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THE RELATION BETWEEN NUTRITIONAL AND HORMONAL EFFECTS ON THE ENZYMES OF NITROGEN METABOLISM

Denise Christowitz

A dissertation presented for the degree of Master of Science in the Faculty of Science at the University of the Witwatersrand.

Johannesburg.

December 1975.

DECLARATION

I declare that the dissertation which is herewith submitted for the degree of Master of Science in the University of the Witwatersrand is entirely my own work and that it has not been previously submitted for a degree in any other university.

D. Christowitz

December 1975.

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Abbreviations used in the text

C.P.S.	- carbamyl phosphate synthetase
O.T.C.	- ornithine transcarbamylase
A.S.S.	- argininosuccinate synthetase
A.S.L.	- argininosuccinate lyase
Arg.	- arginase
AMP	- adenosine monophosphate
ATP	- adenosine triphosphate
N.S.	- not significant
U.S.P.(unit)	- United States Pharmacopoeia (unit)
I.U.	- International Unit

CHAPTER 1 - INTRODUCTION

1.1 Nitrogen Metabolism

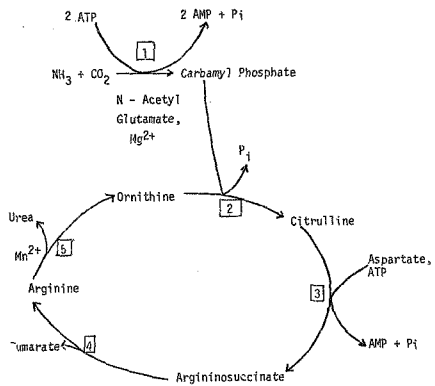
1.1.1 Synthesis of Urea

Urea is the main nitrogenous end product of amino acid catabolism in several groups of vertebrates, including the mammals. Its synthesis represents the conversion of ammonia, the primary but toxic product of amino acid deamination, to a harmless substance excreted by these animals.

Urea is synthesized in the liver by a series of reactions known as the Krebs urea cycle (Krebs and Henseleit, 1932). According to the original scheme of Krebs and Henseleit, urea is formed from ammonia and carbon dioxide by a cyclic process in which ornithine, citrulline and arginine are intermediates. The cycle is now known to involve five enzymic steps (Diagram 1). Ratner (1947) showed that aspartate is the specific nitrogen donor in the conversion of citrulline to arginine. Thus only one half of the nitrogen of urea originates from free ammonia.

The most important donor of amino nitrogen for the formation of aspartate in mammals is glutamate (Berl et al., 1967). Nitrogen is transferred from all amino acids to glutamic acid by transamination with α- ketoglutaric acid. This nitrogen is then converted to urea via glutamic - aspartic transaminase and the urea cycle. These nitrogen-transferring reactions and the generation of oxaloacetate and α-ketoglutarate by way of the citric acid cycle are interdependent (see Diagram 2).

The enzymes of arginine biosynthesis are widely distributed in nature to provide arginine for the synthesis of protein and of phosphogens.



1	Carbamyl Phosphate Synthecase
2	Ornithine Transcarbamylase
3	Argininosuccinate Synthetase
4	Argininosuccinate Lyase
5	Arginase

Diagram 1 - The Urea Cycle and Enzymes of Urea Biosynthesis

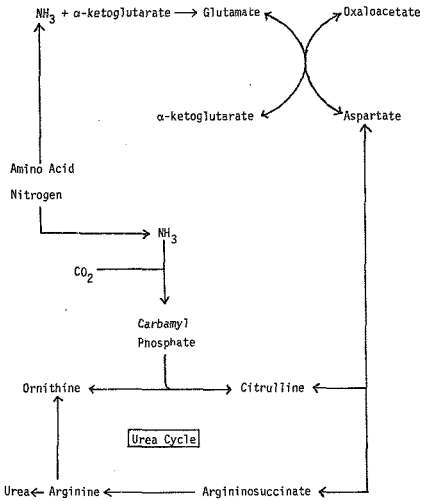


Diagram 2 - Synthesis of Glutamate and Urea Formation

In ureotelic animals, including all mammals and most adult amphibians, the coupling of arginine biosynthesis to a high activity of arginase in the liver results in a functional urea cycle.

1.1.2 The Enzymes of the Urea Cycle

Diagram 1 shows the urea cycle and the reactions catalysed by the five enzymes : carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinate lyase and arginase.

1.1.2.1 Carbamyl Phosphate Synthetases

Grisolia and Cohen (1952) showed that the synthesis of citrulline from ornithine is carried out in two steps each being catalysed by separate enzymes, namely C.P.S. and O.T.C. Carbamyl phosphate synthetase is localised in the mitochondria of ureotelic livers, together with O.T.C. Thus the formation of citrulline occurs via the formation of an intermediate, carbamyl phosphate, which is synthesized from carbon dioxide and ammonia with the aid of the enzyme, C.P.S.

Jones et al. (1955) chemically synthesized carbamyl phosphate and thus elucidated its structure. They also showed that carbamyl phosphate could be used in the first step of two different biosynthetic pathways, namely the synthesis of arginine and urea in the urea cycle, and the synthesis of pyrimidines. Hager and Jones (1967) showed that there are two carbamyl phosphate synthetases, one only being present in ureotelic species. In ureotelic livers the enzyme catalyzing the synthesis of carbamyl phosphate in the urea cycle is known as carbamyl phosphate synthetase I (E.C. 2.7.2.5).

Recently Guthöhrlein and Knappe (1968) have prepared homogeneous

rat liver synthetase with a molecular weight of 250 000 and $S_{20,w}$ and D_{20} values of 10,95 and 4,02, respectively, and a specific activity of 1,67 µmoles/min./mg. The synthetase from vertebrate liver catalyses the overall reaction in which 2 moles of ATP are utilised to form carbamyl phosphate as shown in reaction 1.

$$NH_4 + HCO_3^- + 2\ ATP^{4-} \xrightarrow[\text{acetyl - glutamate}]{Mg^{2+}} \underset{\text{carbamyl phosphate}}{NH_2CO_2PO_3^{2-}} + 2ADP^{3-} + P_i^{2-} + 2H^+ \qquad (1)$$

Guthöhrlein and Knappe (1968) have elucidated the mechanism by which acetylglutamate functions as an allosteric effector. An activation reaction was detected that is acetylglutamate-dependent. ATP and Mg^{2+} ions stabilise the active conformation of the enzyme which is activated from the catalytically inactive conformation.

The reaction resulting in the formation of carbamyl phosphate has been shown by Metzenberg et al. (1958) to consist of at least two steps. The first utilises ATP to activate carbon dioxide, and the second, which is reversible, utilises ATP to form the C-N bond. Jones and Spector (1960) have shown that activated carbon dioxide takes the form of an enzyme - carboxy phosphate, the anhydride, carboxy phosphate, being an intermediate in the reaction. The following sequence has been proposed (Meister, 1965) :

$$E + HCO_3^- + ATP \longrightarrow E - \text{carboxy phosphate} + ADP \qquad (2a)$$

$$E - \text{carboxy phosphate} + NH_4^+ \longrightarrow E - \text{carbamate} + Pi \qquad (2b)$$

$$E - \text{carbamate} + ATP \longrightarrow E + \text{carbamyl phosphate} + ADP \qquad (2c)$$

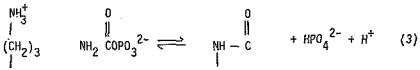
Guthöhrlein and Knappe (1969) have proposed two possibilities concerning the above sequence :

(a) that steps 2a and 2b occur simultaneously as a concerted reaction, and

(b) that 'active CO_2' is formed first in a rapid, reversible step, and that ADP and Pi are released from the enzyme only on reaction with NH_4^+.

1.1.2.2 Ornithine Transcarbamylase

Ornithine transcarbamylase (E.C. 2.1.3.3) catalyses the formation of citrulline from carbamyl phosphate and ornithine as shown in reaction 3.



