, were not significantly different from controls. When the period of fasting was doubled, blood 3-methylhistidine levels were significantly greater than fed animals. Water was available ad libitum to the fasting animals.

## 2:2.2 Surgical Procedures

Birds were immobilized on their backs on a plexiglass surgical board. Animals were then anesthetized by a slow i.v. injection (brachial vein) of sodium pentobarbital until the animal no longer responded to a comb pinch (a dose of approximately 40 mg per kg). The trachea was then exposed, cut through and a length of stiff plastic tubing of a diameter slightly smaller than the trachea, inserted and clamped in place. This tracheal tube was led away to a 'T' junction. One arm of the junction was open to the air, the other was connected in series with a gas humidifier to a flow meter and regulator on a cylinder of compressed air. Gas flow was adjusted to 450-500 ml/min, which escaped through the open side of the 'T'

To this point the animal was breathing the mixture unassisted. The abdominal cavity was then quickly opened with a scissors cut beneath the sternum, and the open side of the 'T' junction closed off. The incision in the abdomen was then enlarged by cutting dorsally through the body wall at the cartilage junctions of the ribs. The inflating air sacs and mesenteries were punctured by scissors cuts to facilitate air flow. After restraint but before anesthesia, a 0.3 ml blood sample was drawn from a brachial vein in a heparinized syringe for blood gas determinations on an I.L. Model 213 analyser. Five minutes after initiation of ventilation, another 0.3 ml blood sample was taken from the

contra-lateral brachial vein and blood gases determined as before.

### 2.2.3 Assays For Tissue Metabolites

A liver sample of approximately two grams was excised from a ventilated bird and immediately (< 2 seconds) freeze clamped with aluminum tongs precooled with liquid nitrogen. A kidney sample of approximately one gram was excised from the same bird immediately after the liver sample was taken and similarly freeze-clamped within 4 seconds of excision.

To obtain levels of metabolites in ischemic liver, a second two-gram piece of liver was excised and freeze-clamped 300 seconds after severance of blood flow. The animals were humanely killed by an intracardiac injection of sodium pentobarbital.

Each frozen liver sample was ground with a pestle in a liquid nitrogen-filled mortar. The powder was scooped into a cooled, tared plastic 50 ml centrifuge tube, weighed and 4 ml of ice-cold 6% perchloric acid per gram of frozen powder was pipetted in. The powder was then immediately homogenized in the centrifuge tube with a loosely fitting, rotating (400 rpm) teflon homogenizing pestle for 5 minutes. The homogenate was centrifuged at 10,000 g (4°C) and the supernatant decanted and neutralized with potassium hydroxide using Universal Indicator (Fisher Scientific Co.)

to indicate pH 7.

Enzymes and biochemicals were obtained from Sigma, St. Louis, Mo. or from Boehringer-Mannheim, (B-M.), Montreal, Quebec. All other chemicals were analytical grade and were obtained from Fisher Scientific, Dartmouth, N.S.

Metabolites were measured in the neutralized perchloric acid extract using the methods described below.

Lactate was determined enzymatically by the method of Lowry & Passonneau (1972), monitoring the production of NADH at 340 nm in a 50 mM tris-hydroxyaminomethane, 50 mM glutamate, 1.5 mM NAD<sup>+</sup> reaction mixture pH 9.9, after the addition of 0.01 ml of beef-heart lactate dehydrogenase (3000 units/ml;Sigma) and 0.01 ml of pig-heart glutamate-pyruvate transaminase (1400 units/ml;Sigma).

Malate was determined, in the same cuvette as lactate, after the first reaction had reached completion by adding simultaneously to the cuvette, 0.01 ml of a pig-heart malate dehydrogenase suspension (6000 units/ml;B-M.) and 0.01 ml of a pig-heart glutamate-oxaloacetate transaminase suspension (2000 units/ml;B-M.) and monitoring the production of NADH at 340 nm.

The five metabolites 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, pyruvate and 2-oxoglutarate were determined sequentially in one cuvette by an enzymatic technique modified from Czok & Lamprecht (1974). The reaction mixture was 40 mM triethanolamine, 7 mM potassium chloride, 10 mM magnesium sulfate, 20 mM ammonium sulfate, 0.5 mM ADP, 0.18 mM NADH, 0.1 mM 2,3-diphosphoglycerate, pH 7.6. The pyruvate concentration was determined by adding 0.002 ml of a 3000 units/ml suspension of beef-heart lactate dehydrogenase and measuring the disappearance of NADH by the decrease in absorbance at 340 nm. The phosphoenolpyruvate concentration was similarly determined by adding 0.004 ml of a 2000 units/ml suspension of rabbit-muscle pyruvate kinase (Sigma) to the cuvette after the first reaction had reached an end point. Likewise, 2-phosphoglycerate, 3-phosphoglycerate and 2-oxoglutarate were determined by adding respectively: 0.02 ml of a 400 units/ml suspension of rabbit muscle enolase (Sigma), 0.003 ml of a 4000 units/ml suspension of rabbit muscle phosphoglycerate mutase (Sigma) and 0.005 ml of a 1000 units/ml suspension of beef liver glutamate dehydrogenase (B-M.) after the completion of each previous reaction.

Fructose-1,6-bisphosphate and the triose phosphates were determined enzymatically by a modification of the method of Michal & Beutler (1974). The reaction mixture was 200 mM tris-hydroxyaminomethane, 0.14 mM NADH, pH 7.5. The

triose phosphates in a sample were determined by measuring the decrease in the absorbance at 340 nm after the addition of 0.01 ml of a mixed suspension of 2-glycerophosphate dehydrogenase (11,000 units/ml) and triose phosphate isomerase (1000 units/ml; Sigma). After completion of the first reaction, fructose-1,6-bisphosphate was similarly determined by the addition of 0.01 ml of a 90 units/ml suspension of rabbit muscle aldolase (B-M.).

Glucose-6-phosphate and fructose-6-phosphate were determined enzymatically by a modification of the method of Lang & Michal (1974). The reaction buffer was 200 mM tris-hydroxyaminomethane, 7 mM magnesium chloride, 0.2 mM NADP<sup>+</sup>, pH 7.5. Glucose-6-phosphate in the sample was determined by measuring the increase in the absorbance at 340 nm after the addition of 0.01 ml of a suspension of yeast glucose-6-phosphate - dehydrogenase (300 units/ml; Sigma). After completion of the first reaction, fructose-6-phosphate was similarly determined by the addition of 0.01 ml of a 3500 units/ml suspension of yeast phosphoglucosomerase (B-M.).

ATP was determined by a modification of the method of Lamprecht & Trautschold (1974). A reaction cocktail consisting of 50 mM tris-hydroxyaminomethane, 1 mM magnesium chloride, 0.5 mM dithiothreitol, 0.5 mM NADP<sup>+</sup> and 1 mM glucose, pH 8.1 was prepared. To 1.5 ml of cocktail was added 0.5 ml of sample and 0.005 ml of yeast

glucose-6-phosphate dehydrogenase (300 units/ml). ATP concentration was determined from the increase in absorbance at 340 nm after the addition of 0.005 ml of yeast hexokinase (1400 units/ml).

ADP and AMP were determined enzymatically by a modification of the method of Jaworek et al. (1974). A reaction cocktail consisting of 50 mM imidazole, 2 mM magnesium chloride, 75 mM potassium chloride, 0.1 mM ATP, 0.3 mM phosphoenolpyruvate, 0.15 mM NADH, pH 7.0 was prepared. To 2.5 ml of cocktail was added 0.5 ml of sample and 0.005 ml of a suspension of beef-heart lactate dehydrogenase (3000 units/ml). After the absorbance at 340 nm had steadied, ADP concentration was determined by adding 0.005 ml of a suspension of rabbit-muscle pyruvate kinase (2000 units/ml) and measuring the decrease in absorbance at 340 nm. AMP concentration was determined after the completion of the previous reaction by adding 0.005 ml of pig-muscle myokinase (8000 units/ml; Sigma), and measuring the decrease in absorbance at 340 nm.

Glucose was determined colorimetrically by a method modified from Raabo & Terkildsen (1960) as reported in Sigma Technical Bulletin # 510, January, 1978. The method uses glucose oxidase to convert quantitatively the D-glucose in a sample to gluconic acid and hydrogen peroxide. The hydrogen peroxide was reacted with ortho-dianisidine in the presence of peroxidase to yield a colored product. The change in



absorbance at 450 nm was measured spectrophotometrically.

Glycogen was determined by a method modified from Hassid & Abraham (1957). One half of a gram of freeze-clamped liver tissue, ground to a powder with a cooled mortar and pestle, was homogenized in 10 volumes of cold 30% potassium hydroxide solution. Two mls of the homogenate was placed in a test tube in a boiling water bath for 2 hours to release the glycogen. The tube was then cooled and to it was added 2.4 mls of cold 95% ethanol to precipitate glycogen. After 20 minutes at 0 degrees celsius, the tubes were centrifuged at full speed in a benchtop centrifuge for 30 minutes at 10,000 X g. The supernatant was then decanted, and the pellet suspended in 2 ml of 4N sulfuric acid. The tubes were then placed in a boiling water bath for 90 minutes, to hydrolyse glycogen to glucose. The contents of the tubes were then neutralized with 4N sodium hydroxide, and the glucose concentration determined as above.

Ammonia was determined enzymatically by a modification of the method of Kun & Kearney (1974). A reaction cocktail consisting of 0.2 M sodium phosphate, 5 mM 2-oxoglutarate, 0.25 mM NADH, pH 7.6 was prepared. To 1.5 ml of cocktail was added 0.5 ml of sample and the ammonia concentration was determined by the decrease in absorbance at 340 nm after the addition of 0.02 ml of beef-liver glutamate dehydrogenase (1000 units/ml in glycerol; Sigma).

Phosphate was determined colorimetrically by the method of Martin & Doty (1949). The sample was reacted with a silicotungstate-molybdate reagent and the product was extracted with a 1:1 mixture of isobutanol:benzene. After reaction with stannous chloride in acidified ethanol the absorbance was measured in a spectrophotometer at 625 nm.

Glutamate was determined with a Beckman model 121M Amino Acid Analyser and Beckman Systems AA computing Integrator for Amino Acid Analyser using a five-buffer single-column method as described by Lee (1974) and modified in Beckman Bulletin # 121M TB-013 (July 1976).

#### 2.2.4 Calculations

Phosphorylation potential is defined as  $ATP / ADP \times P_i$   
(units are in mM<sup>-1</sup>).

Energy Charge is defined as:

$$\frac{ADP + (2 \times ATP)}{2 \times (ATP + ADP + AMP)}$$

The adenylate kinase mass action ratio (M.A.R.) was calculated by the formula:

$$(ATP \times AMP) / (ADP \times ADP)$$

Cytosolic  $NAD^+/NADH$  ratios were calculated from lactate:pyruvate ratios (Williamson et al., 1967). The assumptions made by Williamson et al. (1967) that allow this calculation are: that the lactate dehydrogenase activity in the cell is sufficient to ensure that the reaction is close to equilibrium, that the pH of the cytosol is 7.0, that the concentration of substrates is uniform throughout the tissue and that the content of the metabolite in micromoles per gram of tissue is equivalent to-millimoles per litre. As the activity of lactate dehydrogenase in rat and chicken liver is the same (unpublished observations), and as the other assumptions made by Williamson et al. (1967) seem as likely to be true for the chicken as for the

rat; use of the lactate/pyruvate ratio to calculate the  $\text{NAD}^+/\text{NADH}$  ratio should be valid.

Values were processed for outliers and any outliers dropped before statistical analysis (Dixon, 1953).

Significant differences between means were determined using a two-tailed Student's T-test. Significant differences among means were determined using a Neuman Keuls multiple range test (Steel & Torrie, 1960). Rejection was at the 0.05 level of significance.

### 2.2.5 Sources Of Error

When metabolites are determined in a perchloric acid extract of a freeze-clamped organ, the results are expressed as the content in micromoles per gram wet weight of tissue. When one equates micromoles per gram wet weight with units of concentration (mM), one is ignoring the now well established fact that many metabolites are distributed unequally among the different subcellular compartments (Zuurendonk & Tager, 1974; Tischler et al., 1977). Measurements in whole tissue overlook this sub-cellular distribution and any changes in distribution with fasting could therefore be masked. They also overlook organ heterogeneity such as the also well established difference between rat periportal and perivenous hepatic cells (Bengtsson et al., 1981) or between renal medulla and cortex (Ross & Guder, 1982).

Perchloric acid extraction itself produces artefacts. Recent work has indicated that analytically determined ADP and inorganic phosphate are considerably higher than those measured by NMR which measures free ADP in the cell. This is presumably because perchloric acid hydrolyses some organic phosphates and releases protein bound ADP which is invisible to NMR (Ackerman et al., 1980; Iles et al., 1983). Errors could also be expected to arise in the determination of the adenylates, as the enzyme adenylate kinase may not be destroyed by perchloric acid (Williamson &

Corkey, 1969). This would have the effect of artificially raising the concentration of AMP and ATP if there is an excess of ADP, as suggested above.

Additional variation between data reported here and elsewhere may be expected to arise due to differences in analytical techniques (e.g. nature of the extraction or deproteinization agent used - trichloroacetic acid vs. perchloric acid; conditions of tissue homogenation). It is also expected that there will be variation introduced due to differences in age, strain, tissue dry weights or sex of the birds.

## 2.3 RESULTS

### 2.3.1 Blood Gases

Using the procedure described in the methods section it was possible to maintain blood gases of fowl at physiological levels after the abdominal cavity had been surgically opened (Table 1). The partial pressures of oxygen and carbon dioxide, and the hydrogen ion concentration measured in the blood of the surgically treated fowl after either five or twenty minutes of artificial respiration, were indistinguishable from the values in a conscious animal. Pentobarbital anesthesia resulted in an oxygen tension in venous blood lower than in the conscious animal.

TABLE 1. Blood Gas Partial Pressures and Blood pH of Conscious and Artificially Ventilated Chickens.

	CONSCIOUS	ANESTHETIZED	VENTILATED	
			5 MINUTES	20 MINUTES
pO <sub>2</sub>	58 ± 5 <sup>a</sup>	48 ± 3 <sup>b</sup>	54 ± 7 <sup>ab</sup>	55 ± 4 <sup>ab</sup>
pCO <sub>2</sub>	28 ± 4	29 ± 2	32 ± 4	35 ± 2
pH	7.40 ± 0.06	7.37 ± 0.02	7.36 ± 0.05	7.32 ± 0.01
(n)	(19)	(11)	(12)	(6)

Blood partial pressures of oxygen (pO<sub>2</sub>), carbon dioxide (pCO<sub>2</sub>) and blood pH measured in the wing vein of restrained conscious chickens, restrained anesthetized chickens or in restrained, anesthetized, abdominally opened, artificially ventilated chickens (method detailed in text). Values in the same row bearing different letters were significantly different, Neuman-Keuls multiple range test (P < 0.05). Values are expressed as mean ± S.E.M. for (n) animals.



## 2.3.2 Levels Of Metabolites In Liver And In Ischemic Liver Of Chickens

### 2.3.2.1 Adenine Nucleotide Concentration

ATP levels or the ATP/ADP ratio in freeze-clamped liver of fed chickens (Table 2) were found to be higher than has been reported for chickens by several other investigators (Bannister & Cleland, 1977; Deaciuc & Ilonca, 1981; Barratt et al., 1974; Locke et al., 1972). However total adenylate concentration was found to be the same, or lower which suggests that the smaller ATP levels found by others were a consequence of delay between the humane killing of the animal and freeze-clamping the tissue. This view is supported by the fact that when liver was allowed to remain ischemic for 300 seconds before freeze-clamping, a large drop in the ATP concentration and the ATP/ADP ratio was observed. The two measures of cellular energy level, phosphorylation potential and energy charge, also dropped and there was a coincident rise in the AMP and inorganic phosphate concentrations. However as in the ischemic rat liver (Brosnan et al., 1970) the mass action ratio for the adenylate kinase system remained constant. This latter finding may be due to the observation that adenylate kinase may not be destroyed by perchloric acid (Williamson & Corkey, 1969).

The total content of adenylates in liver fell during ischemia presumably due to the increase in adenylate deaminase activity as a result of the decrease in energy charge (Chapman & Atkinson, 1973). The magnitude of change is comparable to that observed in the rat (Hems & Brosnan, 1970).

#### 2.3.2.2 Metabolites Of Glycolysis

In Table 3 are presented the metabolite concentrations of rapidly freeze-clamped and ischemic freeze-clamped chicken liver. The ratio of the concentration of each metabolite in the ischemic, fed chicken liver divided by its concentration in a fed control is also given. A similar ratio for the rat from earlier published data (Hems & Brosnan, 1970) is also presented for comparison.

In a fowl's ischemic liver glucose, the hexose phosphates, malate, and lactate are increased in the ischemic liver; while the phosphoglycerates, pyruvate and PEP are little changed. In the ischemic chicken liver the relative increases in glucose and lactate are less pronounced than in the rat liver while the relative increase in fructose-1,6-bisphosphate is more pronounced.

The cytosolic  $\text{NAD}^+/\text{NADH}$  ratio is also lower in ischemic compared to control chicken liver, reflecting the shift from aerobic respiration to anaerobic glycolysis of glycogen stores. The increase in malate and the decrease in 2-oxoglutarate also mirror the shift in the cell to a more reduced state.

TABLE 2. Concentrations of Adenine Nucleotides and Phosphate in Liver and in Ischemic Liver of Chickens

	Control		Ischemic
Inorganic Phosphate	4.9 ± 0.9	*	6.6 ± 1.0
AMP	0.49 ± 0.01	*	1.32 ± 0.03
ADP	1.39 ± 0.09	*	1.03 ± 0.05
ATP	1.88 ± 0.07	*	0.44 ± 0.02
ATP/ADP	1.37 ± 0.10	*	0.42 ± 0.02
Total Adenylates	3.77 ± 0.11	*	2.79 ± 0.08
Phosphorylation potential	0.33 ± 0.07	*	0.07 ± 0.01
Energy Charge	0.68 ± 0.01	*	0.34 ± 0.01
Adenylate Kinase M.A.R.	0.51 ± 0.08		0.55 ± 0.05
(n)	(5)		(5)

Metabolite concentrations in freeze-clamped chicken liver taken from artificially ventilated animals and in liver excised from the same animal and left ischemic for 300 seconds before freeze-clamping. Detailed respiration methods are given in the text. Values are expressed as the mean in micromoles per gram of tissue (wet weight) ± S.E.M. Values in the same row marked with an "\*" are significantly different using a paired T-test ( $p < 0.05$ ).

TABLE 3. Concentrations of Metabolites in Liver and in Ischemic Liver of Chickens

	CONTROL	ISCHEMIC	ISCH/CTL (FOWL) (N)	ISCH/CTL (RAT)
GLUCOSE	10.0 ± 1.30	* 23.2 ± 3.8	2.3 (3)	6.7
G6P	0.116 ± 0.029	* 0.380 ± 0.050	3.3 (5)	5.0
P6P	0.033 ± 0.007	* 0.084 ± 0.011	2.5 (5)	4.0
FBP	0.024 ± 0.003	* 0.151 ± 0.018	6.3 (4)	2.6
TRIOSE-P	0.036 ± 0.008	0.058 ± 0.010	1.6 (5)	2.5
3PG	0.048 ± 0.016	0.060 ± 0.016	1.2 (5)	0.2
2PG	0.018 ± 0.002	0.023 ± 0.008	1.3 (5)	0.3
PEP	0.034 ± 0.005	0.028 ± 0.005	0.8 (5)	0.1
PYRUVATE	0.053 ± 0.019	0.027 ± 0.014	0.5 (5)	0.6
LACTATE	0.704 ± 0.20	* 2.51 ± 0.187	3.6 (5)	14.6
AMMONIA	1.57 ± 0.602	1.25 ± 0.128	0.8 (3)	-
GLUTAMATE	5.91 ± 0.46	6.41 ± 0.45	1.1 (3)	-
2-CMOGL	0.68 ± 0.15	* 0.043 ± 0.013	0.1 (5)	-
MALATE	1.50 ± 0.319	* 3.50 ± 0.229	2.3 (5)	-
NAD <sup>+</sup> /NADH (cytosol)	684 ± 77.6	* 97 ± 37.9	0.1 (4)	-

Metabolite concentrations in freeze-clamped chicken liver taken from artificially ventilated animals and in liver excised from the same animal and left ischemic for 300 seconds before freeze-clamping. The ratio of the concentration in ischemic liver over the concentration in control livers for the glycolytic metabolites, is presented for both the chicken and for the rat (Data for the rat from Hems & Brosnan, 1970). Values are expressed as the mean in micromoles per gram of tissue (wet weight) ± S.E.M. Concentration values in the same row marked with a "\*" are significantly different using a paired T-test ( $p < 0.05$ ).