(3)

In 1957, O.T.C., about 92% pure, with a c activity of 200 µmoles/min./mg. at pH 7,3 and 37°C, was p: om rat liver by Reichard (1957). The main component has an $s_{20,w}$ value of 5,6 S. The equilibrium at 37°C greatly favours citrulline formation as is shown by the equilibrium constant :

$$K_{eq} = \frac{(\text{citrulline})\ (P_i)}{(\text{ornithine})(\text{carbamyl phosphate})} = 1 \times 10^5 \text{ at pH } 7,4$$

(Reichard, 1957)

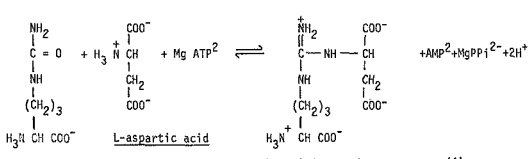
O.T.C. from bovine liver has a specific activity of 880 µmoles/min./mg. protein at pH 8,4 (Cohen and Marshall, 1962). Cohen and Marshall (1962) isolated O.T.C. from bovine liver and found its molecular weight to be 110 000.

From kinetic studies on the purified enzyme (beef liver), Joseph et al. (1964) felt their data supported the hypothesis that the mechanism of action of O.T.C. involves binding of the two substrates (carbamyl phosphate and ornithine) simultaneously at different sites on the enzyme. They further suggested that the carbamyl phosphate binds almost entirely through the phosphate group.

1.1.2.3 Argininosuccinate Synthetase

Ratner and Pappas (1949), and Ratner and Petrack (1951, 1953) identified and separated two enzymes which catalyse the conversion of *citrulline to arginine.* This conversion thus takes place in two steps; firstly, the synthesis of an intermediate, argininosuccinate, from citrulline and aspartate, catalysed by the condensing enzyme, A.S.S., and, secondly, the cleavage of argininosuccinate to form fumarate and arginine, catalysed by A.S.L. (see Diagram 1). These two enzymes occur together in the liver cytosol of *all ureoteli: species and in lesser* amounts in the kidney and other tissues.

In the reaction catalysed by A.S.S. (E.C. 6.3.4.5), 1 mole of is utilised in the reversible condensation between citrulline and aspartate *(Ratner,* 1954) as shown in reaction 4.

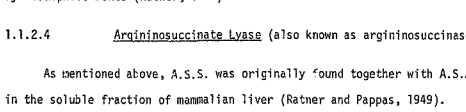


L-citrulline L-aspartic acid L-argininosuccinate (4)

Only the synthetases from hog kidney (Schuegraf et al., 1960) and bovine liver (Rochovansky and Ratner, 1961 and 1967) have been extensively purified. The crystalline enzyme has a specific activity of 3,83 µmoles/min./mg. at 38°C and homogeneous as judged by sedimentation velocity and gel electrophoresis. The enzyme has a molecular weight of 175 000 and is composed of 4 subunits each 45 000 in molecular weight (S.D.S. - gel electrophoresis in the presence of reducing agents). A molecular weight of 90 000 was obtained in the absence of a reducing agent. Thus the enzyme is a tetramer in which two pairs of monomers are cross-linked by disulphide bonds (Ratner, 1973).

1.1.2.4 Argininosuccinate Lyase (also known as argininosuccinase)

As mentioned above, A.S.S. was originally found together with A.S.A. in the soluble fraction of mammalian liver (Ratner and Pappas, 1949). The two enzymes were separated and A.S.L. (E.C. 4.3.2.1) was shown to catalyse the reversible cleavage of argininosuccinate to arginine and fumarate (Ratner and Petrack, 1951, 1953) - see reaction 5.



(5)

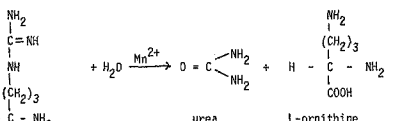
A.S.L. has been partially purified by Ratner et al. (1953) from bovine liver. A.S.L. from bovine liver has a molecular weight of 202 000 at pH 7,5. In Tris buffer, however, dissociation into subunits occurs

and inactivation results. The inactivation is reversible and is prevented by L-arginine, L-argininosuccinate or Pi (Havir et al., 1965). Bovine liver A.S.L. has a specific activity of 15,3 µmoles/min./mg. at pH 7,5 (Havir et al., 1965).

A.S.L. has also been detected in the kidney (Bray and Ratner, 1971) and in brain tissue of mammalian species, including primates (Ratner et al., 1960).

1.1.2.5 Arginase

Arginase (E.C. 3.5.3.1), a soluble cytoplasmic enzyme, is most abundant in the livers of ureotelic animals, where it has the highest in vitro activity of the five urea cycle enzymes. Arginase catalyses the irreversible hydrolysis of L-arginine to ornithine and urea, as shown in reaction 6.



L-arginine urea L-ornithine (6)

Mn^{2+} ions are required for arginase action and stabilisation (Van Slyke and Archibald, 1946). Van Slyke and Archibald (1946) devised the first initial-rate assay and undertook the specification of arginase. Arginase from rat liver has been purified by Schimke (1964b) and by Hirsch-Kolb and Greenberg (1968). Rat liver arginase has a molecular weight of 118 000 in the oligomeric form and an $S_{20,w}$ value of 6,1.

Its specific activity is 790 µmoles/min./mg. protein (Hirsch-Kolb and Greenberg, 1968). Bovine liver arginase has a specific activity of 1040 µmoles/min./mg. at pH 10,2 (Greenberg, 1960).

1.1.3 Intracellular Concentrations of the Urea Cycle Enzymes

In the course of in vivo studies enzyme levels have been determined in both human and rat livers. Table 1 shows the activities of the urea cycle enzymes in rat and human liver.

Table 1 - Activity of Urea Cycle Enzymes in Liver

	Rat[a]		Man[a]	
	µmoles/min./g. wet wt. liver	µmoles/min./mg. protein	µmoles/min./g. wet wt. liver	µmoles/min./mg. protein
C.P.S.	9,98 ± 0,833 (5)	0,134 ± 0,016 (5)	6,00 ± 0.283 (2)	0,052 ± 0,008 (2)
O.T.C.	380 ± 1,20 (5)	5,52 ± 0,317 (4)	141 ± 9,17 (2)	1,85 ± 0,017 (2)
A.S.S.	1,42 ± 0,700 (5)	0,220 ± 0,001 (5)	0,860 ± 0,057 (2)	0,013 ± 0,000 (2)
A.S.L.	2,50 ± 0,107 (2)	0,267 ± 0,083 (2)	2,90 ± 0,100 (2)	0,044 ± 0,004 (2)
Arg.	497 ± 180 (5)	7.00 ± 0 533 (5)	129	1,80

(a) From Brown and Cohen (1960).

Although C.P.S. and O.T.C. occur together in the mitochondria, the level of O.T.C. is many times the level of C.P.S. Consequently, there is an efficient utilisation of carbamyl phosphate. The situation of these two enzymes in one organelle, namely, the mitochondrion provides for an efficient coupling of the two reactions. The fact that ATP is also produced in the mitochondrion by oxidative phosphorylation, allows for an efficient conversion of ammonia to urea and a consequent low level of ammonia.

1.1.4 The Rate-Limiting Step

On the basis of enzyme levels, A.S.S. has been considered to be *the rate-limiting enzyme.* Freedland and Sodikoff (1962) regard A.S.S. as an important 'pacemaker enzyme'. Janssens and Cohen (1966) also *mention* that A.S.S. is rate-limiting in the African lungfish. Ratner (1973) maintains that, since the two synthetase levels (C.P.S. and A.S.S.) are both low, the possibility should be considered, that one or more intracellular substrate levels (ATP, citrulline, aspartate) may be rate-limiting.

There is, however, evidence that C.P.S. may be rate-limiting in certain species. It is the only urea cycle enzyme which shows any change in aestivating animals. The activity is raised six-fold in aestivating animals as has been shown by Balinsky et al. (1967 a, b). On return to water, the activity drops to the normal level.

Arginase does not seem to be rate-limiting to urea synthesis. This is indicated by the very high observed activity, the presence of the enzyme in non-ureotelic species and also by its relatively high Km (0,0116M) for arginine at pH 8,4 (Hunter and Downs, 1945). This is in excess of the intracellular concentration of this substance (30-50 µM). This means that the enzyme is always likely to have 'reserve capacity' to increase its rate in response to any accumulation of arginine (Balinsky, 1970a).

1.1.5 Co-ordinated Changes in the Enzymes of the Urea Cycle

The variables that can affect enzyme levels include, in intact animals, nutritional, hormonal, developmental, thermal, circadian and genetic factors (Schimke, 1973). Some of the factors affecting urea cycle enzyme levels are described below.

1.1.5.1 Changes with Diet

It has been known ever since the work of Lightbody and Kleinman (1939) that increased protein content of the diet more than doubles the rat liver arginase activity per gram dry weight. In a study of the effects of *variation in dietary protein upon hepatic arginase in rat*, Mandelstam and Yudkin (1952) found that the amount of enzyme, both in relation to unit wet weight of liver and total amount of protein, hepatic nitrogen and body weight increases with an increase in dietary protein. Ashida and Harper (1961) fed rats on diets containing 25-70% protein and found that total liver arginase was proportional to the quantity of urea excreted. They also demonstrated that the enzyme change was complete about 9 days after the maximum change in protein content of the diet. Again, daily urea excretion throughout this period was proportional to the rising level of arginase.

Schimke (1962a) in one of the earliest articles on the regulation of a metabolic pathway, demonstrated that all the other enzymes of the urea cycle as well as arginase changed as a co-ordinated group in proportion both to the urea excreted and to the protein consumed. In the study, each group of animals was maintained at each level of protein intake (15% and 60% protein) for two weeks, since four to eight days were required to reach the full increase in activity. Attainment of the new level was independent of growth. There was evidence that the increases were due to increases in the absolute amount of enzymes. Regulation of the urea cycle enzymes by diet was also demonstrated by Das (1971).

Similar adaptive increases have been found by Nuzum and Snodgrass (1971) in primates, including man. Stephen and Waterlow (1968) found that A.S.L. levels are reduced in malnourished children and can be elevated by refeeding.

Co-ordinated changes of the five enzymes suggested that urea formation may be under integrated regulation as a pathway. The activities of other hepatic enzymes showed a varied response to protein increase. Malate and glu ate dehydrogenase activities did not change significantly in animals fed on high protein diets; lactic dehydrogenase decreased, and glucose - 6 - phosphate dehydrogenase increased, although only on the highest protein intake (60%). However, the enzymes closely related to the operations of the urea cycle, glutamic-alanine and glutamic-aspartic transaminases, were increased approximately twofold (Schimke, 1962a).

Amino acid load was found to be a most important contributing factor to regulation of enzyme levels. Schimke (1962b) compared the effects of a period of fasting with changes found after the animals had been maintained on a protein-free diet in which carbohydrate serves as the energy source. Urea excretion was greatly diminished on the protein-free diet and this was linked to the appreciable decreases in the levels of activity of urea cycle enzymes. In contrast urea excretion during fasting was greatly elevated because of the breakdown of tissue protein for energy needs, and this was associated with co-ordinated increases in enzymic activity. The increases occurred during a period in which the total liver protein was being depleted. Many other enzymes decreased in activity during starvation, whereas glutamic dehydrogenase, glutamic-aspartic transaminase, and glutamic-alanine transaminase activities were found to increase (Schimke, 1962b). The latter increases again point to regulation of the whole pathway, as these enzymes are closely related to the urea cycle. Administration of urea cycle intermediates, ornithine, citrulline, arginine and of urea, resulted in no changes in activity, nor were significant changes produced in the intracellular concentrations of these

amino acids by fasting, by a protein-free diet, or by a protein-rich diet (Schimke, 1963).

An alteration in the total amount of arginase can be brought about by a change in the rate of synthesis or in the rate of degradation of the enzyme. Schimke (1964b) has analyzed these two processes through a study of enzyme protein turnover. The rate of incorporation or release of ^{14}C-labelled amino acid was used to determine the rate of synthesis or degradation, and the absolute amount of enzyme. Rates of synthesis and degradation were determined (a) during starvation, when urea excretion and amount of arginase are greatly increased and (b) after a change from a 70% to an 8% protein diet, which results in a decrease of urea excretion and arginase levels.

Schimke found that the balance between synthesis and degradation could be shifted. The increase in arginase levels during starvation was found to be due, primarily, to an increased synthesis of the enzyme (Schimke, 1964b). A change from a high protein (70%) to a low protein (8%) diet caused a rapid decrease in total arginase over a nine-day period (Schimke, 1964b). Schimke found the pattern of change as follows : during the first three days of diet shift, there was a decreased rate of arginine synthesis, and an accelerated rate of degradation. Thus, dietary regulation of enzyme degradation and synthesis has been demonstrated.

1.1.5.2 The Effect of Temperature on Arginase Levels

Rats exposed to cold for several days lost weight and had a decreased nitrogen retention despite their increased food consumption. Knox and Greengard (1965) also found a rise in liver arginase in rats exposed to the cold. This elevation of liver arginase in the cold was thought to be due to increased protein consumption of the animals rather than to adrenal stimulation.

1.1.5.3 Changes during Amphibian Metamorphosis

During metamorphosis, the amphibian undergoes change from the ammoniotelic larva to the terrestrial ureotelic adult. In metamorphosing Rana catesbeiana tadpoles, the urea cycle enzymes showed increased activity, the increase of C.P.S. being the most marked (Brown et al., 1959). Urea excretion increased in proportion to the rise in enzymatic activity. Two other enzymes directly related to the urea cycle, glutamic dehydrogenase and glutamic-aspartic transaminase, underwent a tenfold and fivefold increase, respectively, during metamorphosis, but lactic dehydrogenase and malate dehydrogenase showed no significant changes in activity (Cohen, 1964-1965).

1.1.5.4 Changes during Mammalian Foetal and Neonatal Development

In the rat, it was found that the activity of urea cycle enzymes, was measurable only a few days before birth, and there was a rapid rise one day before birth which continued into the post-natal period for a few days (Räihä and Suihkonen, 1968; Kennan and Cohen, 1959; Charbonneau et al., 1967; Illnerova, 1968). With weaning and the resulting increase in protein consumption, a second rise in activity was found (Greengard et al., 1970 and Illnerova, 1968).

The pattern of change in human development was found to be similar to that in the rat. Individual enzymatic activities could, however, be detected much earlier in faetal life, namely, at eight weeks. Adult levels were almost reached within a few weeks after birth (Kennan and Cohen, 1959, 1961).

1.1.5.5 Response to Hormones

Corticosteroid administration results in tissue breakdown and accelerates gluconeogenesis. Schimke (1963) found that daily administration of large doses of cortisol (25 mg. per 100g. body weight) caused an increase of the urea cycle enzyme levels in proportion to the increase in urea excretion. Adrenalectomy reduced enzymic activities (Schimke, 1963; McLean and Gurney, 1963; Freedland, 1964). This reduction is probably related to decreased gluconeogenesis due to the removal of the source of cortisol.

The presence of an intact adrenal gland, however, was not necessary for the increase in enzyme levels produced by a high protein diet, as shown by Schimke (1963). He found that an abrupt change in dietary protein from 15% to 60% protein, resulted in increased levels of urea cycle enzymes in adrenalectomised rats. This finding paralleled the increases seen in the intact rat.

On the other hand, the extent of change in levels of the urea cycle enzymes on adrenalectomy, was dependent on the dietary protein content. This dependence on dietary protein was reflected in the more extensive drop in activity due to adrenalectomy in animals that had been maintained on a 20-30% protein diet, than in animals maintained on the 15% protein diet (Schimke, 1963).

Glucagon administration increased the activites of the urea cycle enzymes (McLean and Novello, 1965). Although starvation is associated with a physiological increase in glucagon, the aforementioned increases were less marked than the increases produced by starvation and found in alloxan-diabetic animals. Insulin caused an antagonistic effect to

glucagon on C.P.S. and A.S.S., but the arginase levels were unaffected by insulin. Glucagon increased the levels of urea cycle enzymes in perfused rat livers (Penhos et al, 1966, 1968), indicating that the effect of the hormone is directly on the liver. No effect of insulin was seen in vitro., suggesting that its effect may be more complex.

The decreased levels of arginase seen on hypophysectomy (Fraenkel-Conrat et al., 1943) seemed to be a secondary effect as it was less than the decrease with adrenalectomy. This may be due to the opposing actions of Adrenocorticotropic hormone which,like corticosteroids, elevated the level of the enzyme (Beaton, 1963), and growth hormone, which depressed it (Beaton et al.,1953).

Thyroxine is a hormone which has effects on numerous metabolic functions and especially those related to energy utilisation. Studies have been undertaken on the effects of thyroxine on urea cycle enzymes.