### 2.3.3 Levels Of Metabolites In Liver And Kidney Of Fed And Fasted Fowl.

#### 2.3.3.1 Adenine Nucleotide Concentration

In a six day fasted chicken, although the total hepatic adenylates fell as reported in rats (Hems & Brosnan, 1970), there was a rise in the ATP/ADP ratio and energy charge (Table 4) which is similar to the situation in fasting pigeons (Kaminsky et al., 1982). In rats however, the opposite is the case. Measures of cellular energy level drop after a fast (Hems & Brosnan, 1970).

The situation in the chicken kidney, Table 5, is slightly different from that in the chicken liver. The ATP:ADP ratio is lower in kidney than in liver although a higher ratio, (0.70), was found than was reported by Craan et al. (1982), (0.44). Their measurements in an unventilated chicken are presumed to be in an hypoxic tissue as the lactate, was also high (2.4 micromoles/g vs. the 1.1 micromoles/g which was measured in the kidney of chickens under artificial respiration). While a significant drop in the total adenylate concentration was found in the chicken kidney during starvation, the fall is proportional for ATP, ADP, and AMP so there is no change in the measures of the cell energy level - energy charge and phosphorylation potential.

TABLE 4. Hepatic Concentration of Adenine Nucleotides and Phosphate in Fed and Fasted Chickens.

	Fed	Fasted
Inorganic Phosphate	6.82 ± 1.06	* 3.12 ± 0.24
AMP	0.55 ± 0.02	* 0.43 ± 0.04
ADP	1.66 ± 0.12	* 1.21 ± 0.07
ATP	1.90 ± 0.07	* 1.67 ± 0.07
ATP/ADP	1.20 ± 0.07	* 1.41 ± 0.06
Total Adenylates	4.15 ± 0.17	* 3.37 ± 0.18
Phosphorylation potential	0.37 ± 0.04	0.45 ± 0.04
Energy Charge	0.66 ± 0.01	* 0.70 ± 0.01
Adenylate Kinase M.A.R.	0.45 ± 0.04	0.48 ± 0.03
(n)	(16)	(9)

Metabolite concentrations in chicken liver taken from fed or six-day fasted animals. Values quoted are the mean, in micromoles per gram of tissue (wet weight) ± S.E.M. Values in the same row marked with a "\*" are significantly different using a two-tailed T-test ( $p < 0.05$ ).

Table 5. Renal Concentrations of Adenine Nucleotides and Phosphate in Fed and Fasted Chickens.

	Fed		Fasted
Inorganic phosphate	22.0 ± 7.0	*	6.7 ± 1.7
AMP	0.73 ± 0.06	*	0.60 ± 0.02
ADP	2.14 ± 0.24	*	1.44 ± 0.14
ATP	1.83 ± 0.40	*	1.05 ± 0.10
ATP/ADP	0.70 ± 0.05		0.76 ± 0.08
Total Adenylates	4.71 ± 0.52	*	2.97 ± 0.24
Phosphorylation Potential	0.09 ± 0.03		0.14 ± 0.04
Energy Charge	0.60 ± 0.03		0.60 ± 0.02
Adenylate Kinase M.A.R.	0.28 ± 0.03		0.25 ± 0.03
(n)	(7)		(9)

Metabolite concentrations (in micromoles/g ± S.E.M.) in chicken kidney taken from fed or six-day fasted, artificially ventilated animals. Values quoted are the mean, in micromoles per gram of tissue (wet weight) ± S.E.M. Values in the same row marked with a "\*" are significantly different using a two-tailed T-test ( $p < 0.05$ ).



### 2.3.3.2 Metabolites Of Glycolysis

In table 6 are presented the glycolytic metabolite concentrations of freeze-clamped fed, and fasted, chicken's liver. For comparison the ratio of the content of each metabolite in fasted liver divided by its content in fed liver is presented for both the chicken and for the rat. (Ratio for the rat calculated from data presented in Hems & Brosnan; 1970).

The glucose concentration of liver is significantly reduced by fasting in the chicken. The glucose concentration of the liver of a fasted bird falls below the concentration of glucose in whole blood from the portal vein. The situation is similar in the rat.

Of the other metabolites listed in Table 6, only glycogen, fructose-6-phosphate, 2-phosphoglycerate, lactate and malate concentrations showed significant decreases in the fasted chicken, where they were found in lower concentration. In the fasted chicken's liver the cytosolic redox state was seen to become more oxidized, as is shown by the increase in the  $\text{NAD}^+/\text{NADH}$  ratio in the fasted bird. One major difference noted between rat (Hems & Brosnan, 1970) and chicken liver was the significant increase in the PEP concentration of the fowl's liver.

In the fasted chicken the situation in the kidney is different from the fasted liver (Table 6 & 7). Only four values change in the kidney after a fast. The level of 2-phosphoglycerate is lower in the fasted chicken kidney compared to the fed state and the glucose-6-phosphate, malate and 2-oxoglutarate concentrations are elevated. There is no difference between the  $NAD^+/NADH$  ratios for the fed and fasted chicken kidney ( $P > 0.05$ ). Further there is no difference among the means of the  $NAD^+/NADH$  ratios for the fed chicken liver, fed chicken kidney or the fasted chicken kidney (Newman-Keuls Multiple Range test,  $P > 0.05$ ; Steel & Torrie, 1960).

Table 6. Hepatic Concentrations of Metabolites in Fed and Fasted Chickens

FAST/FED	FED CONTROL	*	FASTED	FAST/FED	
				(CHICKEN)	(RAT)
GLYCOGEN	14.0 ± 2.6	*	3.0 ± 0.5	0.2	-
GLUCOSE	9.29 ± 0.60	*	4.23 ± 0.16	0.45	0.29
G6P	0.061 ± 0.010	*	0.045 ± 0.007	0.74	1.2
FGP	0.028 ± 0.003	*	0.017 ± 0.003	0.61	4
FBP	0.019 ± 0.004	*	0.020 ± 0.004	1.1	1
TRIOSE-P	0.025 ± 0.006	*	0.010 ± 0.003	0.4	0.77
3PG	0.052 ± 0.011	*	0.060 ± 0.020	1.2	1.0
2PG	0.024 ± 0.004	*	0.010 ± 0.003	0.42	0.82
PEP	0.037 ± 0.010	*	0.085 ± 0.009	2.3	1.1
PYRUVATE	0.100 ± 0.015	*	0.085 ± 0.020	0.77	0.65
LACTATE	0.860 ± 0.100	*	0.264 ± 0.100	0.30	0.69
AMMONIA	0.54 ± 0.06	*	0.52 ± 0.07	-	-
2-OXOGL	0.53 ± 0.07	*	0.39 ± 0.03	-	-
GLUTAMATE	5.9 ± 0.5	*	6.4 ± 0.5	-	-
MALATE	1.96 ± 0.27	*	0.86 ± 0.08	-	-
NAD <sup>+</sup> /NADP (cytosol)	1060 ± 160	*	2930 ± 830	4.5	0.9

Concentrations of metabolites in liver of artificially ventilated fed or six-day fasted chickens measured, in micromole per gram of tissue (wet weight) ± SEM, N=16 for the fed state and N=9 for the fasted state. The exception was for glycogen which is measured in mg per g ± S.E.M. for N=7 in both fed and fasted conditions. Values in the same row marked with an "\*" are significantly different using a two-tailed T-test ( $p < 0.05$ ).

Table 7. Renal Concentrations of Metabolites in Fed and Fasted Chickens

	FED		FASTED	
GLUCOSE	9.7	± 1.0	8.30	± 0.40
G6P	0.009	± 0.002	* 0.027	± 0.006
F6P	0.008	± 0.003	0.003	± 0.002
FBP	0.017	± 0.005	0.031	± 0.001
TRIOSE-P	0.015	± 0.004	0.006	± 0.004
3PG	0.048	± 0.012	0.019	± 0.003
2PG	0.029	± 0.006	* 0.004	± 0.001
PEP	0.033	± 0.009	0.024	± 0.002
PYRUVATE	0.088	± 0.018	0.064	± 0.013
LACTATE	1.06	± 0.13	1.34	± 0.13
AMMONIA	1.58	± 0.32	1.15	± 0.18
2-OXOGL	0.060	± 0.010	* 0.14	± 0.02
MALATE	0.21	± 0.03	* 0.38	± 0.07
NAD <sup>+</sup> /NADH (cyto)	960	± 280	480	± 120
(n)	(7)		(9)	

Concentrations of metabolites in kidney of ventilated chickens expressed in micromoles per gram of tissue (wet weight) ± SEM. Values in the same row marked with a "\*" are significantly different using a two-tailed T-test ( $p < 0.05$ ).

## 2.4 DISCUSSION

### 2.4.1 Hypoxia

Using the ventilation technique described in this thesis blood gases and pH can be maintained at physiological levels in birds whose abdominal cavities have been surgically opened. The bird's organs are therefore supplied with blood having the normal pH, oxygen tension and carbon dioxide tension up to the time of sampling. This avoids the period of hypoxia which is presumed to have occurred in previous studies of metabolite levels in birds.

Anesthesia lowers oxygen tension in chickens (Table 1) and is reported to lower pH and carbon dioxide tension as well (Besch et al., 1971). Artificial ventilation restores blood oxygen tension to the levels found in a conscious bird.

A major consequence of ischemia (and hence anoxia) in rat liver is the large (> 40%) and rapid (< 60 seconds) drop in ATP concentration and energy level (Hems & Brosnan, 1970). It appears most prior measurements of the ATP concentration of avian liver have suffered from the problem of an anoxic or hypoxic liver at the time of sampling. With the exception of Soling et al., 1973; Dickson & Langslow, 1978; Dickson et al., 1978; and Cramb et al., 1982 the reported hepatic ATP levels or ATP/ADP ratios in vivo for birds have been generally low.

Bannister & Cleland, 1977,	[ATP]= 0.99, ATP/ADP= 0.61;
Deaciuc & Ilonca, 1981,	[ATP]= 1.44, ATP/ADP= 0.61;
Dickson et al., 1978,	[ATP]= 2.46, ATP/ADP= 0.24;
Barratt et al., 1974,	[ATP]= 0.58, ATP/ADP= 0.34;
Locke et al., 1972,	- , ATP/ADP= 0.25;
Rinaudo et al., 1976,	[ATP]= 0.47, ATP/ADP= 0.42)

These latter reports contrast with the findings given in Table 2, of an ATP concentration equal to 1.88, and an ATP/ADP ratio equal to 1.37. Calculated levels of cellular energy measures (Energy Charge and Phosphorylation Potential) are also found to be higher in a ventilated chicken.

The total concentration of adenylates in the liver of the birds used in this study was, in general, lower than that reported by other investigators. Whether this reflects a difference due to strain, diet or analysis is unknown.

As is the case in the rat (Hems & Brosnan, 1970), the rise in the hepatic concentrations of glucose and the hexose phosphates during anoxia is probably a result of glycogen breakdown. It appears the activation of glycogen phosphorylase in the chicken is independent of a 'pull' from phosphofructokinase, as the hexose phosphates accumulate faster than phosphofructokinase can remove them. The rise in the concentrations of AMP and of inorganic phosphate (a substrate of glycogen phosphorylase) should result in an activation of glycogenolysis while the rise in AMP and fall in ATP should result in an activation of phosphofructokinase. The activation of phosphofructokinase

in turn results in an increase of fructose-1,6-bisphosphate, itself an activator of phosphofructokinase (Uyeda, 1979).

The rise in fructose-1,6-bisphosphate should augment glycolysis through a stimulation of chicken liver type M2 pyruvate kinase. Fructose-1,6-bisphosphate at the concentration found in avian liver is known to increase the M2 type (= K-Type) pyruvate kinase's activity by increasing the maximal velocity and lowering the  $K_{.5}$  for phosphoenolpyruvate ( $K_{.5}$  - concentration of substrate at which the velocity is half of  $V_{max}$ ) (Eigenbrodt & Schoner, 1977). That the reaction which is catalysed by pyruvate kinase has been activated in anoxia can be concluded from the fact it is far from equilibrium and yet flux through it increases in the face of decreased substrate levels. [That the reaction catalysed by pyruvate kinase is far from equilibrium can be concluded from the fact the reaction shows a mass action ratio of 0.44 calculated from data in Tables 2 and 3, while the equilibrium constant is 2000 (Rolleston & Newsholme, 1967)]

Another consequence of delay in obtaining a tissue sample from an unventilated bird after cervical dislocation and opening of the abdomen is the rapid change in concentrations of lactate and pyruvate due to hepatic hypoxia (Hems & Brosnan, 1970). The high levels of lactate found by Didier et al. (1981) and Kaminsky et al. (1982), 2.1 and 1.4 micromoles/g, in the livers of quail and pigeons

respectively; and in the liver of chickens by Brady et al. (1978), Bannister & Cleland (1977) and Rinaudo et al. (1976), 1.6, 2.6 and 3.2 micromoles/g, respectively, are likely due to the delay encountered between humanely killing the bird and freeze-clamping a liver sample. Employing the techniques described in this paper it was found that the hepatic lactate levels in chickens were much lower (0.86 micromoles/g) than values reported for non-ventilated birds. In addition, it was found in the ventilated birds that the  $NAD^+/NADH$  ratio in the cytosol calculated from the lactate:pyruvate ratio is much higher than has been previously assumed. This has implications in considering regulation of hepatic gluconeogenesis (see section 4.1).



#### 2.4.2 Fasting

As has been pointed out, one major result of ischemia in liver is the displacement of the phosphorylated adenylates from their in vivo concentrations. In most previous biochemical investigations artificial respiration was not used and it seems likely that the organs of the birds under study were not provided with an adequate supply of oxygen in the period immediately prior to sampling. The shift in the levels of adenylates due to ischemia may have been responsible for masking the increases which are observed in the hepatic ATP/ADP ratio and energy charge after a fast (Table 4).

O'Niell & Langslow (1976, 1978) report a small but significant drop in hepatic hexokinase activity in the chicken and a doubling of glucose-6-phosphatase activity caused by fasting. These authors conclude glucose flux into and out of chicken liver cells is regulated by the activity of glucose-6-phosphatase and the provision of glucose-6-phosphate. This view may have to be re-evaluated in light of the fact that liver glucose decreases markedly in the liver of a chicken fasted for six days. This decrease was similar to the change noted by Hems & Brosnan (1970) in 48 hour fasted rats. As this decrease in liver glucose has important implications for glucose transport from liver to plasma, a second study of the levels of liver glucose in fed, 3-day fasted and 6-day fasted chickens was

carried out. The values observed were  $9.3 \pm 0.6$ ;  $7.6 \pm 0.3$ ; and  $4.9 \pm 0.3$  micromoles per gram respectively (Mean  $\pm$  S.E.M., n=15). This linear decrease in hepatic glucose content over time had a correlation coefficient of 0.7 and an 'F' value of 43. At the same time that levels of glucose in the liver were falling, plasma glucose levels remained at a constant 12 mM. This means if fasted liver was exporting glucose, then it was doing so against a concentration gradient which became steeper the longer the fast continued. This has important implications in that it suggests glucose transport from liver to plasma may be an energy consuming process and conventional ideas about regulation of glucose release/uptake at the hexokinase/glucose-6-phosphatase step may have to be rethought.

There is an increase in the hepatic concentration of PEP with fasting (In fact PEP is the only intermediate measured which increased). This suggests that PEP was not being used as a substrate for gluconeogenesis due to a lack of cytosolic reducing equivalents (as appears to be the case according to the lactate:pyruvate ratio). It could also mean that pyruvate kinase activity was low and PEP concentration built up in the cytosol.

It is also possible that the increase in PEP is due to an increase in the intramitochondrial concentration of PEP without a simultaneous rise in cytosolic PEP. This may imply that the activity of the PEP transporter of the mitochondrion is reduced.

In the liver and kidney of a fasted chicken, there is a fall in the concentrations of inorganic phosphate, AMP and ADP. These compounds are activators of mammalian liver phosphofructokinase. There is also a rise in the hepatic concentration of PEP, a feedback inhibitor of mammalian phosphofructokinase (Uyeda, 1979). This may result in a lower activity of phosphofructokinase and hence a stimulation of gluconeogenesis in the liver and kidney. In addition, the fall during a fast of hepatic and renal AMP, a powerful inhibitor of fructose-1,6-bisphosphatase (Gevers & Krebs, 1966), may further stimulate gluconeogenesis.

The major regulator of phosphofructokinase in mammals appears to be fructose-2,6-bisphosphate (Pilkis et al., 1981; Van Schaftingen et al., 1980; Furuya & Uyeda, 1980). As the tissue concentration of this compound was not measured in the chicken, it cannot be said what effect changes in its level might have had on phosphofructokinase activity.

#### 2.4.3 Effect Of Redox State On Gluconeogenesis

Employing artificial respiration, the hepatic lactate levels in chickens were found to be much lower (0.86) than values reported for non-ventilated birds. It was found in the ventilated birds that the  $\text{NAD}^+/\text{NADH}$  ratio calculated from the lactate:pyruvate ratio in the cytosol was higher than has been previously reported. (Bannister & Cleland, 1977; Deaciuc & Ilonca, 1981; Dickson et al., 1978; Barratt et al., 1974; Locke et al., 1972; Rinaudo et al., 1976).

Unlike the findings of Brady et al. (1978) in chickens and Kaminsky et al., (1982) in pigeons, it was found that the cytosolic  $\text{NAD}^+/\text{NADH}$  ratio of liver increased with fasting, indicating a shift towards a more oxidized NADH system in the cytosol, which is consistent with the observed shift in the blood lactate:pyruvate ratio towards a more oxidized state. (Belo et al., 1976a; Davison & Langslow, 1975; Brady et al., 1978). This implies that the liver of a fasted bird may be dependent upon the availability of reduced substrates for glucose synthesis.

In the kidney however, there was no change in the  $\text{NAD}^+/\text{NADH}$  ratio of the cytosol after a fast. If the availability of reducing equivalents in the cytosol limits gluconeogenesis, then it would be expected that gluconeogenesis from pyruvate in the kidney of a fasted

chicken would be favored over gluconeogenesis from pyruvate in the liver because of the smaller  $NAD^+/NADH$  ratio in the cytosol of the kidney.

In summary this chapter describes a technique for maintaining blood gases at physiological levels in birds throughout a surgical procedure involving an open abdominal cavity. Using this technique it has been shown levels of adenine nucleotides in chicken liver are comparable to those measured in mammals. This latter is in contrast to previous reports, which are believed to have suffered from the problem of low oxygen tension in the avian liver. The same is true for the lower lactate:pyruvate ratios which are reported in liver from a ventilated chicken. The changes reported in metabolite concentrations in ischemic liver are consistent with an acceleration of glycolysis, as a result of glycogen breakdown.

In addition, hepatic levels of metabolites in fasted chickens, in vivo, suggest that gluconeogenesis from amino acids and pyruvate may not be a favored process in the liver. In contrast, metabolite levels in the fasted chicken kidney in vivo, suggest there is no limitation of cytosolic reducing equivalents and therefore no limitation from this factor on gluconeogenesis from amino acids and pyruvate.

CHAPTER 3

ARTERIO-VEINUS DIFFERENCE MEASUREMENTS IN WHOLE BLOOD AND PLASMA.