The most spectacular effects of thyroxine on urea cycle enzymes were seen in amphibian metamorphosis. Thyroxine treatment of premetamorphic tadpoles caused them to undergo premature metamorphosis. At the same time increases in the levels of urea cycle enzymes were seen (Dolphin and Frieden, 1955; Paik and Cohen, 1960; Metzenberg et al., 1961; Wixom et al., 1972). The fact that these increases occurred earlier than they do in normal metamorphosis (Dolphin and Frieden, 1955; Wixom et al., 1972) suggested that these increases are directly induced by thyroxine.

Paik and Cohen (1960) studied the effect of thyroxine administration to tadpoles by immersion in water containing the hormone. They found a lag in the increase of C.P.S. levels during the first four to six days (Paik and Cohen, 1960). Thyroxine administration by injection shortened the

lag phase, but did not abolish it (Paik and Cohen, 1960; Frieden and Westmark, 1961; Kim and Cohen, 1968a, b; Tata, 1965.

The increase in level of C.P.S. has been shown to involve de novo synthesis of the enzyme (Marshall, et al., 1961; Shamb ugh et al., 1969). However, the effect of the hormone does not appear to be a simple one.

Shambaugh et al. (1969, studied the effect of thyroxine on C.P.S. in an organ culture of frog liver in vitro. They found increases in the specific activity of the enzyme in thyroxine-treated preparations. No lag period was observed in vitro. While thyroxine treatment increased the incorporation of ^{3}H-leucine into enzyme protein, the main effect of the hormone appeared to involve a conversion of precursor protein.

During mammalian development, thyroxine also plays a role in the induction of the urea cycle. This is indicated by the observation of Greengard et al.,(1970) that arginase activity in foetal rat liver was increased by thyroxine treatment.

There is also evidence of the effect of thyroxine on the urea cycle in adult rats, though these effects are less straightforward. Nazario and Cohen (1961) found that thyroidectomy had no effect on the activities of C.P.S., O.T.C. and arginase in regenerating rat liver. Grillo and Fossa (1966) showed that the rate of urea synthesis was significantly lower in hypothyroid than normal rats. They reported that the levels of the arginine synthetase system and arginase remained unchanged, while C.P.S. and O.T.C. activities were increased. The level of urea in the blood was increased but less urea was excreted.

Thyroxine administration to normal rats caused an increase in the level of arginase in females (Lightbody et al., 1941), but not in males.

C.P.S. activity was unchanged, and O.T.C. was actually decreased by thyroxine treatment (Grillo, 1964).

Wu et al. (1971) suggested that the effect of thyroxine on arginase is related to that of the corticosteroids. In contrast to the above findings, they found that thyroxine depresses arginase activity in intact rats.

1.1.5.6 Independent Regulation of Arginase

Schimke (1962b, 1963) has produced evidence indicating that the regulation of mammalian arginase is often different from that of the other four urea cycle enzymes. Thus, adrenalectomy produced a large decrease (70-80%) in arginase activity, while the activities of the other enzymes only decreased by approximately 30% (Schimke, 1962b). In another experiment, weanling rats were deprived of arginine. This resulted in decreased urea synthesis. Arginine supplementation to the diet then produced twofold increases in the four enzymes of arginine synthesis (C.P.S., O.T.C., A.S.S. and A.S.L.) whereas arginase activity decreased (Schimke, 1963).

Schimke (1964a) examined the effects of arginine on arginine synthesis in cell cultures of He La and L mammalian cell lines. A.S.S. and A.S.L. activities in cells grown in a limiting concentration of arginine (0,2 mM) were increased as much as 15-fold over the activities in cells grown in a high-arginine medium (2,0 mM). Arginase activity was, however, increased in the high medium. This again points to a different regulation mechanism of arginase.

The independent regulation of arginase is probably connected with the fact that the other four enzymes of the urea cycle serve also

to synthesize arginine, an amino acid needed for protein synthesis. Arginase, on the other hand, only serves the function of urea synthesis.

1.2 General Aspects of Hormonal Control

The general effects of the two hormones used in the present study will be discussed below, together with the effect of removing the endocrine glands responsible for synthesis.

1.2.1 The Adrenocortical Hormones

The adrenal glands lie in relation to the upper poles of the kidneys. Each consists of an inner medulla, which secretes adrenaline and noradrenaline, and an outer cortex which secretes the adrenocortical hormones. The secretion of most adrenocortical hormones is controlled by the pituitary adrenocorticotropic hormone. This in turn is dependent upon the corticotropin releasing factor found in the hypothalamus and transmitted to the adenohypophysis through the portal system of blood vessels which link the median eminence with the anterior lobe of the pituitary gland.

The adrenal steroids influence a wide variety of biochemical and physiological phenomena, some of which are probably a consequence of secondary effects of a primary role, and others which may be even more indirect results of initial actions. The adrenocortical hormones may be classified into two groups according to their metabolic effects and other actions :

(a) The 'glucocorticoid' or carbohydrate-regulating corticosteroids which bring about alterations in carbohydrate, protein and lipid metabolism.

(b) The sodium-retaining hormones, 'mineralocorticoids', e.g. aldosterone.

The administration of cortisol (a glucocorticoid) results in increased glucose release from the liver, increased glycogenesis and augmented gluconeogenesis from amino acids, leading to glycogen deposition in the liver, and a decreased peripheral utilisation of glucose. The augmented glucose release by the liver in response to adrenal steroid administration is probably due to an elevation of glucose- 6 - phosphatase activity. There is an increase in the activities of specific liver transaminases, pyruvate carboxylase and glycogen synthetase activity, resulting in an elevation of glycogen stores (White et al., 1959).

Incorporation of amino acids into protein in non-hepatic tissues is inhibited, but protein synthesis in liver is stimulated. Thus, amino acids are available for gluconeogenesis in the liver and urea synthesis is increased. Cortisol is known to induce several enzymes of nitrogen metabolism. Tryptophan oxygenase (Knox, 1951; Fiegelson et al.,1962), tyrosine transaminase (Kenney and Flora, 1961) and glutamic pyruvic transaminase (Morita and Kawada, 1962) were induced within several hours of cortisol administration. The effect of cortisol on urea cycle enzymes has already been discussed.

Cortisol has been shown to act directly on the liver. Goldstein et al. (1962) showed induction of tyrosine transaminase and tryptophan oxygenase in vitro using perfused livers. The induction of tryptophan pyrolase in vitro seems to be due to an increased amount of enzyme protein (Feigelson and Feigelson, 1964). Thus, the mechanism of action of the steroid hormones is on the protein synthesis machinery. Feigelson and Feigelson (1964) showed increased incorporation of precursors into RNA four hours after hormone administration. Glucocorticoid administration increased the ability of cell-free extracts to incorporate labelled amino acids into

protein (Leou et al., 1962). Cortisol caused a sharp increase in DNA-dependent RNA synthesis in cortical steroid responsive cells.

Cortisol enhances the release of free fatty acids from adipose tissue during fasting or adrenergic stimulation. The action of cortisol on adipose tissue varies considerably in different parts of the body (Wintrobe et al., 1971).

Electrolyte and water metabolism are regulated by the adrenal steroids, particularly in relation to the concentration of sodium and potassium ions in extracellular fluids. Both aldosterone and deoxycorticosterone cause increased reabsorption of sodium, chloride and bicarbonate ions. Sodium retention results in an exchange of intracellular potassium ions for extracellular sodium ions, with the excretion of potassium ions.

Other effects of corticosteroids include a depression of the number of circulating blood eosinophils and lymphocytes and a suppression of the inflammatory response to injury and infection.

1.2.2 The Effects of Adrenalectomy

Two main types of biochemical change result on the removal of the adrenals. The most striking effect is the increased rate of loss of sodium and chloride ions in the urine (Cappell and Anderson, 1971). There is a pronounced ionic imbalance in the blood with deficiency in sodium and an excess of potassium. The excretion of urea is notably diminished and the blood urea rises. The acute salt depletion and secondary extracellular dehydration leads, ultimately, to death. The time of survival may be prolonged by administration of sodium chloride. These changes are the result of loss of the mineralocorticoids and can be corrected

by administration of cortical extract or of aldosterone.

Secondly, the metabolism of carbohydrates is interfered with; the blood sugar level is lowered and the sensitivity to insulin is much increased. This is largely due to the inability to promote the synthesis of glycogen from glucose owing to failure of gluconeogenesis. It is also partly due to excessive utilisation of glucose and diminished intestinal absorption.