### 3.1 INTRODUCTION

Interest in gluconeogenesis from amino acids in chickens has led a number of investigators to examine circulating levels of amino acids in that species. Studies have focused on levels of amino acids as they are affected by diet (Bell et al., 1959; Olsen et al., 1959; Gray et al., 1960; Zimmerman & Scott, 1965; Richardson et al., 1965; Kelly & Scott, 1968; Tasaki & Ohno, 1971; Ohno, 1980; Larpier et al., 1982); as they are influenced by fasting or feeding a non-protein diet (Hill & Olsen, 1963; Zimmerman & Scott, 1967; Boomgaardt & McDonald, 1969; Belo et al., 1976a; Brady et al., 1978) and as they are affected by egg formation in the hen (Taylor et al., 1970).

The number of conclusions that can be drawn from such measurements of metabolites or amino acids in plasma is limited. If a rise in the plasma concentration of a substance is observed, it can be presumed either that production of the substance is increased, its utilization or excretion is diminished, or that it is released from blood cells. The opposite presumptions are made if the concentration of a substance falls in plasma. Johnson & Anderson (1982) have made an attempt to define the relationship between the amino acid composition of the diet and amino acid concentrations in the plasma of the rat but Boomgaardt & McDonald (1969) have shown that there are considerable differences between the chicken and either the

rat or pig in the fasting plasma concentrations of amino acids. The levels of amino acids were seen to be much more variable during a period of fasting in the chicken than in the latter animals. It would seem that in this, as in many other areas of metabolism, the chicken differs significantly from the mammalian "norm".

Another area which has drawn the interest of investigators lies in the distribution of amino acids and metabolites between blood cells and plasma. It has been found in many mammals that the erythrocytes contain little or no glucose (Olmstead, 1935a, 1935b). The same situation was found to exist in pigeons (Vinokurov, 1928; Andreen-Svedberg, 1933) and the domestic fowl (Bendella, 1943; Tapper & Kare, 1956, 1960; Bell, 1956, 1957; Houska, 1969)<sup>6</sup>.

It has also been recognized for some time that amino nitrogen is present in much higher concentration in tissues than in plasma (Van Slyke & Meyer, 1913). It was not until many years later however that the development of new techniques allowed investigators to demonstrate differences in concentration between plasma and blood cells of individual amino acids (Christensen et al., 1947; Johnson &

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6 Consequently it is important to measure glucose in plasma rather than blood to prevent possible errors resulting from age and sex dependent hematocrit changes.



Bergein, 1951; McMenamy et al., 1960). . . Several investigators since then have shown that this difference is true for such a variety of animals as rats (Aoki et al., 1972, 1973; Felig et al., 1973; Soley et al., 1982), dogs (Drewes et al., 1977), sheep, (Heitman & Bergman, 1980), calves (McCormick & Webb, 1982), humans (Hagenfeldt & Arvidson, 1980) and chickens (Bell et al., 1959; Stephens & Evans, 1971). The difference between cells and plasma in the levels of amino acids led Elwyn et al. (1968, 1972), who were working with dogs, to suggest different roles for plasma and erythrocytes in inter-organ transport; that is that plasma carried free amino acids from non-hepatic tissues to the liver, and protein from the liver to the periphery, while free amino acids were carried from the liver to the periphery by erythrocytes.

There is another approach using measurements of blood metabolites to study amino acid metabolism in vivo, that is capable of providing more information than the studies cited above. This involves sampling blood from the afferent vessel(s) and the efferent vessel(s) of an organ and determining if metabolites have been released into, or taken up from the perfusing blood. This is known as an arterio-venous difference. When coupled with measurements of blood flow through the organ, the flux of the substance may also be determined.

The pioneering work in amino acid arterio-venous difference measurements was performed in rats by Flock & Bollman (1962) and has been extended to dogs (Fukuda & Koppie, 1980), sheep (Wolfe et al., 1972), and man (Marliss et al., 1971). Although Knapp (1936) measured glucose arterio-venous differences across the wing of the chicken, there has, as yet, been no report of amino acid arterio-venous difference measurements in birds in vivo. This is most likely due to the difficulties in sampling from abdominal vessels without causing the bird to become anoxic (See Chapter One). The refinement of the technique described in chapter one of this thesis to maintain blood gases of abdominally opened chickens at physiological levels, has allowed the measurement of arterio-venous differences in chicken across liver, muscle and kidney. The results of these measurements are reported and discussed in this chapter.

## 3.2 METHODS AND MATERIALS

### 3.2.1 Experimental Animals

The experimental animals and their care and handling<sup>11</sup> were as described in Section 2.2.1.

### 3.2.2 Analysis

Amino acids were determined on a Beckman model 121M Amino Acid Analyser and Beckman Systems AA computing Integrator for Amino Acid Analyser using a five-buffer single-column method as described by Lee (1974) and modified in Beckman Bulletin # 121M TB-013 (July 1976).

Uric acid was determined using a uricase method as described in Sigma Technical Bulletin #292-UV with reagents obtained in Kit form from Sigma Chemical, St. Louis, Mo. The method uses 0.05 ml of porcine liver uricase (0.3 units/ml) to convert the uric acid (which has a high absorbance at 292 nm) in a sample to allantoin (which has a much lower absorbance at 292 nm). The decrease in absorbance at 292 nm is proportional to the uric acid concentration.

Glutathione levels were determined (in fasted birds only) from a perchloric acid-deproteinized, whole blood extract neutralized immediately before analysis by the method of Akerboom & Sies (1981). Reduced glutathione 'GSH' was determined by measuring the change in absorbance at 240 nm (after the addition of 0.02 ml of a 250 unit/ml solution of yeast glyoxalase (Sigma)) of a pH 7.0 reaction mixture containing 0.1 M phosphate, 1 mM ethylenediamine tetraacetic acid, 5 mM methyl glyoxal and a known volume of sample using the extinction coefficient of 3.37 mM<sup>-1</sup>. Oxidized glutathione, 'GSSG', (glutathione disulfide) was determined in the same cuvette after the completion of the GSH determination by adding 0.01 ml of 10 mM NADPH and 0.01 ml of a 10 units/ml solution of yeast glutathione reductase, and recording the change in absorbance at 340 nm.

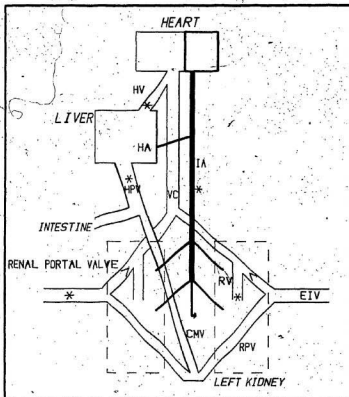
### 3.2.3 Blood Sampling

For the blood metabolite and amino acid determinations, five 2 ml blood samples were taken in ventilated birds using heparinized syringes with a one-inch, 23 gauge needle, from the left hepatic vein (1 cm before it enters the caudal vena cava), the right portal vein (1 cm before it enters the margin of the liver), the right external iliac vein (1 cm before it gives off the caudal renal portal vein), the left caudal renal vein (1 cm before it enters the common iliac vein) and the inferior aorta at the level where the femoral arteries are given off<sup>7</sup>. The vessels were sampled in that order, over a time-period of five minutes. (See Figure 1 for detailed anatomy, Figure 2, for a schematic representation).

This order of sampling was chosen for the following reasons. The left hepatic vein is the vessel of choice for sampling efferent hepatic blood, as the right hepatic veins drain into the posterior vena cava within the margin of the liver where hepatic blood mixes with blood from the vena

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7 It is wise to consult an authoritative anatomy text such as that by Baumel et al., (1979) for the names of vessels. One popular text (McLeod et al., 1964) appears to have confused the positions of the caudal renal portal and caudal renal vein.



HV - HEPATIC VEIN	IA - INFERIOR AORTA
HPV - HEPATIC PORTAL VEIN	RV - RENAL VEIN
HA - HEPATIC ARTERY	RPV - RENAL PORTAL VEIN
VC - VENA CAVA	EIV - EXTERNAL ILIAC VEIN
CMV - COXYCYMESENTERIC VEIN	* - BLOOD SAMPLING SITE

FIGURE 2

SCHEMATIC OF BLOOD VESSELS AND SAMPLING SITES

cava. The left hepatic vein however, leaves the liver as a distinct vessel and therefore contains only efferent hepatic blood. It was decided to first sample the efferent blood from the liver to avoid any effect on venous outflow which might be caused by sampling the hepatic portal vein first. The right hepatic portal vein, sampled second, was chosen because it is larger and more readily accessible than the left hepatic portal vein.

It was decided to sample the inferior aorta blood last to minimize blood loss. Withdrawal of the sampling needle results in considerable hemorrhaging from this vessel. Contralateral external iliac and renal veins were chosen (rather than ipsilateral) to avoid the effects which sampling from both vessels on the same side might have on blood flow or metabolism in the affected kidney. (As blood from the external iliac vein on one side perfuses the kidney via the renal portal veins on the same side and leaves via the renal vein, it is possible that sampling from, say the left external iliac might interrupt flow to the left kidney and the next sample if taken from the left renal vein may not be representative of the blood that might otherwise been

in that vein had the left external iliac vein not been sampled just before.<sup>8</sup>

Following removal of blood samples the animal was humanely killed by an intra-cardiac overdose of sodium pentobarbital.

Each of the five blood samples was divided into one 1.0 ml portion and two 0.5 ml portions for analysis.

1.) For plasma urate, glucose and amino acids the 1.0 ml subsample was placed in a 1.5 ml Eppendorf centrifuge tube and centrifuged for ten minutes at full speed in an Eppendorf centrifuge to obtain plasma. Half of this plasma was retained for the analysis of urate and glucose. The remainder was deproteinized by adding it to 1 ml of cold 10% sulfosalicylic acid which was then vortexed and centrifuged at 15,000 rpm in a Sorvall RC-5B centrifuge for 20 minutes. The supernatant was collected and its pH was adjusted to 2.2 with 3 N lithium hydroxide. An amount of 0.15 N lithium citrate equal to half the volume of the neutralized supernatant was added. The sample was then stored frozen

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<sup>8</sup> Although Odland (1978) reports differences in blood flow between left and right kidneys in hens, Boelkins et al., (1973) in hens and Wolfenson et al., (1978) in males report no significant differences between blood flow to left and right kidneys.



before analysis for amino acids.

2.) For whole blood amino acids, a 0.5 ml blood subsample was added to a ten ml centrifuge tube containing 1 ml of cold 10% sulfosalicylic acid. This was vortexed, and centrifuged at 15,000 rpm in a Sorvall RC-5B centrifuge for 20 minutes. The supernatant was collected and its pH was adjusted to 2.2 with 3 N lithium hydroxide. An amount of 0.15 N lithium citrate equal to half the volume of the neutralized supernatant was added. The sample was then stored frozen before analysis for amino acids.

3.) For whole blood glucose and lactate the last 0.5 ml of the blood sample was added to a ten ml centrifuge tube containing 1 ml of cold 6% perchloric acid. This was then vortexed and centrifuged as above. The supernatant pH was adjusted to pH 7.0 with potassium hydroxide to precipitate potassium perchlorate and centrifuged in a benchtop centrifuge at 5000 rpm. This second supernatant was analysed for lactate concentration.

#### 3.2.4 Hepatic Blood Flow Determination

A preliminary investigation was performed to determine total hepatic blood flow in fed and fasted chickens. Anesthetized, abdominally opened, artificially ventilated, chickens (800 g males), were restrained on their backs and cannulae inserted in left and right brachial veins. The

blood flow determination was carried out by injecting a "loading" dose of 0.5 mg of bromosulphothalein per kg body weight into one vein and, following this dose, infusing bromosulphothalein at a rate of 4.5 mg/hr/kg body weight using a Braun infusion pump (Quigley-Rochester Inc., Rochester, N.Y.). Blood samples (0.5 ml) were withdrawn from the contralateral brachial vein at 10 min intervals and bromosulphothalein concentration in plasma was determined colorimetrically after alkalinization. When the rate of increase of bromosulphothalein in the plasma became constant, blood samples (0.5 ml) were taken from the left hepatic vein, the right hepatic portal vein and the inferior aorta. Bromosulphothalein concentration was determined in plasma. Flow rates of blood were calculated by subtracting the rate of increase of bromosulphothalein in plasma from the infusion rate to determine the hepatic extraction rate and arithmetically arriving at the flow rate of whole blood from knowledge of the afferent-efferent difference across liver (assuming a 30% arterial component, and a cell volume of 40%). The method described above was adapted from methods described by Bradley et al., (1945) and Ossenberg et al., (1974).

### 3.2.5 Calculations

Plasma and whole blood arteriovenous differences for muscle were calculated by subtracting the concentration of the metabolite in the external iliac vein from the concentration in the inferior aorta. For reasons which will be outlined in Section 3.4.1 the plasma and whole blood afferent-efferent differences for the liver were calculated by assuming that the arterial blood perfusing the liver in vivo comprises either 30% or 40% of the total blood perfusing the liver, with blood from the hepatic portal veins supplying the balance. (See discussion in Section 3.4.1)

The plasma and whole blood afferent-efferent differences for the kidney were calculated, for reasons which will be outlined in Section 3.4.1, assuming that the arterial component of renal flow is 50%, 75% or 100% of total renal flow, with the renal portal veins supplying the balance. (See discussion in Section 3.4.1). It was assumed that levels of metabolites in the caudal renal portal vein were equal to the concentrations in the external iliac vein and that blood in the caudal renal portal vein flows into the kidney. Other assumptions that were necessary for these calculations were that the concentration of metabolites in all arteries were equal to those in the inferior aorta. It was also assumed that blood in the hepatic portal vein flows towards the liver and that concentrations of metabolites

were equal in both left and right portal veins. Concentrations of metabolites in all efferent hepatic veins were assumed to be equal to those in the left hepatic vein. Lastly it was assumed that the same patterns of flow exist in both fed and fasted chickens.

Levels of metabolites in the cell and cellular arteriovenous differences were calculated from the concentrations of the metabolite measured in plasma and in whole blood assuming a cell volume of 40% and an interstitial volume of zero.

Forty percent was the average value for the packed cell volume in the cockerels used in this study and is similar to data published by Newell & Shaffner (1950) for cockerels of this age. The small change in red cell volume which takes place in response to different  $pCO_2$  levels in arterial and venous blood, the Hamburger shift (1891), was ignored.

There was no difference between the hematocrits of the five blood samples ( $P > 0.05$ , Neuman-Keuls multiple range test), that is the blood sampling had no significant effect on the packed cell volume. In a separate experiment on similarly treated birds ( Appendix B ), 2 ml. blood samples were drawn from the inferior aorta through an indwelling cannula at two minute intervals for 12 minutes. There was no significant effect of sampling on hematocrit or on plasma glucose in the arterial blood. Further, there was no

significant effect of six days of fasting on blood hematocrit.

The assumption that a hematocrit of 40% is a cell volume of 40% may be an overestimate of the cell volume as Hunsaker (1969) has shown that in human blood, as much as 8% and at least 2.5% of the total plasma is trapped in the red cell mass, depending on the centrifugal force and time of the centrifugation to separate cells and plasma.

Fractional extraction or release for each metabolite was defined as the difference between the afferent and efferent concentration divided by the afferent concentration.

### 3.3 RESULTS

#### 3.3.1 Body And Organ Weight Data.

In table 8, which appears below, the mass of some of the major organs in the fed eight-week-old cockerel are presented.

Table 8. Summary data on organ weights in fed 8-week-old cocks.

Organ	Weight (Mean $\pm$ SEM, n=3)
Whole Animal	770 $\pm$ 36 (g)
Liver + gallbladder	3.48 $\pm$ 0.19
Kidneys	1.05 $\pm$ 0.01
Heart	0.58 $\pm$ 0.01
Proventriculus	0.54 $\pm$ 0.02
Gizzard	2.88 $\pm$ 0.11
Duodenum	0.98 $\pm$ 0.08
Pancreas	0.33 $\pm$ 0.02
(Ileum + jejunum + colon)	2.29 $\pm$ 0.11
Caeca	0.40 $\pm$ 0.04
Testes	0.03 $\pm$ 0.01

Values are in g/100g body weight except for the whole animal body weight in grams.

#### Effect of fasting on relative liver mass Mean $\pm$ SEM (N=6)

Days Fasted	Weight (g/100g body weight)	Dry Weight (g/g tissue wet weight)
0	3.08 $\pm$ 0.21	0.27 $\pm$ 0.01
1	2.26 $\pm$ 0.26	0.28 $\pm$ 0.01
3	1.92 $\pm$ 0.17	0.29 $\pm$ 0.01
5	1.78 $\pm$ 0.15	0.30 $\pm$ 0.01
7	1.62 $\pm$ 0.12	0.28 $\pm$ 0.01

### 3.3.2 Blood Flow

Determination of total hepatic blood flow, using a bromosulphophthalein method, indicated that hepatic blood flow rate decreased in the fasting chicken. A concurrent decrease in the relative size of the liver meant that the flow rate per gram of liver tissue remained the same. The results of the blood flow determination are presented and compared with arterial and hepatic portal flow rates determined by others in Table 9.

Table 9. Total Hepatic Blood Flow, and Portal and Arterial Blood Flow in the Fowl Source

Source		(ml/min/kg body weight)	Blood Flow (ml/min/g tissue)	% of Total
A	Total FED (N=2)	59.0 + 0.93	1.92 + 0.03	100%
	Liver FAST (N=4)	36.0 ± 6.06	1.92 ± 0.53	100%
B	Portal FED	14.8 + 1.15	-	25%
	Vein FAST 24 Hr	14.2 ± 1.51	-	no data
C	Arterial FED	-	1.08 + 0.14	56%
D	Arterial FED	-	0.77 ± 0.09	40%
E	Arterial FED	-	0.56 ± 0.05	29%

Legend

Blood flows determined for liver of the domestic fowl are presented as the mean ± SEM in ml/min/kg or ml/min/g of liver tissue wet weight. Results are presented for the investigations performed in this thesis and from previously published results. The key is presented below.

- A This Thesis. 800 g cockerels. Total flow determined with bromosulphothalein. Fed or fasted 72 hours.
- B Sturkie & Abati (1975). 2.2 kg cocks. Hepatic portal venous flow determined with electromagnetic flow meter.
- C Wolfenson et al. (1978). Adult laying hens. Hepatic Arterial flow determined with radioactive microspheres.
- D Sapirstein & Hartman (1959). Adult laying hens. Hepatic Arterial flow determined by indicator dilution.
- E Boelkins et al. (1973). Adult laying hens. Hepatic Arterial flow determined by indicator dilution.



### 3.3.3 Levels Of Amino Acids In The Circulation

#### 3.3.3.1 Comparison Of Plasma Amino Acids With Previously Published Reports.

Table 10. compares the levels of amino acids found in the arterial plasma of fed and fasted cockerels to the levels reported in chicken plasma by other investigators. With the single exception of arginine, which was higher, the level of each amino acid in the fed state was within the range reported by other investigators. In the fasted state as well, the level of each amino acid was within the range reported for it by others, with the exceptions of alanine and lysine. These two amino acids were each substantially lower than the values reported by others.

Table 10. Amino acid content (mg/100ml) of fowl plasma reported by various investigators. (Legend on following page) "Fed"

	A (0)	B (0)	C (0)	D (8)	E (3)	F (0)	G (0)	H (0)	I (0)	J (0)	K (0)
THR	5.6	5.6	2.6	7.4	12.1	19.6	26.1		3.9	6.4	++
SER	6.4	7.4				10.5	9.7	3.1	12.3		++
GLU	8.2	4.8	3.0	1.9	4.0	5.4	9.4	7.0	3.8		++
GLN	9.5		15.7								-
PRO	4.3			4.3	3.0	12.6			1.0		++
GLY	3.6	4.5	7.7	4.0	4.1	2.1	4.6	4.0	2.8	5.0	++
ALA	4.9	4.5		4.3		4.5	5.9	2.3	3.4		++
VAL	4.9	2.2	3.0	1.8	2.8	6.2	4.5		1.9	5.6	+
MET	1.2		5.1	0.4	0.8	1.6	1.4		0.6	2.5	+
ILE	2.0	1.1	2.2	1.2	1.5	3.8	2.3		1.2	2.4	
LEU	4.5	2.8	3.2	2.3	2.2	5.3	3.1		2.0	3.6	
TYR	3.0		4.5	1.8	1.4	6.4	5.9		1.9	2.4	-
PHE	2.0	2.0	2.8	1.2	1.2	2.0	2.2		1.0	2.5	
TRP	1.2		1.8			1.6	1.3			1.9	-
ORN	0.4			0.6			1.0				+
LYS	7.2	2.8	4.9	2.7	5.2	13.1	20.5			4.4	+
HIS	2.2		2.6	1.7	0.9	1.8	3.8			3.0	trace
CYS	2.3				3.8	1.2	2.6				+
ARG	9.8	3.6	7.3	5.9	2.2	2.6	2.2			9.4	++
ASN	1.8	0.6									

"Fasted"

	A (144)	C (48)	D (36)	E (24)	G (24)	H (72)	J (24)	L (192)
THR	10.6	11.1	11.1	11.4	14.7		10.4	35.5 54.3
SER	5.0		8.3		8.3	5.3		24.5 19.7
GLU	1.4	2.8	2.0	4.4	7.6	4.5		
GLN	7.1	17.2						
PRO	2.2		2.2	2.9			6.2	6.2
GLY	3.5	6.1	3.5	4.4	4.9	2.3	4.5	9.1 10.5
ALA	2.6		3.6		4.8	3.5		12.9 10.9
VAL	4.5	5.2	2.5	4.3	4.2		4.6	5.8 7.0
MET	1.1	1.6	0.8	1.5	1.2		1.5	1.3 2.2
ILE	2.5	3.4	2.0	2.5	3.1		2.6	4.7 3.4
LEU	3.6	5.1	2.7	4.3	3.8		4.3	5.3 6.2
TYR	2.0	2.2	2.1	3.1	2.9		2.4	4.1 3.5
PHE	1.7	2.0	1.0	1.8	2.0		1.8	2.9 3.2
TRP	1.6	1.4			1.1		1.3	
LYS	6.2	24.6	12.5	13.1	22.3		11.2	52.0 36.6
HIS	1.8	2.4	1.2	1.8	3.9		1.5	4.9 3.7
CYS	2.0				4.3	1.1		0.6 0.9
ARG	5.2	2.9	3.3	2.6	3.2		4.4	8.2 6.8
ORN	0.8		0.5		1.4			

Legend For Table 10:

The figure in parentheses at the head of each column designates the period of ~~fasting~~ fasting in hours. Underlined figures in column "A" (Fasted) are significantly different from fed levels. The letter at the head of each column indicates the source of the data. The key is given below.

- A This thesis. 8 week-old, 800g cocks fed 20% protein. Arterial plasma deproteinized with sulphosalicylic acid.
- B Larbier et al., 1982; 320g, 3 week-old broiler cocks, fed 21% protein. Deproteinized with picric acid.
- C Hill & Olsen, 1963; 3 week-old White Plymouth Rock female chicks, fed 15% soya protein or fasted 48 hours. Plasma deproteinized with tungstic acid.
- D Boomgaardt & McDonald, 1969; 6 week-old male chicks, fed 18% protein then fasted 8 hours or 36 hours. Plasma deproteinized with sulfosalicylic acid.
- E Zimmerman & Scott, 1967, crossbred chicks (New Hampshire males x Columbian females), no age or weight given, fed complete amino acid mixture diet then fasted 3 hours or 24 hours. Plasma deproteinized with picric acid.
- F Tasaki & Ohno, 1971, 5 month-old, 1.6kg White Leghorn cocks, fed 21% protein (casein). Deproteinized with picric acid.
- G Stephens & Evans, 1971, 4 week-old chicks, fed, 20% protein (casein) or fasted 24 hours. Deproteinized with sulfosalicylic acid. Amides hydrolyzed to free amino acids.
- H Belo et al., 1976a, 4kg "crossbred" cocks, fed commercial stock diet (soya) or fasted 72 hours. Plasma deproteinized using column chromatography.
- I Richardson et al., 1965, 6.5 week-old chicks, fed 15% protein (soya) Plasma deproteinized with picric acid.
- J Gray et al., 1960, 4 week-old Barred Plymouth Rock cocks. Fed 24% protein (wheat + sunflower meal). Deproteinized with tungstic acid.
- K Bell et al., 1959, Brown Leghorn Adult Cocks, fasted 36 hr. Plasma deproteinized with trichloroacetic acid.
- L Brady et al., 1978, 1.2kg broiler chicks, fed high carbohydrate diet then fasted 1 day or 8 days. Deproteinized with sulfosalicylic acid.

### 3.3.3.2 Effect Of Fasting On Amino Acids In Arterial Blood, Plasma And Cells

This section will discuss the data presented in Table 11. The concentrations of amino acids and metabolites in the four other abdominal vessels sampled in this study are presented in Appendix A.

#### Threonine and Serine

Threonine content of whole blood, plasma and cells increased ( $P < 0.05$ ) after a fast. There was little change in the blood level of serine on fasting.

#### Glutamate

Next to taurine, glutamate was the amino acid found in highest concentration in whole blood of fed chickens.

There was a fall of nearly 40% ( $P < 0.05$ ) in the whole blood level of glutamate on fasting. Most of the change appears to have resulted from the fall in the plasma concentration of glutamate as the cellular levels changed little.

Table 11. Arterial Levels of Amino Acids and Metabolites in Chickens

AA	FED		CELL	FASTED		
	BLOOD	PLASMA		BLOOD	PLASMA	CELL
TAUR	3550 ± 98	193 ± 43	8590 ± 246	3850 ± 160	360 ± 78	9080 ± 409
HPRO	154 ± 18	166 ± 18	114 ± 14	*69 ± 6	*73 ± 34	63 ± 46
THR	329 ± 21	467 ± 27	123 ± 44	*634 ± 124	*893 ± 184	*245 ± 66
SER	581 ± 64	612 ± 62	534 ± 78	437 ± 31	472 ± 41	385 ± 41
ASN	68 ± 9	138 ± 15	UD	45 ± 4	*58 ± 9	24 ± 18
GLU	740 ± 47	555 ± 27	1020 ± 93	*384 ± 25	*98 ± 22	814 ± 63
GLN	444 ± 67	653 ± 96	131 ± 48	370 ± 31	486 ± 52	192 ± 65
PRO	214 ± 17	371 ± 37	UD	*149 ± 11	*174 ± 15	*111 ± 34
GLY	606 ± 33	486 ± 23	786 ± 71	538 ± 26	464 ± 38	650 ± 18
ALA	518 ± 40	545 ± 49	410 ± 29	*323 ± 14	*298 ± 22	360 ± 28
VAL	336 ± 30	472 ± 56	133 ± 39	519 ± 76	386 ± 64	*719 ± 98
CYS	98 ± 6	203 ± 15	UD	*53 ± 2	161 ± 26	UD
MET	14 ± 9	77 ± 5	UD	*67 ± 7	76 ± 13	*53 ± 5
ILE	138 ± 18	188 ± 34	62 ± 9	173 ± 24	191 ± 25	*145 ± 31
LEU	244 ± 28	343 ± 47	96 ± 11	236 ± 28	275 ± 29	*177 ± 31
TYR	148 ± 12	164 ± 12	125 ± 25	*106 ± 6	*108 ± 11	103 ± 16
PHE	93 ± 5	124 ± 6	46 ± 11	-84 ± 10	105 ± 12	53 ± 12
BALA	403 ± 22	UD	1010 ± 56	361 ± 33	UD	902 ± 83
TRP	29 ± 2	60 ± 4	UD	31 ± 4	79 ± 9	UD
ORN	21 ± 4	33 ± 7	3 ± 5	*49 ± 10	59 ± 16	34 ± 16
LYS	312 ± 68	529 ± 140	UD	239 ± 30	340 ± 39	*48 ± 17
HIS	65 ± 5	105 ± 8	4 ± 10	60 ± 7	85 ± 11	22 ± 8
3MH	15 ± 2	24 ± 3	2 ± 1	*48 ± 8	*79 ± 14	UD
ANS	596 ± 71	UD	1330 ± 90	695 ± 70	UD	1740 ± 174
CARN	1640 ± 147	UD	4091 ± 369	1310 ± 245	UD	*2666 ± 155
ARG	258 ± 25	464 ± 44	UD	*158 ± 14	*245 ± 15	2 ± 1
GLUC	ND	13400 ± 430	-	ND	12500 ± 340	-
GLYC	12 ± 1	ND	-	*61 ± 17	ND	-
LACT	2340 ± 290	ND	-	2360 ± 170	ND	-
GSH	ND	ND	-	1222 ± 86	ND	-
GSSG	ND	ND	-	385 ± 58	-	-
URIC	ND	470 ± 41	-	ND	*621 ± 63	ND
NH3	235 ± 47	343 ± 63	UD	222 ± 6	*230 ± 33	*263 ± 63

The data are presented as the concentration of the amino acid in nanomoles per ml ± S.E.M. Values marked with an "\*" are significantly different from fed levels in that blood fraction. "ND" indicates the metabolite was not determined. "UD" denotes the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means the cellular content could not be calculated as the substance was not measured in both blood and plasma. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7. For amino acids of fasted animals N=5 and for glucose and uric acid, N=14, for glycerol, N=2, and for lactate, N=11.

### Glutamine and Asparagine

Only a slight (statistically insignificant) decrease was observed on fasting in the glutamine and asparagine concentrations in arterial whole blood. No significant change was seen in the plasma and cellular glutamine concentrations on fasting.

### Proline and Hydroxyproline

The significant drop in whole blood levels of proline was a result of the drop in plasma concentration as cellular levels were found to increase on fasting - from undetectable levels in the fed bird to just over 100 nmol/ml in the fasted bird. Hydroxyproline levels decreased significantly in whole blood, primarily as a result of the drop in plasma levels as cellular levels were statistically unchanged after a six-day fast.

### Glycine and Alanine

The amino acid glycine was found in the third highest concentration in whole blood of a fed chicken. Little change was seen in the circulating levels of this amino acid on fasting. Whole blood and plasma alanine however decreased ( $P < 0.01$ ) on fasting.

### Branched Chain Amino Acids

Whole blood and plasma branched chain amino acids changed little during a fast, though the cellular levels increased ( $P < 0.05$ ).

### Methionine and Cysteine

Whole blood methionine, while low in concentration in the fed chicken, increased slightly ( $P < 0.05$ ) on fasting. Whole blood cysteine on the other hand decreased. Cellular methionine also increased on fasting, indicating the change seen in whole blood methionine was a result of the change in the cellular content of this amino acid. There was no cysteine measured in the blood cells in either the fed or fasted state.

### Aromatic Amino Acids

Tryptophan and phenylalanine changed little in whole blood or plasma. No tryptophan was observed in cells. No change was seen in cellular phenylalanine in this study.

### Basic Amino Acids

Lysine and histidine changed little in whole blood after a fast, Cellular levels of these two amino acids were low. Lysine was seen in this study to increase in concentration with fasting. The difference noted between this thesis and reports in the literature is quite high

levels of lysine in the circulation after a fast may be explained by the observations of Wang & Nesheim, (1972) who showed that lysine degradation via hepatic lysine-ketoglutarate reductase shows strain differences. As is later seen in Table-12, the afferent-efferent difference for lysine across the liver of fasted chickens is positive and significant ( $P < 0.05$ ) suggesting that hepatic degradation is not impaired in this strain.

Whole blood and plasma arginine were seen to decrease on fasting ( $P < 0.01$ ). The fall seen in whole blood was a result of a decrease in the amount of arginine carried in plasma. Cellular levels in fasted birds, were negligible and unaffected by fasting.

These three basic amino acids are found in much greater concentration in plasma than in the cells. As these three amino acids often share a common transporter (Christensen & Antoniolli, 1969), their low concentration in cells may indicate a low activity of the transporter in the erythrocyte membrane or high red cell metabolism of these amino acids. Lerner et al. (1983) reported that in the 4 week-old chick, the lysine transporter activity at physiological concentrations of lysine is about half of glycine transport activity and only a quarter of leucine transport activity.



### 3-Methylhistidine

3-Methylhistidine levels increased ( $P < 0.01$ ) in whole blood and in plasma after a fast pointing to an increased breakdown of muscle protein (if excretion is not impaired). 3-Methylhistidine levels were negligible in cells.

### Anserine, Carnosine and Taurine

Anserine (Beta-alanyl-N-methyl-histidine) and carnosine (Beta-alanyl-histidine) were absent from plasma and highly concentrated in cells. Taurine while present in plasma, was very much more concentrated in the cells. There was little change in the concentration of these peptides and the amino acid, taurine, in the blood after a six-day fast, although cellular carnosine levels dropped slightly ( $P < 0.05$ ).

### Other Metabolites<sup>9</sup>

Ammonia distribution between cells and plasma in the

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9 Aspartate levels are not reported as the aspartate peak is masked by the glutathione peak in the amino acid chromatography. Alternate measurements of this amino acid in a preliminary study showed it to be of sufficiently low concentration in the circulation of the birds used in this investigation that it was not deemed justified to measure it in this study. The same was true for pyruvate levels in the circulation.

blood of the fowl was quite variable (see large standard error). This may indicate difficulties with the assay procedure, and the fact that the cellular concentration is calculated from the plasma and whole blood concentrations.

As noted by other investigators, plasma glucose levels remained constant during a fast (Hazelwood & Lorenz, 1959; Brady et al., 1978) as did blood lactate.

Plasma urate levels were seen to rise ( $P < 0.01$ ) after a fast. This is similar to the observations of Henry et al. (1934), Okumura & Tasaki (1969) and Homma & Sato, (1960) although, unlike the observations of Bell et al. (1959).

Glycerol levels were found to be low in the circulation in the fed chicken, and although the level increased significantly after six days of fasting, the amount of glycerol present in the blood, is still amongst the lowest of the metabolites measured.

### 3.3.4 Arterio-Venous Difference Measurements In Fed And Fasted Chickens

#### 3.3.4.1 Liver

There was a significant positive afferent-efferent difference across livers of fed birds for glutamate, asparagine, proline, leucine, isoleucine, phenylalanine, tryptophan, arginine, lactate and glucose.

The situation in fasted liver was different from the fed state. The number and amount of amino acids removed from whole blood increased. There were significant positive afferent-efferent differences for threonine, serine, glutamine, glycine, alanine, tyrosine, phenylalanine, ornithine, lysine, arginine, lactate, glucose and a negative arterio-venous difference for urate. With a few differences, a similar situation was seen in plasma, suggesting the extraction from whole blood was a result of the removal of the amino acid from the plasma compartment of blood.

The fractional extractions of amino acids and metabolites by liver in the fed bird ranged between ten and twenty percent. In most cases the fractional extraction of the amino acid by liver was greater after a six-day fast. This was true for serine, glutamine, alanine, phenylalanine, ammonia, threonine, glycine, tyrosine, ornithine, lysine and urate (Table 13).

Table 12. Significant Differences Across Liver in Fed and Fasted Chickens

AA	Arterial	FED		FASTED	
		BLOOD	PLASMA	BLOOD	PLASMA
TAUR	30%	(186 + 140)	-85 + 35	(229 + 258)	(-47 + 59)
	40%	(177 + 131)	-85 + 36	(194 + 263)	(-37 + 53)
HPRO	30%	(-18 + 12)	(3 + 18)	(15 + 7)	(-2 + 6)
	40%	(-19 + 12)	(1 + 18)	(14 + 6)	(-2 + 7)
THR	30%	(23 + 12)	55 + 12	75 + 14	(113 + 58)
	40%	(22 + 11)	55 + 11	75 + 14	(107 + 56)
SER	30%	(41 + 23)	89 + 23	112 + 10	154 + 34
	40%	(44 + 21)	93 + 20	111 + 14	155 + 37
ASN	30%	9 + 3	(9 + 21)	(16 + 8)	17 + 7
	40%	(7 + 3)	(21 + 9)	(15 + 8)	17 + 6
GLU	30%	76 + 26	108 + 8	(35 + 16)	24 + 3
	40%	75 + 24	107 + 9	(31 + 15)	21 + 3
GLN	30%	(6 + 28)	72 + 27	121 + 28	174 + 56
	40%	(6 + 27)	72 + 24	117 + 32	172 + 58
PRO	30%	21 + 5	50 + 19	(67 + 29)	32 + 12
	40%	19 + 4	49 + 18	(64 + 29)	(28 + 12)
GLY	30%	(64 + 27)	102 + 21	85 + 15	122 + 40
	40%	(59 + 25)	97 + 19	79 + 16	116 + 40
ALA	30%	(62 + 44)	161 + 43	128 + 17	169 + 36
	40%	(57 + 41)	153 + 40	122 + 19	162 + 38
VAL	30%	(22 + 10)	36 + 14	(93 + 44)	(34 + 16)
	40%	(22 + 10)	38 + 13	(87 + 41)	(32 + 15)
MET	30%	(-2 + 7)	22 + 5	(10 + 5)	21 + 8
	40%	(-2 + 6)	21 + 5	(9 + 4)	(19 + 9)
ILE	30%	15 + 4	25 + 9	(22 + 13)	22 + 7
	40%	14 + 4	24 + 8	(20 + 13)	(19 + 8)
LEU	30%	26 + 6	45 + 13	(33 + 16)	36 + 9
	40%	25 + 6	44 + 12	(32 + 16)	33 + 9
TYR	30%	(14 + 6)	28 + 6	28 + 7	37 + 8
	40%	(13 + 6)	28 + 5	27 + 7	36 + 9
PHE	30%	14 + 5	27 + 5	25 + 5	36 + 8
	40%	13 + 4	27 + 4	25 + 6	36 + 8
BALA	30%	(17 + 14)	UD	(6 + 61)	UD
	40%	(16 + 12)	UD	(1 + 7)	UD
TRP	30%	3 + 1	4 + 1	(-1 + 2)	(16 + 8)
	40%	3 + 1	(4 + 2)	(-1 + 2)	(16 + 8)
ORN	30%	(1 + 1)	(0 + 2)	12 + 5	(5 + 9)
	40%	(1 + 1)	(0 + 2)	12 + 5	(3 + 9)
LYS	30%	(33 + 15)	60 + 17	57 + 18	(64 + 32)
	40%	(31 + 14)	58 + 16	55 + 19	(57 + 31)

Table 12. (continued) Significant Differences Across Liver in Chickens

		FED		FASTED	
AA	Arterial	BLOOD	PLASMA	BLOOD	PLASMA
HIS	30%	(7 + 3)	16 + 2	(12 + 19)	(43 + 18)
	40%	(6 + 3)	15 + 2	(11 + 19)	(39 + 17)
CYS	30%	(19 + 8)	83 + 12	(6 + 5)	28 + 8
	40%	(18 + 8)	81 + 12	(6 + 5)	26 + 8
3MH	30%	(1 + 1)	(2 + 1)	(5 + 4)	(9 + 8)
	40%	(1 + 1)	(2 + 1)	(5 + 4)	(8 + 7)
ANS	30%	(9 + 19)	UD	(29 + 49)	UD
	40%	(10 + 18)	UD	(23 + 49)	UD
CARN	30%	(68 + 60)	UD	(111 + 121)	UD
	40%	(67 + 57)	UD	(102 + 120)	UD
ARG	30%	24 + 10	60 + 9	22 + 8	(20 + 13)
	40%	24 + 10	62 + 8	21 + 7	(17 + 13)
GLYC	30%	10 + 3	ND	17 + 3	ND
	40%	7 + 2	ND	15 + 3	ND
NH3	30%	(34 + 33)	103 + 21	(62 + 52)	(24 + 60)
	40%	(38 + 33)	103 + 25	(57 + 52)	(-21 + 58)
GSH	30%	ND	ND	(-28 + 165)	ND
	40%	ND	ND	(-55 + 232)	ND
GSSG	30%	ND	ND	(15 + 16)	ND
	40%	ND	ND	(30 + 21)	ND
GLUC	30%	ND	876 + 290	ND	555 + 148
	40%	ND	994 + 299	ND	565 + 144
LACT	30%	523 + 199	ND	1110 + 130	ND
	40%	534 + 182	ND	1103 + 130	ND
URIC	30%	ND	(26 + 24)	ND	-139 + 35
	40%	ND	(39 + 24)	ND	-132 + 35

Afferent-efferent differences (Paired T-test,  $P < 0.05$ ) across liver in fed and six-day fasted fowl. Differences across liver are shown for the two assumptions that the arterial component of blood flow is either 30% or 40% of the total. The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. Where the difference is not significant, the difference is in parentheses. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7. For amino acids and peptides of fasted animals N=5 and for glucose and uric acid, N=14, for glycerol N=2, and for lactate, N=11.