Adrenalectomy usually leads to no change or a decrease in the level of enzyme activity. In general, those enzymes that increase after administration of corticosteroids are decreased on adrenalectomy.

In rodents, particularly the rat, there is often accessory adrenal tissue capable of producing large amounts of adrenocortical hormones (Yatvin, 1970). This accessory tissue has considerable regeneration capacity. One must thus be careful in the interpretation of results in studies of this species.

1.2.3 The Thyroid Hormones

The thyroid gland is located in the anterior aspect of the neck immediately below the thyroid cartilage of the larynx. It consists of two lateral lobes connected by the isthmus. The gland is composed of follicles, each of which is a cyst-like structure surrounded by epithelial cells which secrete the thyroid hormones. Secretion of the thyroid hormones is controlled mainly by the thyroid-stimulating hormone of the anterior pituitary.

Thyroxine and its variously related iodinated forms comprise the thyroid hormone system. Thyroxine and triiodothyronine accelerate cellular reactions in practically every organ and tissue of the body as reflected in an increase in basal metabolic rate. More than 100 enzyme

systems have been reported to be altered in activity by thyroxine administration. These include increased activities of glucose - 6 - phosphatase (Syepesi and Freedland, 1969), glucose - 6 - phosphate dehydrogenase (Freedland, 1965) and NADP - cytochrome C reductase (Suzuki et al., 1967). It is debatable whether many of the enzymatic effects are due to direct action of the hormone (White et al., 1959).

A critical level of thyroid activity is essential for growth and normal nitrogen metabolism, but excessive amounts of thyroxine induce a net protein catabolic state. The growth-promoting action of the growth hormone was shown to be potentiated by thyroxine treatment (Simpson et al., 1950) as was the catabolic effect of the adrenal steroids (Wells and Kendall, 1940).

Thyroxine stimulates de novo synthesis of protein in liver mitochondria and microsomes. Tata and his associates (Tata, 1968; Tata et al., 1963) have described the effects of thyroid hormones on RNA and protein synthesis in liver and other tissues. On thyroxine stimulation, the protein-synthesizing capacity became evident 30 hours after administration of the hormone, suggesting that a 'priming' of the protein-synthetic machinery took place. This 'priming' started with the stimulation of nuclear DNA-dependent RNA polymerase. The next step was seen in a six-fold increase in RNA turnover. There was also an increased incorporation of labelled amino acids by the microsomes. Thus the thyroxine expression took place via the normal functioning of protein synthetic processes. Thyroid hormone may affect genetic transcription at the RNA polymerase level (Widnell and Tata, 1963, 1966).

Sokoloff and Kaufman (1961) have demonstrated that the mitochondria play a key role in protein synthesis stimulated by thyroxine. Mitochondria

and an oxidative substrate are essential for the thyroxine effect on amino acid incorporation into protein.

Thyroxine was shown to promote protein synthesis and increase retention of nitrogen in physiological amounts, as when hypothyroidism is being corrected. When excessive thyroxine was administered, however, protein catabolism was accelerated and a negative nitrogen balance resulted, with increased urea excretion. Protein synthesis was inhibited and there was an increase in the concentration of free amino acids in plasma, liver and muscle (Williams, 1968).

1.2.4 Effect of Thyroidectomy on Enzyme Levels *in vitro*

A number of studies have been directed towards the changes in enzyme levels occurring one to six weeks after thyroidectomy. Just as with treatment with thyroid hormones, many secondary effects possibly occur as a result of thyroidectomy prior to enzyme analysis (Pitot and Yatvin, 1973). In general, most of the effects of thyroidectomy are manifested in significant decreases in both mitochondrial and cytosol enzymes. Exceptions are the three enzymes in cysteine metabolism. They were shown to double in thyroidectomised rats (Chatagner and Jolles-Bergeret, 1963; Chatagner and Durieu-Trautman, 1965), as did threonine dehydratase under similar conditions (Ku et al., 1969). Genetic transcription in the liver is thought to be affected by thyroidectomy since RNA polymerase was found to decrease by some 40% after thyroidectomy (Widnell and Tata, 1963, 1966).

1.3 In vitro Studies

There are many advantages from an experimental viewpoint arising from the ability to culture and study cells and tissues in isolation.

The tissue may be removed from the controlling influences of other tissues in the body and consequently, specific effects observed. Hormonal effects can be narrowed down to the target organ involved. The amino acid pool can be varied in an attempt to correlate the concentration of a given substrate with the activity of specific enzymes.

The three main culture methods are known as cell culture, tissue culture and organ culture. In cell culture, the tissue is intentionally disorganised at the start by disrupting it into individual cells. Cell growth takes place in vitro. In both tissue and organ culture, very small fragments of tissue are placed in a nutrient medium. In organ culture, the maintenance or growth of tissues, or the whole or parts of an organ, in such a way as to allow differentiation and preservation of structure and metabolism, is stressed (Paul, 1970). On the other hand in tissue culture, conditions to preserve normal tissue architecture are not required. Thus cells which are motile might migrate from the tissue fragment which becomes disorganised (Paul, 1970).

1.3.1 Liver Culture

Chem (1954) grew embryonic rat liver fragments in a fluid serum medium for 6-13 days. The liver showed differentiation but at a slower rate than in vivo. Trowell (1959) attempted organ cultures of adult rat liver in a synthetic medium. He found that all cells had degenerated within four days, but obtained better results with foetal liver.

1.3.2 Urea Cycle Enzymes in Tissue Culture

Hillis and Bang (1962) tested the functional capacity of human embryonic organ cultures by measuring the arginase activity. The cultures

were kept alive for three weeks, and the arginase activity was found to decrease fairly linearly with the age of the culture. Significant activity was still found at two weeks.

Schimke (1963) studied the effect of arginine on A.S.S., A.S.L. and arginase in cell cultures of He La and L mammalian cell lines, and found significant activities of all three enzymes. He was not, however, able to demonstrate a direct effect of corticosteroids on arginase in vitro.

1.4 The Present Study

The aim of the present work was to investigate the responses of the five urea cycle enzymes to variations in dietary protein and to hormonal influence in rats fed on diets of different protein content. The hormones studied were cortisol and thyroxine. To test the specificity of the target site, the study was extended to an investigation of in vitro hormonal effects.

CHAPTER 2 - EXPERIMENTAL

2.1 Materials

2.1.1. Chemicals

The British Drug Houses Ltd., Poole, England supplied the dipotassium hydrogen phosphate, potassium-dihydrogen phosphate, ammonium bicarbonate, magnesium sulphate, L-ornithine HCl, L-aspartic acid, potassium sulphate, copper sulphate (hydrated), sulphuric acid, calcium gluconate, potassium chloride, casein (light white, vitamin-free), calcium lactate, iron citrate, nigrisine and thyroxine. Sodium chloride and iron III chloride were obtained from Hopkin and Williams, Essex, England while the sodium-potassium tartrate was supplied by May and Baker Ltd., England.

The Sigma Chemical Company, St. Louis Missouri, U.S.A. supplied the ATP, N-acetyl glutamate, di-lithium carbamyl phosphate, L-citrulline, urease, argininosuccinate, L-arginine HCl, bovine serum albumin and hydrocortisone. Grand Island Biological Company, New York, U.S.A. supplied the medium 199 (+ Hanks' balanced salt solution — arginine) and the whole egg ultrafiltrate. Millipore filters 1,2μ, 0,65μ 0,45μ and 0,22μ) were obtained from the Millipore Filter Corporation, Bedford, Massachusetts, U.S.A.

E. Merck Company, Darmstadt, Germany supplied the manganese sulphate, N-glycyl glycine, sodium hydroxide, tris (hydroxymethyl) aminomethane, phosphoric acid, diacetyl monoxime, ethanol (absolute), urea, perchloric acid, ether, glucose, disodium hydrogen phosphate, calcium phosphate

and potassium iodide. Sodium carbonate was obtained from Riedel - de Haën, Hannover, Germany. Schuchardt, München, Germany supplied the 1-phenyl-1,2-propanedione-2-oxime.