Table 13. Fractional Extraction/Release of Amino Acids and Metabolites From Whole Blood (or from plasma for Urate and Glucose) by Liver in Fed and Fasted Chickens.

AA	FED		FASTED	
	Arterial 30%	Arterial 40%	Arterial 30% <sup>f</sup>	Arterial 40%
TAUR	(5%)	(4%)	(5%)	(4%)
HPRO	(-10%)	(-15%)	(18%)	(17%)
THR	(7%)	(6%)	14%	13%
SER	(7%)	(8%)	28%	28%
ASN	13%	(9%)	(33%)	(30%)
GLU	10%	10%	(9%)	(8%)
GLN	(3%)	(2%)	34%	33%
PRO	9%	8%	(37%)	(36%)
GLY	(9%)	(9%)	15%	14%
ALA	(10%)	(7%)	36%	34%
VAL	(7%)	(7%)	(15%)	(14%)
CYS	(16%)	(16%)	(11%)	(11%)
MET	(-3%)	(-4%)	(12%)	(11%)
ILE	10%	11%	(12%)	(11%)
LEU	10%	11%	(13%)	(12%)
TYR	(10%)	(9%)	25%	24%
PHE	14%	13%	28%	28%
BALA	(4%)	(5%)	(-3%)	(-4%)
TRP	13%	14%	(-4%)	(-2%)
ORN	(3%)	(3%)	24%	25%
LYS	(9%)	(9%)	23%	22%
HIS	(11%)	(8%)	(11%)	(10%)
ANS	(2%)	(3%)	(3%)	(3%)
CARN	(4%)	(4%)	(4%)	(4%)
ARG	9%	9%	12%	12%
NH3	3%	3%	28%	27%
LACT	22%	23%	26%	26%
GLUC	7%	8%	9%	9%
URIC	(7%)	(10%)	24%	23%

Fractional extractions or releases for liver of fed and fasted cockerels for the two assumptions that arterial flow comprises 30% or 40% of total arterial flow. Where the afferent-efferent difference from Table 12 is not significant, the fractional extraction is enclosed in parentheses.

### 3.3.4.2 Kidney

In fed birds the kidney was different from the liver in that a smaller number of amino acids showed a positive afferent-efferent difference<sup>10</sup> in whole blood and there was a negative afferent-efferent difference for ammonia.

In the fasted bird, the kidney showed a positive afferent-efferent difference in blood for a larger number of amino acids, notably the gluconeogenic amino acids alanine, arginine, glycine, the gluconeogenic dipeptide carnosine, and a large increase in the afferent-efferent difference for lactate.

The largest changes were seen in the glucose and urate afferent-efferent differences. The kidney in the fasted bird showed a large negative afferent-efferent difference for glucose, while the fed kidney did not. The kidney of the fasted bird also showed a large positive afferent-efferent difference for urate while in the fed bird a lesser, significant difference was seen (Table 14).

Taurine, which is not considered a gluconeogenic amino acid, was extracted from blood by both fed and fasted

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10 It is assumed for purposes of this discussion that renal portal flow comprises 50% of total renal flow (See Section 3.4.1).

kidneys in fairly high amounts. It would seem that this sulfur-containing amino acid is excreted in the urine.

In the kidney of the fed bird, the fractional extraction from plasma of urate was 44%. In the fasted bird however, a higher amount of urate (74%) was extracted from plasma. This high extraction rate reflects the high tubular secretion of urate (Shannon, 1938).



Table 14. Significant Differences Across Kidney in Fed and Fasted Chickens

AA	% Arterial	FED BLOOD	PLASMA	FASTED BLOOD	PLASMA
TAUR	50%	(581 + 345)	(-1 + 9)	(370 + 204)	(10 + 8)
	75%	(610 + 346)	(-1 + 11)	346 + 126	(6 + 12)
	100%	(640 + 352)	(-2 + 13)	321 + 69	(2 + 15)
HPRO	50%	29 + 9	(10 + 19)	(-10 + 18)	(6 + 6)
	75%	19 + 5	(4 + 22)	(-11 + 15)	(5 + 10)
	100%	10 + 2	(-2 + 25)	(-11 + 12)	(5 + 14)
THR	50%	(14 + 13)	(0 + 7)	(28 + 17)	(-47 + 28)
	75%	(18 + 14)	(-2 + 9)	(22 + 16)	(-69 + 25)
	100%	(22 + 15)	(-4 + 12)	(16 + 22)	-92 + 25
SER	50%	(-57 + 55)	-175 + 46	-78 + 22	-185 + 22
	75%	(-43 + 62)	-172 + 42	-84 + 19	-195 + 18
	100%	(-29 + 69)	-169 + 38	-91 + 20	-205 + 16
ASN	50%	(2 + 4)	17 + 7	(1 + 4)	(1 + 6)
	75%	(5 + 5)	(11 + 9)	(-2 + 4)	(-6 + 6)
	100%	(0 + 6)	(6 + 10)	(-6 + 4)	(-13 + 7)
GLU	50%	(-30 + 43)	-118 + 22	(33 + 12)	(-15 + 8)
	75%	(-9 + 49)	-100 + 21	(12 + 10)	(-12 + 7)
	100%	(13 + 57)	-81 + 19	(13 + 11)	(-7 + 6)
GLN	50%	62 + 20	64 + 16	(33 + 17)	(25 + 45)
	75%	(50 + 26)	(20 + 17)	(5 + 18)	(-17 + 38)
	100%	(38 + 33)	(-24 + 21)	(-22 + 20)	(-59 + 33)
PRO	50%	(10 + 8)	(24 + 24)	(-2 + 10)	(-14 + 9)
	75%	(16 + 7)	(19 + 25)	(-5 + 12)	(-24 + 11)
	100%	22 + 7	(15 + 27)	(-8 + 14)	(-33 + 14)
GLY	50%	(21 + 22)	(0 + 12)	52 + 13	(11 + 12)
	75%	(27 + 24)	(-3 + 15)	33 + 13	(-14 + 9)
	100%	(33 + 26)	(-6 + 19)	(14 + 14)	39 + 12
ALA	50%	58 + 11	99 + 27	75 + 19	70 + 36
	75%	56 + 13	(72 + 31)	44 + 17	(26 + 30)
	100%	53 + 19	(44 + 35)	(12 + 16)	(-18 + 26)
VAL	50%	(24 + 13)	49 + 7	(40 + 30)	(-19 + 14)
	75%	31 + 12	53 + 11	(38 + 34)	(-21 + 14)
	100%	38 + 12	57 + 16	(36 + 38)	(-23 + 16)
CYS	50%	(-4 + 6)	(-10 + 6)	9 + 1	(-8 + 12)
	75%	(-4 + 7)	(-6 + 5)	11 + 2	(-9 + 13)
	100%	(-4 + 8)	(-3 + 5)	13 + 3	(-10 + 14)
MET	50%	(-8 + 10)	(-7 + 5)	(2 + 4)	(-10 + 12)
	75%	(-7 + 12)	(-8 + 4)	(-4 + 4)	(-16 + 12)
	100%	(-7 + 15)	-10 + 4	-10 + 4	(-23 + 12)
ILE	50%	(4 + 5)	(8 + 8)	(11 + 8)	(-21 + 18)
	75%	(7 + 5)	(10 + 8)	(9 + 8)	(-21 + 18)
	100%	(9 + 5)	(13 + 8)	(7 + 8)	(-21 + 20)

Table 14. (continued) Significant Differences Across Kidney in Fed and Fasted Chickens

AA	% Arterial	FED		FASTED	
		BLOOD	PLASMA	BLOOD	PLASMA
LEU	50%	(10 + 7)	(22 + 14)	(13 + 6)	(-11 + 8)
	75%	(14 + 7)	(28 + 12)	(10 + 6)	(-12 + 8)
	100%	(19 + 8)	34 + 10	(7 + 7)	(-12 + 9)
TYR	50%	(3 + 6)	(2 + 4)	(5 + 2)	(-1 + 2)
	75%	(5 + 9)	(2 + 4)	(3 + 3)	(-3 + 2)
	100%	(8 + 10)	(2 + 4)	(0 + 4)	(-5 + 3)
PHE	50%	(1 + 3)	(2 + 3)	6 + 2	(0 + 2)
	75%	(3 + 4)	(4 + 3)	(4 + 2)	(-1 + 3)
	100%	(5 + 5)	(6 + 4)	(2 + 3)	(-3 + 4)
BALA	50%	(24 + 20)	UD	(-37 + 31)	UD
	75%	(28 + 22)	UD	(-57 + 38)	UD
	100%	(32 + 25)	UD	(-76 + 49)	UD
TRP	50%	(3 + 3)	(5 + 3)	(4 + 3)	(16 + 9)
	75%	(3 + 3)	(5 + 3)	(2 + 3)	(17 + 7)
	100%	(2 + 3)	(5 + 3)	(1 + 3)	18 + 6
ORN	50%	(4 + 2)	(7 + 3)	(-1 + 4)	(-9 + 10)
	75%	(3 + 2)	(5 + 3)	(-1 + 4)	(-15 + 11)
	100%	(2 + 3)	(4 + 2)	(-2 + 5)	(-21 + 12)
LYS	50%	(-1 + 13)	(7 + 17)	(1 + 6)	-26 + 10
	75%	(1 + 15)	(4 + 22)	(-9 + 8)	-46 + 14
	100%	(3 + 17)	(2 + 26)	(-18 + 11)	-65 + 20
HIS	50%	(-1 + 3)	-8 + 3	(-14 + 10)	(-32 + 27)
	75%	(-2 + 4)	-10 + 3	(-18 + 10)	(-42 + 26)
	100%	(-2 + 5)	-12 + 4	(-22 + 10)	(-53 + 24)
3-ME	50%	(1 + 1)	(1 + 1)	(0 + 1)	(-4 + 2)
	75%	(1 + 1)	(1 + 2)	(0 + 3)	(-5 + 2)
	100%	(1 + 1)	(0 + 2)	(0 + 4)	(-5 + 2)
ANS	50%	(32 + 30)	UD	(67 + 32)	UD
	75%	(42 + 36)	UD	(48 + 30)	UD
	100%	(52 + 43)	UD	(28 + 32)	UD
CARN	50%	(69 + 73)	UD	120 + 37	UD
	75%	(88 + 83)	UD	108 + 37	UD
	100%	(107 + 98)	UD	97 + 38	UD
ARG	50%	(4 + 11)	(12 + -14)	34 + 10	(35 + 19)
	75%	(7 + 13)	(14 + 14)	25 + 7	(23 + 16)
	100%	(10 + 15)	(16 + 15)	16 + 5	(10 + 13)
GLYC	50%	(-4 + 18)	ND	62 + 8	ND
	75%	(-17 + 20)	ND	(34 + 20)	ND
	100%	(-31 + 23)	ND	(6 + 31)	ND
NH3	50%	-58 + 23	(-93 + 60)	-123 + 44	-122 + 19
	75%	(-47 + 35)	(-74 + 64)	-113 + 42	-108 + 24
	100%	(-36 + 48)	(-54 + 70)	-102 + 40	-95 + 31

Table 14. (continued) Significant Differences Across Kidney in Fed and Fasted Chickens

GLUC	50%	ND	(-73 + 139)	ND	-512 + 136
	75%	ND	(255 + 140)	ND	-462 + 139
	100%	ND	584 + 161	ND	-345 + 164
GSH	50%	ND	ND	-299 + 71	ND
	75%	ND	ND	-446 + 105	ND
	100%	ND	ND	-593 + 139	ND
GSSG	50%	ND	ND	94 + 37	ND
	75%	ND	ND	137 + 50	ND
	100%	ND	ND	180 + 63	ND
URIC	50%	ND	179 + 24	ND	409 + 47
	75%	ND	212 + 24	ND	431 + 48
	100%	ND	245 + 27	ND	453 + 49
LACT	50%	(95 + 222)	ND	356 + 121	ND
	75%	(37 + 173)	ND	(246 + 132)	ND
	100%	(-21 + 145)	ND	(136 + 147)	ND

Legend for Table 14.

Afferent-efferent differences across kidney in fed and six-day fasted fowl. Differences across kidney are shown for the three assumptions that the arterial component of kidney blood flow is either 50%, 75% or 100% of the total. The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates the metabolite was not determined. "UD" indicates that the concentration was below detectable levels. Where the difference is not significant, ( $P > 0.05$ ), the difference is enclosed in parentheses. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=2, and for lactate, N=7. For amino acids and peptides of fasted animals N=5 and for glucose and uric acid, N=14, for glycerol, N=2, and for lactate, N=11.

Table 15. Fractional Extraction/Release of Amino Acids and Metabolites From Whole Blood (or from plasma for Urate and Glucose) by Kidney in Fed and Fasted Chickens.

AA	FED		FASTED	
	Arterial 50%	Arterial 100%	Arterial 50%	Arterial 100%
TAUR	(17%)	(18%)	(9%)	8%
HPRO	15%	7%	(-27%)	(-17%)
THR	(4%)	(6%)	(5%)	(3%)
SER	(-13%)	(-9%)	-19%	-22%
ASN	(0%)	(-5%)	(0%)	(-13%)
GLU	(-5%)	0%	(3%)	(3%)
GLN	12%	(5%)	(8%)	(-6%)
PRO	(5%)	10%	(-1%)	(-7%)
GLY	(3%)	(5%)	9%	(3%)
ALA	11%	10%	19%	(4%)
VAL	(9%)	13%	(7%)	(5%)
CYS	(-4%)	(-4%)	18%	23%
MET	(-62%)	(-50%)	(3%)	(-17%)
ILE	(5%)	(9%)	(8%)	(5%)
LEU	(6%)	(10%)	(6%)	(4%)
TYR	(2%)	(4%)	(5%)	0%
PHE	(1%)	(5%)	8%	(3%)
BALA	(6%)	(7%)	(-9%)	(-27%)
TRP	(8%)	(5%)	(8%)	(-1%)
ORN	(20%)	(12%)	(2%)	(2%)
LYS	(3%)	(5%)	(2%)	(-6%)
HIS	(-3%)	(-5%)	(-17%)	(-35%)
ANS	(4%)	(6%)	(8%)	(3%)
CARN	(4%)	(5%)	10%	8%
ARG	(2%)	(5%)	19%	10%
NH3	-36%	(-32%)	-54%	-40%
URIC	44%	52%	74%	73%
LACT	(4%)	(1%)	15%	(6%)
GLUC	(-1%)	.4%	-4%	-3%

Fractional extractions or releases for kidney of fed and fasted cockerels for the two assumptions that arterial flow comprises 50% or 100% of total arterial flow. Where the afferent-efferent difference from Table 14 is not significant, the fractional extraction is enclosed in parentheses.

#### 3.3.4.3 Muscle

In fed chickens the amino acids proline, leucine and valine showed a positive arteriovenous difference in blood across muscle. There was also a significant uptake of glucose and urate from plasma. These results for glucose are not dissimilar from the observations of Knapp (1936) who measured a significant positive arteriovenous difference of glucose across wing muscle.

In the fasted chicken there were a number of changes in the types of amino acids showing arteriovenous differences. The muscle of fasted birds showed a negative arteriovenous difference for glutamine, alanine, glycine, methionine, lysine, phenylalanine, tyrosine, histidine and lactate in blood (Table 16). There was an increase in the fractional release from muscle of glutamine, glycine, alanine, methionine, tyrosine, phenylalanine, lysine, histidine and lactate (Table 17).

Table 16. Significant Differences Across Muscle in Fed and Fasted Chickens

AA	FED		FASTED	
	BLOOD	PLASMA	BLOOD	PLASMA
TAUR	(117 ± 163)	(-2 ± 11)	(-99 ± 340)	(-15 ± 14)
HPRD	[-38 ± 18]	(-22 ± 19)	(-2 ± 13)	(-1 ± 17)
THR	(16 ± 13)	(-8 ± 14)	(-25 ± 46)	(-90 ± 32)
SER	(55 ± 40)	(11 ± 19)	(-26 ± 33)	(-40 ± 25)
ASN	(-4 ± 6)	-21 ± 8	[-12 ± 6]	-26 ± 7
GLU	[86 ± 43]	74 ± 14	(0 ± 15)	(17 ± 9)
GLN	(-48 ± 30)	-177 ± 34	-109 ± 17	-167 ± 40
PRD	24 ± 5	[-18 ± 8]	(-14 ± 10)	[-39 ± 14]
GLY	(25 ± 22)	(-12 ± 17)	-75 ± 17	-101 ± 30
ALA	(-10 ± 30)	-109 ± 32	-125 ± 20	-177 ± 32
VAL	28 ± 5	(16 ± 18)	(-7 ± 20)	(-7 ± 8)
CYS	(-1 ± 6)	(14 ± 12)	[7 ± 3]	(-5 ± 6)
MET	(2 ± 12)	(-6 ± 6)	-25 ± 3	-26 ± 7
ILE	[10 ± 5]	10 ± 4	(-7 ± 8)	(0 ± 15)
LEU	18 ± 7	[24 ± 13]	(-11 ± 6)	(-3 ± 15)
TYR	(8 ± 8)	(0 ± 4)	-11 ± 3	(-9 ± 7)
PHE	(8 ± 4)	8 ± 3	-8 ± 3	(-6 ± 6)
BALA	(16 ± 20)	ND	(-81 ± 64)	ND
TRP	(-2 ± 2)	(0 ± 5)	(-5 ± 3)	(3 ± 10)
ORN	[-3 ± 2]	-5 ± 2	(-1 ± 3)	-25 ± 9
LYS	(6 ± 12)	(-10 ± 22)	-39 ± 13	-77 ± 33
HIS	(-2 ± 5)	-10 ± 4	-16 ± 4	-43 ± 13
3MH	(1 ± 1)	(-1 ± 2)	(0 ± 2)	(-2 ± 2)
ANS	(41 ± 38)	ND	[-77 ± 36]	ND
CARN	(76 ± 91)	ND	(-46 ± 28)	ND
ARG	(13 ± 12)	(8 ± 8)	[-36 ± 16]	-49 ± 20
GLYC	(-54 ± 22)	ND	(-113 ± 77)	ND
NH3	(43 ± 55)	(79 ± 41)	41 ± 16	(55 ± 34)
GLUC	ND	1320 ± 210	ND	(332 ± 180)
GSH	ND	ND	(18 ± 70)	ND
GSSG	ND	ND	[116 ± 53]	ND
LACT	(-233 ± 292)	ND	-441 ± 111	ND
URIC	ND	131 ± 34	ND	87 ± 29

Arteriovenous differences across muscle in fed and six-day fasted fowl. The data are presented as the concentration of the amino acid in nanomoles per ml ± S.E.M. "ND" denotes the metabolite was not determined. Where the arteriovenous difference is significant ( $P < 0.05$ ) the data are presented unbracketed. Where the difference is marginally significant ( $0.05 < P < 0.1$ ) the data are bracketed. Where the data are insignificant ( $P > 0.1$ ) the data are enclosed in parentheses. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7. For amino acids and peptides of fasted animals N=5 and for glucose and uric acid, N=14, for glycerol N=2, and for lactate, N=11.

Table 17. Fractional Extraction/Release of Amino Acids and Metabolites From Whole Blood (or from plasma for Urate and Glucose) by Muscle in Fed and Fasted Chickens.

AA	FED	FASTED	
	BLOOD	BLOOD	
TAUR	(3.3%)	(-2.6%)	
HPRO	(-2.5%)	(-2.9%)	
THR	(4.9%)	(-3.9%)	
SER	(9.5%)	(-6.0%)	
ASN	(-5.9)	[-27.0%]	
GLU	[11.6%]	0	
GLN	(-10.8)	-29.5%	
PRO	11.2%	(-9.4%)	
GLY	(4.1%)	-13.9	
ALA	(-1.9%)	-38.7%	
VAL	8.3%	(-1.3%)	
CYS	(-1.0%)	[13.2%]	
MET	(14.0%)	-37.3%	
ILE	[7.2%]	(-4.0%)	
LEU	7.4%	(-4.7%)	
TYR	(5.4%)	-10.4%	
PHE	(8.6%)	-9.5%	
BALA	(4.0%)	(-22.0%)	
TRP	(-6.9%)	(-15.0%)	
ORN	(-14.3%)	(-2.0%)	
LYS	(1.9%)	-16.3%	
HIS	(3.1%)	-26.7%	
3MH	6.7	0	
GLUC	9.6%	(2.6%)	(measured in plasma)
LACT	(10.0%)	-18.7%	
URIC	38.0%	(14.0%)	(measured in plasma)

Fractional extractions for muscle of fed and fasted cockerels. Where the fractional extraction is significant ( $P < 0.05$ ) the data are presented unbracketed. Where the extraction is marginally significant ( $0.05 < P < 0.1$ ) the data are bracketed. Where the data are insignificant ( $P > 0.1$ ) they are enclosed in parentheses. In the fed state,  $N=6$  for amino acids, for glucose and uric acid,  $N=14$ , and for lactate,  $N=7$ . For amino acids of fasted animals  $N=5$  and for glucose and uric acid,  $N=14$  and for lactate,  $N=11$ .

### 3.4 DISCUSSION

#### 3.4.1 Consideration Of The Validity Of The Study

Several authors have reported estimates of blood volume in chickens (Adult - 5.6% of body weight, Bond & Gilbert, 1958; Adult - 6.8%, Wels et al., 1967; Adult - 6.5%, Immature (chick)- 12%, Medway & Kare, 1959; Immature (800g male) - 10.6%, Newell & Shaffner, 1950). If the figure of Newell & Shaffner (1950) is used to calculate blood volume for the birds used in this study, the 10 mls of blood drawn in the five samples represents 12% of the total blood volume. In the fed chicken and 14% of the total blood volume of the fasted chicken. Blood loss of this magnitude is known to result in significantly reduced blood pressure in hens (Wyse & Nickerson, 1971). Larger blood losses (22% of blood volume in hens, 30% in roosters) are reported to cause a significant reduction in hematocrit (Ploucha et al., 1981; Wyse & Nickerson, 1971). Blood losses in the range of 30% to 70% of the initial blood volume are reported to cause an increase in blood glucose in hens (Ploucha et al., 1981). It was shown by Davison (1975) that removal of 6% (2ml) of the total blood of fed or fasted six-week-old chicks by frontal heart puncture had no effect on hematocrit or plasma glucose measured 30 minutes after the first sample. One hour after a total of 12% of the total blood volume had been withdrawn (2 ml sample at time zero and a second 2 ml sample taken 30 minutes later) there was a significant reduction in



hematocrit and an increase in plasma glucose.

Aside from the effects of blood sampling; handling and restraint of the chickens is reported to cause an initial hyperglycemia (Honeywell, 1921; Freeman & Manning, 1976), and an increase in blood pressure and heart rate (Whittow et al., 1965). Anesthesia is known to cause a decrease in blood pressure (Ray & Pedde, 1969).

From the foregoing discussion it would seem that the procedures used in this thesis are not without effects on blood glucose, hematocrit and blood pressure. The fact that I was unable to see a significant effect of hemorrhage due to blood sampling on blood glucose or hematocrit however, shows that the changes in these parameters were small and within the normal range exhibited by individuals. The likelihood that blood pressure is diminished by blood sampling cannot be discounted however. It is possible too, that changes in patterns of blood flow may be affected by the blood sampling. As this was not measured directly, observations on other factors may allow conclusions regarding these parameters.

One of the important factors to consider when afferent-efferent difference measurements across liver and kidney are made in the domestic fowl is the relative proportion of arterial and portal flows to those organs. Estimations of the arterial component of liver flow range

between 30% and 40% (Sapirstein & Hartman, 1959; Purton, 1975). As I have no basis to choose between the two, data for afferent-afferent differences were presented for the two extremes.

Estimates of the arterial component of kidney flow cover a wider range, from 33% (Sperber, 1960) to 50% (Skadhauge, 1973). Additional estimates can be calculated from other published data. The total renal blood flow in chickens is approximately 67 ml/min/kg (Skadhauge, 1973; Sperber, 1960; Orloff & Davidson, 1959; Nechay & Nechay, 1959; Osbaldiston, 1969). Sturkie et al. (1977) measured a total flow in the external iliac vein of 16.4 ml/min/kg and estimated that 70% of this blood perfuses the kidneys via the renal portal system. Using his values in concert with total renal flow yields an arterial component of 83%. Odland (1978) reports that only 44% of iliac blood perfuses the kidneys. Using this value in concert with the data of Sturkie et al. (1977) yields an arterial component of 89%. Several other investigators have measured arterial flow to the kidney. Using their arterial flow data in concert with the total flow rate above, yields arterial components of kidney flow of 49% (Sapirstein & Hartman, 1959), 31% (Wolfenson et al., 1978), 61% (Merrill et al., 1981); 33% (Wolfenson et al., 1982), and 33% (Boelkins et al., 1973). The average value for all of these estimates is 50%, though only one (Merrill et al., 1981 - 61%) is in male chickens.

Further evidence of how the total kidney blood flow is divided between arterial and portal components are the data which are presented in Table 18.

As the calculated arterial component of total renal flow is increased from 50% to 100%, the amount of glucose equivalents taken up from afferent blood accounts for less of the glucose carbon released from kidney. If the arterial percentage is kept below 70% (or 80% if the 0.1 level of significance is taken) the amount of glucose released from kidney can be accounted for by the amount of gluconeogenic carbons taken up. However when these calculations are performed assuming that arterial flow comprises 80%, or more, of the total renal flow, with the remainder arising from the renal portal system, then it can be seen that only a small portion of the glucose release is accounted for by the amount of gluconeogenic carbons taken up. These biochemical data suggest that the renal portal system contributes 30% or more, of the total renal blood flow but this does not preclude the need for more definitive physiological investigations on this subject.

A controversy surrounds blood flow in the chicken kidney concerning the role of the coccygeomesenteric vein. Experiments by Akester (1967) and Purton (1975), demonstrated that in the fowl, blood flow could be in either direction in this vessel, while Purton (1970), Sturkie et al., (1977) and Sturkie & Abatie (1975) report that blood

Table 18. Carbon Balance Across Kidney of Fasted Chicken

PANEL A.  
Arterial  
Component

	Afferent - Efferent Difference							
	PLASMA		WHOLE BLOOD					
	GLUC	LACT	GLY	ALA	CYS	CARN	ARG	PHE
50%	-512	357	52	75	9	120	34	6
60%	-479	313	44	62	10	115	30	5
70%	-445	(269)	37	50	11	111	26	-
80%	-412	-	29	-	11	106	23	-
90%	-379	-	-	-	12	102	19	-
100%	-346	-	-	-	13	97	16	-

PANEL B.

	GLUCOSE RELEASED (PER ML BLOOD)	GLUCOSE EQUIV. TAKEN UP P <.05 (P <.1)	% ACCOUNTED FOR P <.05 (P <.1)
50%	307	326	106%
60%	287	289	101%
70%	267	117 (251)	44% (99%)
80%	247	84	34%
90%	227	66	29%
100%	207	63	30%

Panel A. shows the significant afferent-efferent differences across kidney for glucose and for the major gluconeogenic substrates which are taken up by the kidney (Units in nanomoles per ml of plasma for glucose and per ml of whole blood for the substrates). Panel B. indicates the amount of glucose released for different assumed contributions of arterial and renal portal flow, and the amount of glucose equivalents taken up by the kidney for each assumed flow pattern. Unbracketed numbers are calculated using the 0.05 level of significance. Bracketed numbers are calculated using the 0.1 level of significance.

flow in the coccygeomesenteric vein is directed cranially. The problem of flow in this vessel is not yet resolved. For the purposes of this thesis, the results of Purton (1970) and of Sturkie and co-workers have been accepted.

#### 3.4.2. Levels Of Amino Acids

The concentration and composition of circulating amino acids in the post-prandial chicken is probably determined at least to some extent, as it is in rats, by the interaction between the amino acid content of the diet and the chicken's requirements of amino acids for protein synthesis (Johnson & Anderson, 1982).

Differences in the age and sex of the birds used, in the composition of the diets, in the deproteinizing reagents and in analytical techniques will all contribute to the variation among the different reports. It should not be surprising therefore that there are minor differences between the arterial plasma levels of amino acids reported here and those reported by the other investigators which are summarized in Table 10.

As noted by Hill & Olsen (1963) the effect of fasting on the concentration of circulating amino acids will be modulated to some extent by the amino acid content of the diet previously consumed and by the duration of the fast. This consideration aside, any change in the circulating

concentration of amino acids on fasting does reflect the interaction between the release of amino acids from skeletal muscle and the removal of the amino acids from the circulation by consuming tissues. The fate of an amino acid removed from the circulation may be for use as a substrate for catabolic (oxidation) or anabolic processes (gluconeogenesis, protein synthesis) or for excretion.

#### 3.4.3 Blood Flow

It is useful when measuring arteriovenous differences, if possible, to measure simultaneously the rate of blood flow through the vessels, so that the metabolite flux can be calculated. In the experiments described in this thesis, blood flows were not determined simultaneously with the arteriovenous differences for to have done so would have been impractical.

Blood flows through the liver were however determined in a separate series of experiments on fed animals. Using the bromosulphothalein method (Bradley et al., 1945; Ossenberg et al., 1974) total hepatic flow rates were found to be compatible with values reported in the literature for arterial flow using radioactive microsphere methods (Wolfensen et al., 1978) or indicator dilution techniques (Sapirstein & Hartman, 1959, Boelkins et al., 1973).

The total hepatic flow rate measured here is four times as large as blood flow in the hepatic portal vein, determined using electromagnetic probes (Sturkie et al., 1977; Sturkie et al., 1978; Sturkie & Abati, 1975). If Sturkie and coworker's figures are correct it would imply that the hepatic portal contribution to total liver flow is only 25%. This may say something about the difference between, on one hand the microsphere, indicator dilution, and bromosulphthalein methods of determining blood flow, and on the other hand, the electromagnetic probe method used by Sturkie. The latter authors (Sturkie & Abati, 1975) report no effect of fasting on blood flow in the portal vein. This failure of fasting to affect the amount blood flow per gram of hepatic tissue was also seen in the results of the determinations of flow using bromosulphthalein. Vogel & Sturkie, (1963) report a 48% decrease in cardiac output in chickens fasted for 8 days. In this study, blood flow to liver (ml/min/kg) decreased by 38% (Table 9). Liver mass as a proportion of the whole animal (g/kg) was also diminished so that there was no decrease of blood flow per gram of liver.

Implicit in the discussion in the next section is the yet unproven assumption that blood flow per gram of kidney & muscle tissue also does not change on fasting. It must be remembered<sup>o</sup> that if fasting causes a change in the rate of blood flow through an organ, then any conclusions drawn

concerning a change in the magnitude of an arteriovenous difference on fasting may be invalid. However, if a change in the sign of an arteriovenous difference is observed on fasting, then only a reversal of blood flow can invalidate the conclusions drawn.

#### 3.4.4 Arteriovenous Difference Measurements

Taking into account the caveats of the preceding section, it seems not unreasonable to refer to a positive arteriovenous difference as an uptake and a negative arteriovenous difference as a release. Although these terms may not be technically correct, their use in the body of this thesis implies the aforementioned cautions.

##### 3.4.4.1 Liver

The liver takes up approximately the same number and amount of amino acids in the fed and fasted states (Table 12), although the liver is generally more efficient in removing amino acids from the circulation in the fasted state as evidenced by the increase in the fractional extraction for most amino acids. The large amount of urate produced by the liver in the fasted state suggests that the fate of the amino acids taken up is deamination. Nitrogen released as urate accounts for about 40% of the amino nitrogen taken up. The fate of the carbon skeletons of the



amino acids is less certain. Some carbon undoubtedly is released in the form of ketone bodies, while some fraction is incorporated into peptides, proteins, glucose, glycogen and nucleic acids or oxidized to carbon dioxide.

Deamination does not appear to be the fate of the amino acids taken up in the fed state; as no urate is released and as many of the amino acids taken up are essential amino acids, it appears that their fate is to be substrates for protein synthesis. As no urate is released by the liver of fed birds, this indicates that renal synthesis of uric acid predominates over hepatic synthesis in the fed state.

The liver takes up nearly the same amount of glucose per gram of tissue in the fasted as in the fed state. This is a remarkable observation as the liver of the fasted chicken appears to be removing glucose from the circulation while the liver of a fasted mammal is considered a prime gluconeogenic organ. It is even more perplexing as in the chicken there is simultaneously a large uptake of lactate. In light of the results of the liver blood flow determinations using hepatic bromosulphthalein uptake, (Sect 3.3.2), it is difficult to present the easiest argument i.e. that there might be a problem with the arteriovenous measurement itself. It does not appear that the recumbant position of the animal results in greatly altered or reversed hepatic blood flow.

One alternative available to explain the observed hepatic uptake of glucose is to assume that the glucose uptake which is seen is a metabolic artefact. It is possible that the surgery and stress of blood sampling affects the metabolism of glucose in the liver of the chicken much more than other metabolites. In that case it might be hypothesized that glucose oxidation or glycogen synthesis is stimulated in these livers. (See more complete discussion in Section 4.1)

#### 3.4.4.2 Kidney

The afferent-efferent difference across the kidney is easier to verify than the arteriovenous difference across the liver. In the kidney there is an internal marker for the goodness of the afferent-efferent difference, namely urate extraction. As the kidneys are known to excrete urate, a negative afferent-efferent difference would indicate incorrect vessels are being sampled. This is a real possibility in the chicken's kidney wherein there lies a multiplicity of vessels from which to sample. As, in all cases reported in this thesis, urate was seen to be extracted from plasma, it is concluded that no serious problems existed with the afferent-efferent difference calculated for the kidney.

Judging from the afferent-efferent difference on the fed kidney, it seems that this organ in the fed animal is much less involved in exchanging amino acids with the circulation than is liver or muscle. A significant afferent-efferent difference is seen for only a few amino acids (Hpro, Ala, Val).

The situation in fasted kidney is quite different. Glucose is released from the kidney, and significant amounts of gluconeogenic amino acids are taken up. Assuming that the renal portal component is at least 30% of total kidney flow, the amount of carbon taken up as amino acids, carnosine<sup>11</sup>, and lactate from blood is approximately equal to the amount of carbon released in the plasma as glucose. The amino acid serine is released into plasma. It seems that this amino acid is released from the kidneys of most animals (Pitts & MacLeod, 1972; Squires et al., 1976).

Using data presented for chickens of this age by Riesenfeld (1979) of a glucose turnover of 44 micromoles/min/kg, a recycling rate of 50% of the turnover, a body lipid decrease of 0.59 g/hr and assuming that all

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11 Carnosine (Beta-alanylhistidine) is hydrolysed to beta-alanine and histidine by the enzyme carnosinase, present in significant quantities in chicken kidney, although low in activity in chicken liver (Wolos & Piekarska, 1975).

lipids are triglycerides with a molecular weight of 850; and if all this potential glycerol were converted to glucose, then glycerol's contribution to new glucose synthesis can account for, at most, 24% of the total glucose turnover. In a fasted chicken, while glycerol is taken up by liver in this study, the afferent-efferent difference is small (17 nmol/ml). This is only an eighth of the afferent-difference of alanine across liver. Thus it would appear that glycerol is of minor importance as a glucose precursor in the liver in vivo. The afferent-efferent difference of glycerol across kidney is larger (62 nmol/ml), and similar to the afferent-efferent difference for alanine. Thus glycerol may be a significant gluconeogenic precursor in kidney.

It should be pointed out that the uptake of amino acids and lactate by the kidney of a fasted chicken occurs mostly from the renal portal blood. This blood in turn, comes from the external iliac vein which drains the leg. If an afferent-efferent difference is calculated from the concentrations in the inferior aorta and the renal vein alone there are very few amino acids for which a significant difference is observed, although a large amount of glucose is still seen to be released. It seems therefore, that the renal portal circulation carries amino acids from the leg directly to the site of glucose synthesis in the kidney. There may be an advantage to the chicken in this

arrangement, as if amino acids were released into the general circulation there would be a dilution effect, and facilitated transport into the renal tissue would be energetically more expensive.

Assuming that the arterial blood flow is 50% of the total renal blood flow (Skadhauge, 1973) and using the measured afferent-efferent difference of  $-512$  nanomoles/ml of plasma in the fasted kidney, a cell volume of 40%, and a flow rate of  $67$  ml/min/kg, the calculated renal glucose production is  $34$  micromoles/min/kg. This figure ( $34$ ) compares with the reported range for glucose turnover in the fowl ( $26-52$ ) (Annison et al., 1966; Brady et al., 1978; Riesenfeld et al., 1981). It suggests that the kidney may be responsible for the production of at least 66% and perhaps 100% of the glucose synthesized by the fowl under these conditions. The afferent-efferent difference measured across kidney may not reflect all of the glucose production by kidney. If the distal tubules are a glucose consumer in the chicken as they are in the rat (Ross & Guder, 1982), then glucose production by kidney may be underestimated by an afferent-efferent difference measurement.

#### 3.4.4.3 Muscle

The major amino acids released from muscle of a fasted chicken are glutamine, alanine and glycine. The uptake of glucose by the leg of a fasted chicken is not significant. This is similar to the situation in the muscle of fasted rats (Aikawa et al., 1973).

Presumably the carbon skeletons of these amino acids derive from the other amino acids liberated from protein as a result of muscle proteolysis but which are not released into the circulation. If this is the case, then it would be similar to the situation in the mammalian (rat) muscle (Chang & Goldberg, 1978). It can be seen from the data presented in Table 19 that the amino acids, alanine and glutamine<sup>12</sup> make up a disproportionate amount of the amino acids which are released from muscle.

The amino acid composition of rat diaphragm does not change after a 48 hr. fast (Chang & Goldberg, 1978). If the same is true for chicken muscle, the pattern of amino acid release cannot be due to preferential breakdown of proteins high in glutamine and alanine. The additional glutamine and alanine must be synthesized in the muscle.

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<sup>12</sup> Although no data are available for chickens, in rats glutamine makes up about 6% of muscle protein (Kominz et al., 1954).

Table 19. Total Amino Acid Content of Chicken Muscle

	Church '70		Harvey '70	F.A.O. '70
	BROILER	HEN	DARK MEAT (FAT-FREE)	FLESH
SER			24.8	7.44
ALA			28.3	4.6
THR	7.37	7.62	26.7	6.67
GLU	22.5	23.3	91.3	20.4
GLY	18.3	19.7	63.9	7.2
VAL	8.6	8.9	33.4	8.7
MET	3.6	3.7	14.7	3.4
ILE	8.3	8.6	35.4	8.2
LEU	11.4	11.8	46.6	11.2
TYR	4.0	4.1	13.9	3.7
PHE	4.9	5.1	19.2	4.8
TRP	1.2	1.3	4.4	1.0
LYS	9.9	10.3	34.4	8.7
HIS	2.8	2.9	9.7	2.5
CYS	2.3	2.4	7.4	2.2
ARG	6.2	6.4	26.4	5.3
PRO			29.7	7.2
ASP				13.8
TOTAL	111.4	116.1	510.2	127.0

% OF TOTAL  
MUSCLE OUTPUT

PERCENTAGE PRESENT IN MUSCLE

	TINKER (P <)		HARVEY '70	Church '75		F.A.O. '70
	.05	.1	DK MEAT	Hen	Broiler	Flesh
GLN	26%	20%	-----	not given	-----	
ALA	30%	23%	5.5%	- not given	-	3.6%
GLY	18%	14%	12.5%	17.0%	16.4%	5.7%
MET	6%	5%	2.9%	3.2%	3.2%	2.7%
TYR	3%	2%	2.7%	3.6%	3.6%	2.9%
PHE	2%	1%	3.8%	4.4%	4.4%	3.8%
LYS	9%	7%	6.7%	8.9%	8.9%	6.8%
HIS	4%	3%	1.9%	2.5%	2.5%	2.0%
ASN		3%	-----	not given	-----	
CYS		1%	1.5%	2.1%	2.1%	1.7%
ARG		7%	5.2%	5.5%	5.6%	4.2%
ANS		14%	-----	not given	-----	
TOTAL	100%	100%	43%	47%	47%	33%

Legend to Table 18.