Anchor, Industria, S.A. supplied the food yeast. Penicillin G was obtained from Glaxo-Allenbury, Johannesburg, S.A. Goldfields Veterinary Supplies, Johannesburg, S.A. supplied the Hibitane, Thalamonal, Lethidrone and Hexachlorophene soap, while Contrad was obtained from Hickman and Kleber, Durban, S.A. Lederle Laboratories, Isando, S.A. supplied the Gevral Vitamin-Mineral Nutritional supplement. Cane sugar, cornstarch, lard and methylated spirits were obtained from O.K. Bazaars, Ltd., S.A. Terramycin was supplied by Psizer Laboratories Pty., Ltd., Pietermaritzburg, S.A. South African Commercial House, S.A. supplied the Hycolin.

Dr. Sarah Ratner (Public Health Research Institute, New York) kindly supplied some of the argininosuccinate.

2.1.2 Experimental Animals

Male Sprague-Dawley rats were obtained from Frankenwald, University of the Witwatersrand, Johannesburg, S.A. Before the rats were used for experiments, they were maintained on a normal diet of mouse pellets supplied by the Delmas Milling Company, Ltd., S.A.

2.1.3 Biological Material

All liver tissue was obtained from six-week old, male, Sprague-Dawley rats weighing approximately 100 g. The animals were killed by cervical dislocation and the livers were promptly removed. The enzymic extracts were prepared on the removal of the liver and the enzymes immediately assayed.

Bovine serum was prepared from blood obtained from the Johannesburg Municipal abattoir, S.A.

2.2 Methods

2.2.1 Feeding of Animals

All rats were fed a 'normal' diet consisting of mouse pellets, prior to being put on diets of different protein content for experimental purposes. The mouse pellets contained not less than 16% protein (estimated at approximately 20% protein); not less than 2,5% fat and 0,7% phosphorus, and not more than 6% fibre and 1,8% calcium.

Different (isocaloric) diets consisting of 10%, 34% and 75% protein were prepared using vitamin-free, light white casein as the protein source. The methods used were based on those of Professor Sonia Walker, Medical School, University of the Witwatersrand, S.A. (personal contact). The food was prepared in dry bulk and stored in the cold until needed. Before the food was given to the rats, it was mixed to a crumbly texture with water so that it could be easily handled by the rats.

All feeding was done ad libitum and the rats were maintained on their respective diets for at least seven days prior to experimentation.

2.2.1.1 Preparation of the 10% Protein Diet

2.2.1.1.1 Contents - The diet included the following (percentages by weight) :

sucrose	62%
cornstarch	10%
casein	5%
fat	10%
food yeast	10%
Steenbock salts	3%

A commercially prepared vitamin-mineral nutritional supplement was added to the above. One capsule was used per 200 g of food. The contents of one 'Gevral' vitamin capsule were :

Vitamin A acetate	5 000 U.S.P. units
Vitamin D	500 U.S.P. units
Thiamine mononitrate (B_1)	5 mg.
Riboflavin (B_2)	5 mg.
Pyridoxine HCl (B_6)	0,5 mg.
Vitamin B_{12}	1 µg.
Ascorbic Acid (C)	50 mg.
d-α-Tocopheryl Acetate (E)	10 I.U.
Niacinamide	15 mg.
Calcium Pantothenate	5 mg.
Calcium (as $CaHPO_4$)	145 mg
Phosphorus (as $CaHPO_4$)	100 mg.
Elemental Iron (as Ferrous Fumarate)	10 mg.
Magnesium (as MgO)	1 mg.
Potassium (as K_2SO_4)	5 mg.
Iodine (as KI)	0,1 mg.
Copper (as CuO)	1 mg.
Manganese (as MnO_2)	1 mg.

Zinc (as ZnO)	0,5 mg.
L-lysine HCl	25 mg.
Choline Bitartrate	50 mg.
Inositol	50 mg.

Lard was used as the source of fat and cane sugar as the sucrose source. This carbohydrate was supplemented with cornstarch as a sugar content exceeding 62% (by weight) resulted in food which the rats found unpalatable. The food yeast was obtained commercially and contained approximately 50% protein.

The Steenbock salts contained the following :

sodium chloride	23,36 parts
magnesium sulphate	24,60 "
disodium hydrogen phosphate	35,80 "
dipotassium hydrogen phosphate	69,60 "
calcium phosphate	68,80 "
calcium lactate	15,40 "
potassium citrate	0,16 "
iron citrate	5,98 "

2.2.1.1.2 Method - The fat was melted and all dry ingredients, except the casein, were added and thoroughly mixed. Thereafter the casein was slowly added with constant stirring to ensure an even constituency.

The food was stored in the cold until required, when it was prepared as described above (see 'Feeding of Animals').

2.2.1.1.3 Preparation of the 34% Protein Diet

The procedure as described for the 10% protein diet was followed

but with certain modifications :

(i) 29% casein (by weight) was used instead of 5%

(ii) 48% sucrose (by weight) was used instead of 62% and the cornstarch was omitted.

2.2.1.3 Preparation of the 75% Protein Diet

The method used to prepare the 10% protein diet was employed, with the following alterations :

(i) 70% casein (by weight) was used instead of 5%

(ii) 7% sucrose (by weight) was used instead of 62% and the cornstarch was omitted.

2.2.2 Measurement of the Activities of the Urea Cycle Enzymes

2.2.2.1 Preparation of Enzymic Extracts

Freshly excised liver was blotted and weighed. The liver tissue was then sliced and suspended in 25 volumes of cold, freshly prepared phosphate buffer (10 mM; pH 7,4) and homogenised for thirty seconds using an Ultra-Turrax homogenizer. This homogenate was divided into three parts.

One part was diluted with two volumes of the phosphate buffer. This was the A.S.S. extract with a final concentration of one in 50 (W./V.), which was assayed for A.S.S. activity.

A second part, to be assayed for A.S.L. activity, was diluted with four volumes of manganese sulphate (10 mM) to give a final extract concentration of one in 100 (W./V.). A portion of this extract was

further diluted with three volumes of manganese sulphate (10 mM), to yield the arginase extract with a final extract concentration of one in 300 (W./V.).

The remaining buffer extract contained the mitochondrical enzymes, C.P.S. and O.T.C. Mitochondria were ruptured by freeze-thawing. Further dilution with 10 volumes of the phosphate buffer yielded the C.P.S. extract with a final extract concentration of one in 250 (W./V.). The latter was diluted with 50 volumes of buffer to give the O.T.C. extract with a final extract concentration of one in 5 000 (W./V.).

All extracts were kept cold until incubation. Enzyme assays were immediately carried out.

The protein concentration of a sample of homogenate with a final dilution of one in 2,000 (W./V.) was measured by the Lowry method (Lowry et al., 1951), as described in 'Estimation of Proteins'.

2.2.2.2 Enzyme Assay Methods

Assay methods based on those of Brown and Cohen (1959) were used for C.P.C., O.T.C., A.S.L. and arginase assays. A.S.S. was assayed using a method based on the one developed by Ratner (1955) and later modified by Wixom et al. (1972). Each determination was done in triplicate.

The extracts were incubated in excess of all necessary reagents. All tubes were shaken to ensure proper mixing. After termination of the enzymic reactions, the fractions were frozen to store until colorimetric determinations were made of citrulline production (in the case of C.P.S. and O.T.C.); citrulline depletion (for A.S.S.), or urea production (in the case of A.S.L. and arginase).

The levels of the enzymes were related to the amount of product

(citrulline or urea) produced or depleted. The amount of product was determined colorimetrically as described under 'Colorimetric Determinations'.

Activity was expressed in international units, one unit being that amount of enzyme per gram wet weight of liver which catalyzed the production (or depletion) of 1 µmole of product per minute under assay conditions.

Specific activity was expressed as the activity (as defined above) per mg. of protein.

2.2.2.3 Assay Systems

2.2.2.3.1 Carbamyl Phosphate Synthetase

The assay system consisted of 50 µmoles of ammonium bicarbonate, 5 µmoles of L-ornithine, 5 µmoles of N-acetyl-L-glutamate, 10 µmoles of magnesium sulphate, 5 µmoles of ATP, approximately 150 units of partially purified beef liver ornithine transcarbamylase (see preparation method, following), plus extract in a final volume of 1,0 ml. It was necessary to adjust the pH of the first four reagents to 6,8 before adding the rest of the components of the assay system. This was done by gassing equal volumes of the first four reagents with CO_2 for approximately 30 minutes. ATP and O.T.C. extract were then added in the following proportions : two volumes gassed mixture : one volume ATP: one volume O.T.C. extract.

After equilibration of the sample tubes and contents in the water - bath, the reaction was started by adding the C.P.S. extract. After incubation at 37°C for 15 minutes, the reaction was terminated by the addition of 5,0 ml. of perchloric acid solution (1M). Control tubes

(zero time) received 5,0 ml. of the perchloric acid solution before the addition of enzyme extract.

2.2.2.3.1.1 Preparation of Beef Liver O.T.C. Extract

The O T.C. extract used in the C.P.S. assay system was obtained from a stock supply which was prepared as follows :

Beef liver was homogenised in three volumes of cold water. The homogenate was strained through a cheesecloth and frozen using a dry ice and acetone mixture. After thawing, it was centrifuged at 12 000 to remove cell debris. The supernatant was heated at 60°C for 20 minutes and then centrifuged at 12 000 r.p.m. to remove denatured protein. It was stored at -18°C and diluted as required before use.

2.2.2.3.2. Ornithine Transcarbamylase

The assay system consisted of 10µ moles of L-ornithine (pH 8,0), 45 µmoles of N-glycyl glycine buffer (pH 8,3), 10 µmoles of freshly prepared di-lithium carbamyl phosphate solution, and enzyme extract in a final volume of 2,0 ml.

The enzyme extract was added to the other components in the sample tubes which had been equilibrated to 37°C. After incubating at this temperature for 10 minutes, the reaction was terminated by the addition of 2,0 ml. of perchloric acid solution (1M). Control tubes (zero time) received 2,0 ml. of the perchloric acid solution before the addition of enzyme extract.

2.2.2.3.3 Argininosuccinate Synthetase

The assay system consisted of 1 µmole of L-citrulline (pH 7,5),

5 µmoles of L-aspartate (pH 7,5), 5 µmoles of magnesium sulphate, 5 µmoles of ATP, 100 µmoles of tris (hydroxymethyl) aminomethane, approximately 1,0 unit of urease, plus enzyme extract in a final volume of 1,0 ml.

The reaction was started by adding enzyme extract to the sample tubes and contents equilibrated to 37°C. After 20 minutes incubation, the reaction was terminated by the addition of 4,0 ml. of perchloric acid (0,5M). Control tubes (zero time) were similarly treated, but the citrulline was not added until the reaction had been terminated.

2.2.2.3.4 Argininosuccinate Lyase

The assay system consisted of 2 µmoles of argininosuccinate (pH 7,0), 50 µmoles of potassium phosphate buffer (pH 7,0), excess arginase, and enzyme extract in a final volume of 1,0 ml. The argininosuccinate was obtained as the barium salt. Potassium sulphate was used to precipitate the barium, after which the pH of the supernatant was adjusted to 7,0 using the phosphate buffer.

The reaction was started by adding enzyme extract to the equilibrated sample tubes containing the other assay components. The system was incubated at 37°C for 30 minutes. The reaction was terminated by the addition of 2,0 ml. of perchloric acid (1M). Control tubes (zero time) received 2,0 ml.of the perchloric acid before the addition of enzyme extract.

Arginine formed by the cleavage of the argininosuccinate was converted by the excess arginase (present in the homogenate) to urea.

2.2.2.3.5 Arginase

The assay system consisted of 25 µmoles of L-arginine (pH 9,5) and

the arginase extract made up to a final volume of 2,5 ml.

After the tubes plus L-arginine had been equilibrated to 37°C, the enzyme extract was added to start the reaction. Incubation at 37°C was for 10 minutes. The reaction was terminated with 5,0 ml. of perchloric acid (0,5M). Control tubes (zero time) received 5,0 ml. of the perchloric acid before the addition of enzyme extract.

2.2.2.4 Colorimetric Determinations

The stored fractions were thawed and centrifuged at 3 000 r.p.m. for approximately 10 minutes to remove any precipitated protein. The clear supernatant solutions were analyzed for either citrulline or urea according to a method based on that of Archibald (1944) as modified by Ratner (1955).

2.2.2.4.1 Citrulline Determination

Two ml. of sample was added to 2 ml. of acid mixture consisting of one part sulphuric acid : three parts phosphoric acid : six parts water, and containing iron III chloride (0,5 mM). To this was added 1 ml. of aqueous diacetyl monoxime (0,75% (W./V.)) in the dark. The tubes were stoppered with rubber bungs which allowed the release of pressure, shaken and placed in rapidly boiling water for 15 minutes in the dark. The tubes were then promptly removed and cooled in crushed ice for five minutes.

The tubes were then protected from the light by placing in a nigrisine solution. The optical density of the coloured product produced between citrulline and diacetyl monoxime was read at 490 nm. on an Hitachi colorimeter, using a water blank.

A standard curve for citrulline in the C.P.S., O.T.C. and A.S.S. assays was constructed using citrulline as a standard (see Figure 1).

Figure 1 : Standard Curve for Citrulline Assay

A standard solution of citrulline adjusted to pH 7,0 was used to construct the standard curve.

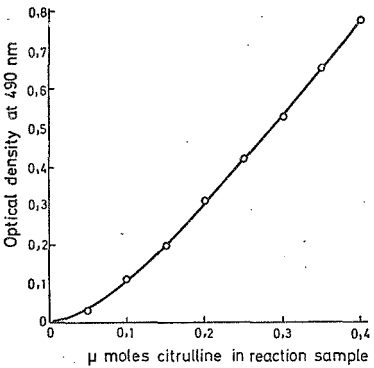


Fig. 1 - Standard Curve for Citrulline Assay

2.2.2.4.2 Urea Determination

Two ml. of the A.S.L. assay sample or 0,5 ml. of the arginase assay sample was added to 5 ml. of acid mixture (prepared as for citrulline colour determinations - see 'Citrulline Determination' above). To this was added, in the dark, 0,5 ml. of 1-phenyl-1,2-propanedione-2-oxime solution in absolute alcohol (3% (W./V.)). The tubes were stoppered, shaken and placed in rapidly boiling water for one hour. The tubes were then promptly removed and cooled in crushed ice for five minutes.

The colour produced with urea is light sensitive and the coloured solutions were thus protected from light by placing in nigrisine solution. The optical density of the coloured product formed between urea and 1-phenyl-1,2-propanedione-2-oxime was read at 540 nm. on an Hitachi colorimeter, using water as a blank.

Two standard curves, for urea in A.S.L. and arginase assays, respectively, were prepared using urea as a standard (see Figures 2 and 3).

2.2.2.5 Estimation of Proteins

Proteins were measured by a modification of the method used by Lowry et al. (1951) as follows :

Reagent A : 2% (W./V.) sodium carbonate in 0.1N sodium hydroxide
Reagent B : 0,5% (W./V.) copper sulphate (hydrated) and
1,35% (W./V.) sodium-potassium tartrate
Reagent C : 2 ml. of reagent B plus 48 ml. of reagent A.

Figure 2 : Standard Curve for Urea in A.S.L. Assay

A standard solution of urea adjusted to pH 7,0 was used to construct the standard curve.

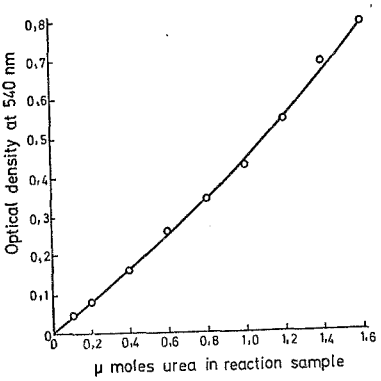


Fig. 2 - Standard Curve for Urea in A.S.L. Assay

Figure 3 : Standard Curve for Urea in Arginase Assay

A standard solution of urea adjusted to pH 9,5 and combined with components of the arginase assay mixture was used to construct the standard curve.

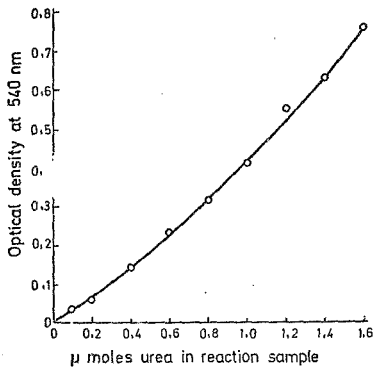


Fig. 3 - Standard Curve for Urea in Arginase Assay

To 1,0 ml. of the protein solution was added 5 ml. of reagent C. The mixture was shaken. After 10 minutes 0,5 ml. of 1N Folin Ciocateau reagent was added, with vigorous shaking. The colour was allowed to develop for 30 minutes and was read at 750 nm. against a reagent blank.