The figures in the top half of the table indicate the amino acid content of chicken muscle as reported by previous investigators. Units are in millimoles per 100 grams of tissue. In the bottom half of the table, in the first two columns, the amount to which an individual amino acid contributes to the total release of amino acids is shown. The first column is for a significance level of 0.05 while the second column is for a significance level of 0.1. The remaining four columns indicate the relative amounts of these amino acids present in muscle tissue.



That muscle protein degradation is an important source of circulating amino acids in the fasted chicken is corroborated by the observation that there is no significant release of amino acids from muscle in the fed state, while there is a significant release of several amino acids from muscle in the fasted state. If constant blood flow is assumed in muscle between fed and fasted chickens, this means that there is a substantial increase in the amount of amino acids released from muscle.

That muscle protein degradation is occurring in the fasted chickens can be surmised from the observed increase in plasma 3-methylhistidine (Table 10), an indicator of protein degradation (Hillgartner et al., 1981; Ward & Buttery, 1978). Half-lives of soluble proteins are known also to be decreased by fasting in the breast muscle of quail (Bush & Marquardt, 1978). It is likely that the same holds true for the chicken.

It should be mentioned that the metabolic fate of branched chain amino acids may not be as distinctive in the chicken as in the human. In the human branched chain amino acid transaminase activity is high in muscle and low in liver while branched keto-acid dehydrogenase activity is low in muscle and high in liver. This means that in the human, the branched chain amino acids liberated by muscle proteolysis are transaminated in the muscle and the corresponding keto-acids are released for oxidation in the

liver. In the chicken the difference in the activities of liver and peripheral branched chain amino acid transaminase and branched chain ketoacid dehydrogenase is less pronounced (Featherston & Horn, 1973).

#### 3.4.4.4. Urate

The significant uptake of urate by muscle tissue in the fed and fasted chicken is difficult to explain. One possibility is that xanthine dehydrogenase in muscle is able to catalyse the conversion of urate to xanthine. The equilibrium constant of the reaction is 966 (calculated from electrochemical half reactions, White et al., 1978). Reversal of this reaction is thermodynamically more favourable than is the reaction catalysed by lactate dehydrogenase (Equilibrium constant = 0.00011), and thus it might be concluded that the reaction catalysed by xanthine dehydrogenase is also reversible. Another possibility is that there is reverse flow in the caudal renal portal vein into the external iliac vein, and the blood sampled in the external iliac, is blood which has perfused the kidney. A third possibility, is that there is a significant activity of uricase in the hindquarters and the urate taken up is oxidized to allantoin. While these alternatives seem unlikely, a better explanation does not readily spring to mind.

The source of urate in a fed animal also is not demonstrated by these investigations. While kidney extracts urate from plasma, neither liver nor hindquarters, release urate into plasma. The most likely alternate source of plasma urate in the fed animal is the breast musculature, by far the largest muscle mass in the chicken. The gut is unlikely to be a source of plasma urate, as there is no significant difference across viscera when an arterial-venous difference is calculated using the urate concentrations in the inferior aorta and the hepatic vein (data not shown),

#### 3.4.4.5 Glutathione

In the early stages of data collection and analyses, I was not aware of the literature reports which indicated that there was a significant renal portal blood supply to the kidney. Inferior aorta - renal vein differences calculated for the kidney in a fasted chicken, indicated that there was a significant release of glucose without a concurrent uptake of gluconeogenic substrates. Fonteles & Leibach (1982) had shown that glutathione was an effective glucose precursor in the isolated perfused rat kidney. It was postulated therefore, that glutathione, present in high concentration in chicken blood, might be a potential glucose precursor in fasted chicken kidney. An investigation of this possibility however, indicated that glutathione uptake could not provide

the carbon for glucose synthesis. The amount of glutathione disulfide (GSSG) taken up by kidney was not significantly different from the amount of reduced glutathione (GSH) released ( $P > 0.05$ ). As glutathione did not appear to provide carbon precursors for glucose synthesis in the fasted kidney, the investigation was not extended to the fed kidney. There was no uptake or release of glutathione or glutathione disulfide by the liver of fasted chickens.

It does appear that glutathione disulfide in the circulation is reduced to glutathione in muscle and kidney tissue. This situation is unlike that in fed rats reported by Anderson et al. (1980). In rats glutathione (GSH) and glutathione disulfide (GSSG) were taken up by kidney while muscle took up glutathione only. Anderson et al. (1980) did not report hepatic portal vein concentrations of glutathione, and thus an arterio-venous difference across liver could not be calculated.

CHAPTER 4  
GENERAL DISCUSSION

#### 4.1 GLUCOSE SYNTHESIS IN THE LIVER.

The literature cited in this thesis spans over 170 years, a long history of investigation into intermediary metabolism in the fowl. These investigations have determined that the Class Aves is quite different from the Class Mammalia in anatomy, physiology and metabolism. The avian respiratory and circulatory systems (Section 1.5) and the manner in which nitrogen is excreted (Section 1.3) have evolved along a path quite unlike the mammalian.

For over a century, an area that has perplexed scientists, perhaps more than any other area of investigation in birds, and the area wherein lies the topic of this thesis, is avian glucose metabolism. Glucose metabolism has been the recipient of many hours of study from metabolic biochemists (Sections 1.1, 1.6), endocrinologists (Section 1.2) and enzymologists (Section 1.4). Although much has been discovered in this field, there are still many unanswered questions about carbohydrate metabolism in birds. The objective when this thesis was undertaken was to answer the related questions: (1) In what organ or organs is glucose produced in the fasted fowl, (2) what are the substrate(s) used for glucose synthesis and (3) which tissue provides the substrate(s) for gluconeogenesis.

Chapter Two of this thesis describes an older technique adapted to maintain blood gases at physiological levels in the circulation of an anesthetised, supine fowl, whose abdominal cavity has been opened for blood and tissue sampling. Using this model, levels of metabolites were determined in perchloric acid extracts, of rapidly freeze-clamped liver and kidney. It appears from the results that the conditions in the liver of a fasted cockerel were not favourable for the synthesis of glucose from amino acids and pyruvate due to a fairly oxidized  $NAD^+/NADH$  redox state in the liver cytosol. In the kidney of a fasted bird however, the  $NAD^+/NADH$  redox couple is more reduced. Another indication that gluconeogenesis in the liver of a fasted chicken may be inactive is that the glucose level in the liver of a six day fasted fowl is only half the level in the liver of a fed bird and much less than plasma glucose. In the kidney the glucose levels remain unchanged during a fast.

There is some indication in the literature that it is the low availability of cytosolic NADH which is rate-limiting for hepatic gluconeogenesis in birds. Lactate has been found to be a much better gluconeogenic precursor than alanine or pyruvate in the perfused liver (Ogata et al., 1982; Sugano et al., 1982; Deaciuc & Ilonca, 1981; Soling et al., 1973) and in hepatocytes (Brady et al., 1979; Watford et al., 1981; Bannister & O'Neill, 1981; Dickson,

1983; Dickson & Langslow, 1978). However, in vivo the difference in the ability of lactate and the ability of alanine or pyruvate to elevate plasma glucose is not as pronounced (Sarkar, 1971; Davison & Langslow, 1975; Langslow et al. 1970). The fact that alanine and pyruvate seem to be better glucose precursors in vivo than in hepatocytes or in perfused liver, suggests that there may be an additional non-hepatic source of glucose where cytosolic reducing equivalents are not in such short supply as in the liver. Brady et al. (1977), who also suggested that organs other than the liver contribute to the conversion of amino acids to glucose, found, using radioactive tracers, that the estimate of alanine conversion to glucose in vivo was higher than in isolated hepatocytes.

The low rates of hepatic gluconeogenesis from pyruvate and amino acids have been postulated to be due to the intracellular location of chicken liver PEPCK. It has been shown (Watford et al., 1981; Bahnister & O'Neill, 1981; Tinker et al., 1983a), that there is little or no phosphoenolpyruvate carboxykinase in the cytosol of the liver of chickens and no induction with fasting. With no phosphoenolpyruvate carboxykinase in the cytosol, the carbon skeleton for gluconeogenesis must leave the mitochondria not as reduced malate, but as the more oxidized phosphoenolpyruvate (See pathway in Figure 3). If reducing equivalents are limited in the cytosol, as the data in



Chapter Two indicate they are during a fast, then it is not surprising that gluconeogenesis from lactate or glycerol, which provide their own cytosolic reducing equivalents, progresses at a greater rate in vitro, than gluconeogenesis from amino acids or pyruvate.

Soling (1974) suggests that the rate limiting step for gluconeogenesis from pyruvate in pigeons is the transport of reducing equivalents out of the mitochondria. There is support for this proposition in the data reported in Table 4, in that a two-fold reduction in the hepatic concentration of malate is observed which may suggest a limit on the availability of malate. But whatever the kinetic rate-limiting step may be, it seems probable that the cytosolic availability of NADH plays an important role.

The significance of the cytosolic  $\text{NAD}^+/\text{NADH}$  ratio in hepatic gluconeogenesis is further supported by the data of Ochs & Harris (1978). Using their data the calculated cytosolic  $\text{NAD}^+/\text{NADH}$  ratio in fed chicken hepatocytes, incubated without exogenous substrate, was seen to drop from 2420 to 259 when the hepatocytes were incubated with 10.6 mM lactate plus 2.6 mM pyruvate. At the same time the hepatocytes were seen to change from net glucose users to net glucose producers with the addition of substrate and the diminished  $\text{NAD}^+/\text{NADH}$  ratio.

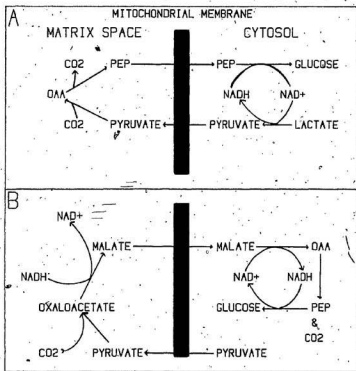


FIGURE 3  
 GLUCONEOGENIC PATHWAYS WITH  
 MITOCHONDRIAL (A) OR CYTOSOLIC (B) PEPCK

The dependence of gluconeogenesis on the redox state of the cytosol is further illustrated by another study of Ochs & Harris (1980). They reported that the addition of aminoxyacetate, (a transaminase inhibitor preventing the transfer of reducing equivalents between cytosol and mitochondria via the malate-aspartate shuttle), to hepatocytes provided with exogenous lactate reduced the  $NAD^+/NADH$  ratio still further to 13.6. Rather than causing an increase in gluconeogenesis from lactate however, an inhibition was seen. This was hypothesized to be due to a shift in the lactate dehydrogenase equilibrium and consequent diminished availability of pyruvate. Bannister & O'Neill (1981) likewise found that in chicken hepatocytes, a decrease in the cytosolic  $NAD^+/NADH$  ratio inhibited gluconeogenesis from lactate and glycerol but stimulated gluconeogenesis from pyruvate. Ogata et al. (1982) also found inhibition of gluconeogenesis from glycerol under similar conditions in chicken hepatocytes.

As gluconeogenesis in isolated chicken hepatocytes appears sensitive to the cytosolic  $NAD^+/NADH$  ratio, and as it has been found that the  $NAD^+/NADH$  ratio of fasted chicken liver, in vivo, is much higher than the conditions under which glucose is known to be formed in hepatocytes; it seems likely the availability of cytosolic reducing equivalents is a prime factor affecting hepatic

gluconeogenesis in vivo.

It is possible that a major role of hepatic gluconeogenesis in the chicken is to regulate plasma lactate levels. Riesenfeld et al. (1982) have shown that the intestine of the fowl attenuates potential perturbations in plasma glucose caused by the ingestion of carbohydrate, by converting the glucose to lactate. Perhaps one function of the fowl's liver in the post-prandial state may be to convert this lactate to glycogen or glucose and avoid any perturbations in blood lactate caused by feeding. The same reasoning would hold, in either the fed or fasted state, for the attenuation of a rise in blood lactate caused by an increase in its production during anaerobic glycolysis in tissues.

The liver of fed and fasted chickens is a consumer of glucose and amino acids (Chapter 3). While it appears that the amino acids taken up by the liver of a fed bird are used for biosynthetic processes, in the fasted bird, the concomitant release of urate from liver indicates that amino acids are degraded in this tissue. The source of urate in the circulation of a fed chicken is less clear. Urate is not released by the liver of a fed chicken. Barratt et al. (1974) and Badenoch-Jones & Buttery (1975) have reported that in perfused chicken liver or in hepatocyte preparations, when insulin is included in the medium, uricogenesis is stimulated. It is odd that urate release by

the liver is insignificant as insulin levels should not be low in a post-prandial chicken (Langslow et al., 1970).

It is not clear however what is happening to the lactate, glucose and carbon skeletons of the amino acids taken up by the fasted liver. The fate of the glucose taken up may be either incorporation into glycogen or lipid (the stress of an intracardiac saline injection is known to increase glycogen deposition in the fasting fowl, Davison & Langslow, 1975) or the fate of the glucose may be oxidation to acetyl CoA. Likewise, the fate of the lactate taken up by the liver of a fasting chicken may be incorporation into glycogen or lipid, or oxidation to acetyl CoA.

If incorporation into glucose or glycogen is the fate of the lactate which is taken up, it is in line with the hypothesis formulated in Section 2.4.3 of this thesis, concerning the effect of an oxidized NADH system in the cytosol on gluconeogenesis, but it does not explain the fate of the amino acids taken up.

If however, oxidation to acetyl CoA is the fate of the lactate, it implies that pyruvate dehydrogenase is not inactivated in the liver of a fasted chicken. The latter is not the case however as when I measured the amount of active pyruvate dehydrogenase in the fed and fasted states in the chicken, I found the percent active in each nutritional state to be  $91 \pm 3\%$  and  $10 \pm 2.6\%$ , of total activities of

4.47  $\pm$  .40 and 4.5  $\pm$  .40 micromoles/min/g. respectively (mean  $\pm$  S.E.M.).

An explanation of the fate of the carbon, taken up by the liver of a fasted bird may be found by considering that about five minutes elapsed between removing the bird, conscious, from the cage and finishing the task of securing it on its back. Another 2-3 minutes elapsed while the pentobarbital was injected into a wing vein and a surgical plane of anesthesia was attained. Approximately two more minutes elapsed before the abdominal cavity was opened and the ventilation begun. The bird was then maintained for five minutes under artificial ventilation before the first sample was taken. In all, about fifteen minutes elapsed between the first stress of handling and the sampling of abdominal blood vessels or freeze-clamping of liver or kidney tissue.

When the conscious bird is first removed from the cage and restrained on its back, it might be presumed that liver glycogen is depleted and blood glucose is increased over basal resting levels through enhanced gluconeogenesis and glycogenolysis (Freeman & Manning, 1976).

One explanation why the liver was taking up glucose, is that when the samples were taken, the bird was not in a steady-state. It is possible that glucose uptake by liver is the response of a chicken recovering from stress.

Glucose, amino acids and lactate may be removed from the circulation by liver for hepatic glycogen synthesis. This would imply that in the livers of the chickens at the time that the samples were taken, gluconeogenesis was active, as suggested in the discussion in Section 2.4.2. For glycogen deposition to occur from glucose synthesized in the liver and glucose taken up from the blood, glucose-6-phosphatase must be relatively inactive compared to the activities of hexokinase and glycogen synthase. While the activity of glucose-6-phosphatase and glycogen synthase are not known, if all the hexokinase reported to be in liver (Wals & Katz, 1981) is active, there is more than three times the activity needed to phosphorylate all of the glucose taken up.

The liver afferent-efferent differences for lactate, alanine, glutamine (1111, 128, 121 nmol/ml of blood respectively) and glucose (555 nmol/ml plasma) can be used to calculate the uptake of theoretical glucose equivalents using the liver blood flow rate of 1.92 ml/min/g (plasma flow = 1.15 ml/min/g)(Table 9) and a liver weight of 11.6 g in a six-day fasted bird weighing 650 grams (Table 8). The resulting figure for glucose equivalent uptake is 22.5 micromol/min/liver. If all this were converted to glycogen (which it probably is not as liver requires energy too), then the rate of glycogen deposition at the time of measurement would be 3.64 mg/min/liver or 0.31 mg/min/g of liver. However, even though pyruvate dehydrogenase is only

10% active, flux through this enzyme could account for 2.5 micromoles of glucose equivalents/min/liver. Thus, glycogen deposition per liver would be decreased by 0.4 mg/min/liver or 0.03 mg/min/g of liver and the estimate of the maximal rate of glycogen deposition is 3.2 mg/min/liver or 0.27 mg/min/g of liver. As the amount of glycogen in the liver five minutes after the initiation of ventilation is 35 milligrams (3 mg/g of liver), it is obvious that glycogen deposition cannot have been progressing at this rate for very long.

It is possible that this "recovery" from the stresses of the handling and surgical procedures, and the initiation of glucose uptake and glycogen synthesis began immediately after the bird was anesthetized. I consider it more likely however, that the "recovery" began after the initiation of ventilation (five minutes before the samples were taken) because the blood oxygen tension is significantly lower in the anesthetized bird while in the bird which has been ventilated for five minutes, the blood oxygen tension is not significantly lower than the conscious animal's (Table 1).

If the assumption that the liver is not in steady-state is correct then the measurements of afferent-efferent differences in this study may not reflect the normal condition in an unstressed chicken in vivo. One can only speculate what the case might be in an unperturbed chicken. The intracellular location of PEPCK will not have changed



and thus it might reasonably be expected that cytoplasmic availability of reducing equivalents would still be limiting for glucose synthesis from amino acids and pyruvate in the liver. Thus while gluconeogenesis from lactate in liver would be of physiological importance, pyruvate and amino acid gluconeogenesis should be low. One would like to believe that in the unperturbed chicken, glucose is not taken up by the liver, for this contradicts much that we know about intra-organ metabolite relationships in other animals. However the present study is the first to directly address this question in birds.

#### 4.2 GLUCONEOGENESIS IN THE KIDNEY

In light of the many reports of low rates of hepatic gluconeogenesis from amino acids in vitro, the question arises as to the source of new glucose in a fasting chicken. The Cori cycle is reported to be active in the fowl, (Brady et al., 1978; Belo et al., 1976a) and although it is reported to account for 50% of the glucose turnover in the fasted chicken (Riesenfeld et al., 1981) it cannot replace glucose lost through oxidation. It is a pathway for the recycling of lactate. It has been hypothesized that the reason that the liver of a chicken is able to produce glucose at significant rates from lactate while it is less able to do so from amino acids in vivo and in vitro, is due to the intramitochondrial location of PEPCK (Section 1.6.2).

The situation may be different in the kidney. There is a significant amount of cytosolic phosphoenolpyruvate carboxykinase in renal tissue, which is increased by fasting (Shen & Mistry, 1979; Watford et al., 1981). Chicken kidney tubules are also able to produce glucose at appreciable rates from pyruvate and amino acids (Wittman & Weiss, 1981; Watford et al., 1981). I have found by in vivo measurements of kidney afferent-efferent differences that glucose production occurs in fasted chickens and glucose uptake in fed chickens. (Sect. 3.3.2.2). Thus it would appear that the kidney is an important glucose producer in the fasted chicken.

In this thesis it is reported that in the chicken there is no change in the  $NAD^+/NADH$  ratio of the renal cytosol with fasting, and further, the  $NAD^+/NADH$  ratio in kidney cytosol is the same as in the cytosol of fed chicken liver. This would suggest that reducing equivalents are not limiting in the chicken kidney cytosol in either the fed or the fasted state.

The increase observed in glucose-6-phosphate in the kidney of a fasted chicken may result as the organ changes from a glycolytic tissue in the fed state to a gluconeogenic tissue in the fasted state. The glucose-6-phosphatase activity in fasted chicken kidney is reported to increase on starvation (Shen & Mistry, 1979) although there is an another report of a decrease in renal glucose-6-phosphatase

(Evans & Scholz, 1971). If the findings of Shen & Mistry (1979) are correct and if the activity of renal hexokinase does not change, then net renal glucose synthesis will be higher in the fasted than in the fed condition. As in a fasted chicken the glucose concentration in kidney tissue water, (content per gram divided by water content of tissue) is approximately equal to the concentration of glucose in arterial plasma, then the kidney should have less difficulty in releasing glucose into the bloodstream than would the liver, where tissue glucose is lower than the circulating levels.

From the afferent-efferent difference results, it appears that the kidney is the primary gluconeogenic organ in the fasted cockerel. Glucose is released by the kidney of a fasted bird and sufficient substrate in the form of amino acids, carnosine and lactate are taken up from blood to account for its biosynthesis.

If the kidney is a major producer of glucose in the fasted chicken this poses the question why this should be so. One possibility is suggested by the fact that the processes of uricogenesis and gluconeogenesis, both located in the liver in chickens, are energy requiring processes, expensive in their ATP requirements. For the bird there may be some metabolic advantage which is not yet clear, in compartmenting these two processes in separate organs.

It is intriguing to note while as much as 17% of the urate excreted in the urine may be synthesized in the kidneys of a fed bird, renal synthesis of urate in a fasting bird is much reduced<sup>13</sup> (Chin & Quebbeman, 1978) or non-existent (Martindale, 1976).

In the kidney, gluconeogenesis from amino acids, lactate and carnosine, may also be affected by the stress of the procedure used in this study. However, judging from the arterio-venous difference data, the effects of the procedure appear not to have had the same effect on kidney as on liver. Glucose is released by kidney, and sufficient substrate is taken up to account for its synthesis.

A possibility that remains however, is that gluconeogenesis in the kidney is turned on by stress, just as glycogen synthesis appears to be turned on in the liver. But, if the kidney does not make glucose under physiological conditions, and the liver is only able to produce glucose

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13 Although Chin & Quebbeman (1978) show that urate synthesis is reduced in the fasted kidney and accounts for only 15 % of the total urate synthesized in the fasted chicken, as the kidney tissue mass is approximately 20% of the total urate synthesizing tissue (liver + kidney), this means that the kidney synthesizes about as much urate per gram of tissue as does liver.

from reduced substrates (lactate and glycerol), it raises the question as to which organ is the primary gluconeogenic organ in the fasting chicken. As significant rates of gluconeogenesis have not been reported in any other organ, in mammals or birds there would appear to be no other source of glucose except for kidney. Thus I would conclude that when the metabolite level and arterio-venous differences measurements were made, metabolism in the kidney was little changed from metabolism in that organ under physiological conditions. Therefore, I conclude that the kidney in the fasted cockerel is the primary gluconeogenic tissue.

CHAPTER 5  
BIBLIOGRAPHY

## 5.1 LIST OF REFERENCES

(Abbreviations Used are from the World List of Scientific Periodicals, 4th Edition).

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APPENDIX A

A.1 AMINO ACIDS AND METABOLITES IN FOUR ABDOMINAL VESSELS  
OF FED AND FASTED, ARTIFICIALLY RESPIRATED CHICKENS.

Table A. Hepatic Vein Amino Acid and Metabolite Concentrations in Fed Birds.

AA	BLOOD	PLASMA	CELL
TAUR	3430 + 140	282 + 38	7900 + 180
HPRO	178 + 30	177 + 21	132 + 5
THR	309 + 24	410 + 28	177 + 48
SER	517 + 59	496 + 67	551 + 58
ASN	67 + 12	116 + 10	UD
GLU	672 + 29	452 + 32	943 + 57
GLN	443 + 79	577 + 85	153 + 27
PRO	205 + 21	329 + 44	UD
GLY	573 + 25	424 + 19	770 + 70
ALA	490 + 45	438 + 59	551 + 56
VAL	315 + 31	421 + 49	173 + 29
CYS	87 + 8	138 + 7	UD
MET	16 + 6	63 + 3	UD
ILE	127 + 19	171 + 29	69 + 9
LEU	226 + 31	307 + 44	121 + 22
TYR	136 + 5	134 + 11	111 + 6
PHE	80 + 5	96 + 6	54 + 9
BALA	390 + 23	UD	975 + 58
TRP	26 + 3	57 + 71	UD
ORN	22 + 3	36 + 6	UD
LYS	293 + 67	483 + 125	UD
HIS	62 + 5	98 + 8	UD
3MH	14 + 2	22 + 2	UD
ANS	579 + 72	UD	1450 + 180
CARN	1570 + 140	UD	3920 + 350
ARG	234 + 24	392 + 33	UD
GLYC	24 + 8	-	-
NH3	175 + 21	240 + 33	113 + 52
LACT	1740 + 390	ND	-
URIC	ND	352 + 26	-
GLUC	ND	11800 + 280	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7.

Table A. (cont) Hepatic Vein Amino Acid and Metabolite Concentrations in Fasted Birds.

AA	BLOOD	PLASMA	CELL
TAUR	3860 + 250	334 + 86	9160 + 550
HPRO	63 + 9	72 + 44	UD
THR	587 + 115	822 + 151	234 + 78
SER	332 + 69	312 + 81	362 + 55
ASN	36 + 10	44 + 12	UD
GLU	381 + 37	93 + 20	UD
GLN	273 + 59	331 + 110	185 + 61
PRO	103 + 23	169 + 12	UD
GLY	498 + 47	388 + 64	71 + 22
ALA	241 + 33	181 + 52	331 + 33
VAL	472 + 45	364 + 57	633 + 56
CYS	45 + 4	149 + 26	UD
MET	62 + 5	69 + 11	52 + 19
ILE	161 + 22	191 + 35	115 + 55
LEU	215 + 21	260 + 35	148 + 43
TYR	83 + 6	78 + 9	92 + 23
PHE	59 + 3	69 + 6	45 + 9
BALA	396 + 41	UD	990 + 104
TRP	29 + 2	62 + 6	UD
ORN	34 + 7	67 + 17	UD
LYS	200 + 32	324 + 56	UD
HIS	52 + 15	68 + 5	UD
3MH	42 + 6	74 + 14	UD
ANS	704 + 60	UD	1760 + 150
CARN	1250 + 136	UD	2825 + 155
ARG	147 + 8	242 + 11	UD
GLYC	56 + 14	ND	-
NH3	212 + 79	236 + 60	UD
LACT	1300 + 120	ND	-
URIC	ND	717 + 88	-
GLUC	ND	11850 + 320	-
GSH	1860 + 183	ND	-
GSSG	183 + 12	ND	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. For amino acids of fasted animals N=5 and for glucose and uric acid, N=14, for glycerol, N=2 and for lactate, N=11.

TABLE B. Hepatic Portal Vein Amino Acids and Metabolite Concentrations in Fed Birds.

AA	BLOOD	PLASMA	CELL
TAUR	3650 + 140	198 + 28	8870 + 450
HPRO	163 + 22	186 + 9	105 + 20
THR	333 + 23	464 + 40	170 + 50
SER	549 + 51	573 + 71	556 + 64
ASN	80 + 10	136 + 13	UD
GLU	751 + 46	562 + 30	1000 + 100
GLN	452 + 65	647 + 81	219 + 115
PRO	232 + 26	382 + 50	UD
GLY	650 + 43	544 + 36	807 + 76
ALA	567 + 57	622 + 42	515 + 102
VAL	338 + 31	451 + 60	186 + 32
CYS	108 + 8	229 + 12	UD
MET	14 + 7	88 + 9	UD
ILE	144 + 21	198 + 35	76 + 10
LEU	256 + 36	360 + 50	136 + 21
TYR	150 + 10	162 + 13	133 + 16
PHE	93 + 6	122 + 6	57 + 11
BALA	408 + 26	UD	1020 + 65
TRP	29 + 3	63 + 7	UD
ORN	24 + 3	37 + 6	UD
LYS	331 + 80	549 + 141	UD
HIS	71 + 2	117 + 7	UD
3MH	15 + 2	23 + 3	UD
ANS	585 + 62	UD	1460 + 150
CARN	1650 + 140	UD	4125 + 350
ARG	257 + 22	446 + 29	UD
GLYC	43 + 16	ND	-
NH3	198 + 33	343 + 41	111 + 56
LACT	2230 + 245	ND	-
URIC	ND	337 + 26	-
GLUC	ND	12330 + 300	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7.

Table B. (cont) Hepatic Portal Amino Acid and Metabolite Concentrations in Fasted Birds.

AA	BLOOD	PLASMA	CELL
TAUR	4200 + 282	255 + 112	10110 + 650
HPRO	82 + 13	69 + 42	103 + 56
THR	674 + 113	953 + 197	255 + 72
SER	448 + 73	464 + 79	423 + 83
ASN	56 + 8	63 + 7	47 + 15
GLU	430 + 31	125 + 21	886 + 70
GLN	404 + 46	514 + 79	238 + 30
PRO	180 + 18	211 + 14	132 + 41
GLY	602 + 49	532 + 46	709 + 64
ALA	389 + 37	372 + 33	413 + 52
VAL	584 + 55	403 + 49	856 + 116
CYS	51 + 4	183 + 19	UD
MET	74 + 8	96 + 14	40 + 4
ILE	187 + 20	224 + 33	132 + 21
LEU	254 + 23	304 + 34	179 + 22
TYR	113 + 5	117 + 9	108 + 14
PHE	84 + 7	105 + 11	53 + 7
BALA	420 + 35	UD	1130 + 31
TRP	27 + 3	77 + 9	UD
ORN	45 + 10	78 + 22	UD
LYS	264 + 33	409 + 48	UD
HIS	66 + 4	122 + 21	UD
3MH	46 + 9	83 + 17	UD
ANS	750 + 53	UD	1875 + 132
CARN	1400 + 80	UD	2886 + 198
ARG	174 + 12	269 + 16	UD
GLYC	78 + 28	ND	-
NH3	288 + 49	204 + 21	414 + 118
LACT	2440 + 175	ND	-
URIC	ND	561 + 62	-
GLUC	ND	12530 + 252	-
GSH	2086 + 180	ND	-
GSSG	183 + 12	ND	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. For amino acids of fasted animals N=5 and for glucose and uric acid, N=14 for glycerol, N=2, and for lactate, N=11.

Table C. Iliac Vein Amino Acid and Metabolite Concentrations in Fed Birds.

AA	BLOOD	PLASMA	CELL
TAUR	3440 + 130	195 + 34	8190 + 360
HPRO	193 + 33	188 + 15	207 + 60
THR	314 + 16	474 + 30	113 + 51
SER	526 + 37	600 + 56	469 + 55
ASN	72 + 4	160 + 12	UD
GLU	654 + 27	481 + 30	886 + 62
GLN	492 + 46	830 + 110	UD
PRO	190 + 19	389 + 42 <sup>7</sup>	UD
GLY	581 + 42	498 + 31	710 + 74
ALA	528 + 42	654 + 44	406 + 55
VAL	308 + 32	457 + 52	117 + 50
CYS	99 + 12	190 + 10	UD
MET	12 + 5	83 + 6	UD
ILE	128 + 19	178 + 36	100 + 17
LEU	226 + 32	318 + 58	100 + 26
TYR	140 + 4	163 + 8	109 + 11
PHE	84 + 5	116 + 7	UD
BALA	388 + 29	UD	970 + 73
TRP	31 + 2	60 + 5	UD
ORN	24 + 4	38 + 6	UD
LYS	305 + 72	539 + 137	UD
HIS	66 + 6	116 + 9	UD
3MH	14 + 2	25 + 3	UD
ANS	556 + 54	UD	1390 + 130
CARN	1560 + 160	UD	3900 + 400
ARG	245 + 30	455 + 42	UD
GLYC	67 + 22	ND	-
NHS	264 + 27	264 + 27	121 + 67
LACT	2580 + 410	ND	-
URIC	ND	340 + 26	-
GLUC	ND	12230 + 266	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7.



Table C. (cont) Iliac Vein Amino Acid and Metabolite Concentrations in Fasted Birds.

AA	BLOOD	PLASMA	CELL
TAUR	3950 + 340	375 + 86	9300 + 760
HPRO	71 + 15	74 + 46	UD
THR	659 + 112	984 + 210	174 + 76
SER	464 + 56	512 + 59	392 + 70
ASN	57 + 7	84 + 7	UD
GLU	384 + 30	81 + 19	UD
GLN	477 + 46	653 + 77	214 + 50
PRO	163 + 10	214 + 26	88 + 30
GLY	613 + 39	565 + 47	685 + 35
ALA	448 + 30	476 + 38	406 + 23
VAL	526 + 62	393 + 60	725 + 78
CYS	46 + 2	166 + 23	UD
MET	92 + 7	102 + 17	78 + 12
ILE	180 + 17	191 + 26	163 + 20
LEU	247 + 25	278 + 34	171 + 10
TYR	117 + 6	118 + 13	117 + 14
PHE	93 + 11	111 + 15	65 + 9
BALA	442 + 36	UD	1106 + 90
TRP	36 + 4	76 + 16	UD
ORN	50 + 12	84 + 22	UD
LYS	278 + 35	417 + 58	UD
HIS	76 + 6	128 + 24	UD
3MH	48 + 8	82 + 13	UD
ANS	771 + 79	UD	1930 + 200
CARN	1350 + 230	UD	2820 + 170
ARG	193 + 12	294 + 12	UD
GLYC	174 + 94	ND	-
NH3	200 + 20	173 + 18	300 + 20
LACT	2800 + 190	ND	-
URIC	ND	533 + 64	-
GLUC	ND	12180 + 293	-
GSH	1928 + 20	ND	-
GSSG	170 + 45	ND	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. For amino acids of fasted animals N=5 and for glucose and uric acid, N=14, for glycerol, N=2 and for lactate, N=11.

Table D. Renal Vein Amino Acid and Metabolite Concentrations in Fed Birds.

AA	BLOOD	PLASMA	CELL
TAUR	2910 + 380	195 + 37	6280 + 1130
HPRO	145 + 18	167 + 24	114 + 44
THR	307 + 18	471 + 31	109 + 51
SER	610 + 47	781 + 80	521 + 30
ASN	69 + 4	132 + 13	UD
GLU	727 + 39	636 + 24	885 + 85
GLN	406 + 40	677 + 96	UD
PRO	192 + 18	356 + 51	UD
GLY	573 + 39	492 + 31	704 + 93
ALA	465 + 32	500 + 45	514 + 37
VAL	298 + 41	415 + 53	180 + 21
CYS	102 + 10	206 + 11	UD
MET	21 + 8	86 + 5	UD
ILE	128 + 22	175 + 30	71 + 13
LEU	226 + 35	309 + 42	126 + 28
TYR	141 + 8	162 + 9	118 + 13
PHE	88 + 5	118 + 5	51 + 7
BALA	371 + 24	UD	930 + 60
TRP	27 + 2	55 + 3	UD
ORN	18 + 4	29 + 8	4 + 1
LYS	309 + 82	527 + 134	UD
HIS	67 + 5	118 + 8	UD
3MH	14 + 2	24 + 4	UD
ANS	545 + 42	UD	1360 + 105
CARN	1530 + 140	UD	3820 + 350
ARG	248 + 32	447 + 38	UD
GLYC	56 + 48	ND	-
NH3	396 + 58	397 + 58	253 + 39
LACT	2370 + 250	ND	-
URIC	ND	226 + 31	-
GLUC	ND	12750 + 405	-

The data are presented as the concentration of the amino acid in nanomoles per ml + S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured in both blood and plasma. In the fed state, N=6 for amino acids, for glucose and uric acid, N=14, for glycerol, N=3, and for lactate, N=7.

Table D. (cont) Renal Vein Amino Acid and Metabolite Concentrations in Fasted Birds.

AA	BLOOD	PLASMA	CELL
TAUR	3530 ± 190	358 ± 84	8280 ± 500
HPRO	80 ± 13	68 ± 42	UD
THR	618 ± 123	985 ± 179	UD
SER	528 ± 27	677 ± 41	305 ± 56
ASN	50 ± 5	71 ± 11	UD
GLU	371 ± 25	105 ± 27	770 ± 65
GLN	390 ± 38	545 ± 74	158 ± 81
PRO	157 ± 15	208 ± 16	102 ± 8
GLY	524 ± 29	503 ± 39	555 ± 32
ALA	311 ± 19	317 ± 35	302 ± 24
VAL	483 ± 60	409 ± 59	594 ± 102
CYS	40 ± 2	171 ± 27	UD
MET	77 ± 6	99 ± 15	44 ± 15
ILE	165 ± 26	212 ± 26	UD
LEU	229 ± 30	288 ± 30	140 ± 39
TYR	106 ± 8	114 ± 10	96 ± 13
PHE	82 ± 11	108 ± 14	44 ± 10
BALA	439 ± 31	UD	1096 ± 78
TRP	30 ± 2	61 ± 4	UD
ORN	51 ± 13	80 ± 27	16 ± 3
LYS	257 ± 38	405 ± 50	UD
HIS	82 ± 16	138 ± 28	39 ± 16
3MH	48 ± 10	85 ± 15	UD
ANS	666 ± 62	UD	1666 ± 154
CARN	1210 ± 32	UD	2410 ± 77
ARG	142 ± 13	235 ± 20	UD
GLYC	55 ± 47	ND	-
NH3	344 ± 59	322 ± 28	378 ± 112
LACT	2230 ± 110	ND	-
URIC	ND	168 ± 30	-
GLUC	ND	12860 ± 274	-
GSH	1934 ± 16	ND	-
GSSG	160 ± 52	ND	-

The data are presented as the concentration of the amino acid in nanomoles per ml ± S.E.M. "ND" indicates that the metabolite was not determined. "UD" denotes that the concentration was too low to be determined or was calculated to have a negative sign. A dash, "-", means that the cellular content could not be calculated as the substance was not measured, in both blood and plasma. For amino acids of fasted animals N=5 and for glucose and uric acid, N=14 and for lactate, N=11.

APPENDIX B

B.1 EFFECT OF BLOOD SAMPLING ON HEMATOCRIT AND PLASMA GLUCOSE.

CHICKEN 1

TIME MIN.	PLASMA GLUCOSE			MEAN ± SD	HEMATOCRIT
	i	ii	iii		
0	14.9	14.7	15.7	15.1 ± 0.5	35%
2	15.5	16.0	16.1	15.9 ± 0.3	38%
4	16.1	16.2	16.2	16.2 ± 0.1	34%
6	15.5	15.7	15.8	15.7 ± 0.2	34%
8	15.5	15.2	16.3	15.7 ± 0.6	33%

CHICKEN 2

TIME MIN.	PLASMA GLUCOSE			MEAN ± SD	HEMATOCRIT
	i	ii	iii		
0	13.7	13.8	13.6	13.7 ± 0.1	32%
2	14.2	14.3	14.7	14.4 ± 0.3	32%
4	13.8	13.9	14.7	14.1 ± 0.5	32%
6	14.0	14.2	14.2	14.1 ± 0.1	31%
8	14.2	14.1	14.4	14.3 ± 0.2	31%

PLASMA GLUCOSE RANDOMIZED COMPLETE-BLOCK ANALYSIS OF VARIANCE,  
 $F=(6.313,4,4)$  (Not significant at  $P < 0.05$ )

HEMATOCRIT ONE WAY ANALYSIS OF VARIANCE  $F=(0.43,4,5)$  (Not significant at  
 $P < 0.05$ )

Juvenile cockerels (5 weeks old) were anesthetized, restrained on their backs, and artificially ventilated as described in the methods in Chapter 2. A cannula was placed in the inferior aorta at the level of the femoral arteries and a 2 ml blood sample was withdrawn. Four more 2 ml samples were taken over a period of 8 minutes. The plasma was obtained and analysed for glucose as described in the methods of Chapter 3. A sample of the blood was collected in a heparinized microhematocrit tube, centrifuged at  $12,000 \times g$  and hematocrit was calculated using the ratio of the volume of red cell mass divided by the total blood volume. Analysis of variance indicated that there was no effect of sampling on either hematocrit or plasma glucose.

APPENDIX C

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C.1 INDEX

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