A standard curve was prepared using bovine serum albumin as a standard (see Figure 4).

2.2.2.6 Standard Curves

Standard curves were prepared for citrulline determinations, urea determinations (using A.S.L. and arginase assay components; urea replacing enzyme extract) and protein determinations (using bovine serum albumin as a standard). Suitable dilutions were made and the colours developed as described under 'Colorimetric Determinations' and 'Estimation of Proteins'. The colours were read at the appropriate wavelengths and suitable standard curves were constructed (see Figures 1, 2, 3 and 4).

2.2.3 Hormone Treatment

2.2.3.1 Cortisol

A solution of cortisol was prepared by suspending an insoluble hydrocortisone in water with 0,1% polyoxyethylene sorbitan monolaurate.

Cortisol (20 mg. per 100 g. body weight) was injected into the peritoneum either as a single injection to test short-term effects (after eight hours and 24 hours) or daily.

2.2.3.2 Thyroxine

A solution of thyroxine was prepared by dissolving thyroxine in

Figure 4 : Standard Curve for Protein Estimation Using Bovine Serum Albumin

A standard solution of bovine serum albumin was used to construct the standard curve.

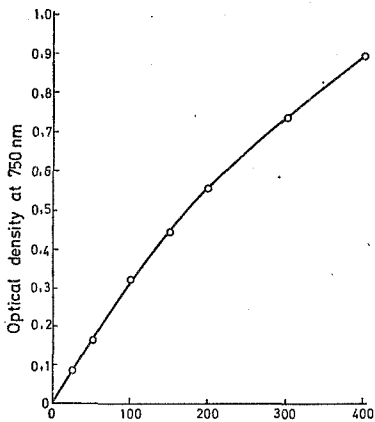


Fig. 4 - Standard Curve for Protein Estimation using Bovine Serum Albumin

water using a minimum amount of sodium hydroxide.

One mg. of thyroxine per 100 g. body weight was injected into the peritoneum and short-term effects (after eight hours and 24 hours) were investigated. The effect of 2 mg. of thyroxine per 100 g. body weight was also studied in a similar fashion.

2.2.4 . Operative Procedures

Procedures described by D'Amour and Blood (1959) were followed. Sham operations were performed on control rats.

2.2.4.1 Adrenalectomies

2.2.4.1.1 Method

Instruments, swabs and paper towels were sterilised by steam prior to operations.

The animals, weighing approximately 100 g. were anaesthetised with Thalamonal (0,1 ml. to 0,15 ml. per 100 g. body weight) injected intra-muscularly. Ether was at hand in the event of the anaesthetic wearing off before completion of the operation.

The operation site was wiped with hexachorophene soap; shaved and sterilised with a Hibitane solution made up in the following volumetric proportions : 1 Hibitane : 1½ water : 7½ methylated spirits. During the operation, the instruments were kept sterile in the same Hibitane solution.

With the rat in the prone position, a dorsal mid-line incision was made approximately 2 cm. long, extending from the tenth thoracic to the third lumbar vertebrae. Both adrenals could be removed from

this central skin incision, on either side of the vertebral column.

After removal of the adrenals, the wounds were stitched. The animals were injected intra-muscularly with the anaesthetic antidote Lethidrone (0,1 ml. per 100 g. body weight).

2.2.4.1.2 Post-operative Care of Animals

The conscious animals were put in a clean, dry cage and kept warm for several hours.

Initially, the mortality rate of adrenalectomised rats within the first 72 hours was high. The rats were, at first, given drinking water which contained 0,3% (W./V.) potassium chloride and 0,3% (W./V.) sodium chloride. This was not sufficient to prevent loss of life. Subsequently, the rats were given drinking water containing 5% (W./V.) glucose and 0,9% (W./V.) sodium chloride, as well as ample sodium chloride as a lick. This maintained life for at least three days.

During this period it was necessary to renew drinking water at least twice a day as the animals lost a great deal of water. Food was given as usual. The cages were kept as dry as possible.

2.2.4.2 Thyroidectomies

2.2.4.2.1 Method

Preparations for an aseptic operation; anaesthetisation and cleansing of the operation site were carried out as for the adrenalectomies.

The anaesthetised rat was strapped in the prone position on a sterilised wooden board. The thyroids were removed from a ventral mid-line incision made through the skin of the neck. Care was taken not to touch the recurrent laryngeal nerve.

After removal of the thyroids, the wound was stitched. Lethidrone (0,1 ml. per 100 g. body weight) was injected intra-muscularly as an anaesthetic antidote.

2.2.4.2.2 Post-operative Care of Animals

The conscious animals were put into a clean, dry cage and kept warm for several hours.

Post-operatively, the animals were given a 1% (W./V.) solution of calcium gluconate for drinking water to avoid parathyroid tetany, as the parathyroids were removed together with the thyroids. Terramycin was added to the drinking water to counteract respiratory infection.

Basal metabolic rates were determined two weeks after thyroidectomy to assess the success of the thyroidectomy.

2.2.5 Organ Cultures

2.2.5.1 Method

Prior to culturing, all apparatus and instruments were sterilised by steam sterilisation. Both were washed in Contrad for at least 24 hours, thoroughly rinsed in tap water followed by triple distilled water and dried before sterilisation.

Culturing took place in a sterile room under a sterile perspex hood, both being irradiated by an ultraviolet lamp when not in use. The working area and hood were washed with Hycolin, and the latter with 70% ethyl alcohol before and after use. Before entering the culture room, a sterile hood, gown, foot covers and mask were donned. Sterile conditions were maintained as far as possible.

Immediately before culturing, the culture medium was freshly prepared. The basic culture medium consisted of Medium 199 (without arginine) (Morgan et al, 1950); Hanks' balanced salt solution (Paul, 1970); 10% whole egg ultrafiltrate and 20% bovine serum. (Several other culture mediums were tried using different concentrations of serum (0%, 10%, 30%, 45%, 55%); adding insulin and cyclic AMP; replacing Medium 199 by Earle's balanced salt solution (Paul, 1970) or Hanks' balanced salt solution. The medium which gave the best recovery of enzyme activity was, however, as above : . dium 199 (— arginine) Hanks' balanced salt solution + 20% bovine serum + 10% whole egg ultrafiltrate).

Medium 199 (+ Hanks' balanced salts -arginine) was obtained commercially in powder form. It was dissolved in cold triple-distilled water and the pH was adjusted to 7,2 with the addition of 0,35 g. of sodium bicarbonate per litre. Better recovery of activity was obtained when the medium was freshly prepared. The Medium 199 solution was filtered through a series of sterile millipore filters (using a Millipore stainless steel pressure filter unit) into a sterile flask. The following filter sequence was used : fibreglass prefilter; 1,2μ millipore filter; 0,65μ millipore filter; 0,45μ millipore filter; 0,22μ millipore filter. Each filter was separated from the next by a double layer of dacron gauze.

Once the Medium 199 solution had been filtered, the addition of commercially prepared whole egg ultrafiltrate, bovine serum (prepared as set out below) and other components took place in the tissue culture room under aseptic conditions. A 'washing medium' containing penicillin G (600 mg. per 200 ml.) plus basic culture medium was prepared as well as the mediums containing hormones - 20 mg. of cortisol per 100 ml. of medium,

and 1 mg. of thyroxine per 100 ml. of medium, respectively. Both hormones were added in the form of a stock solution which was filtered through a 0,45μ filter in a Swinnex filter unit with the aid of a syringe. All media were prepared immediately prior to killing the rat.

Once the rat was killed, a wide area of skin in the region of the abdomen was removed. The latter was washed with 70% ethyl alcohol and the liver removed. The excised liver was placed into a sterile beaker and promptly transferred to the sterile room where it was washed several times with 'washing medium' to remove all traces of blood. This was followed by washing in the medium in which the liver was to be cultured.

Using a scalpel, thin slices of liver were cut (approximately 0,6 mm. thick). The central well of a Conway unit was filled with culture medium and 2-3 slices of liver (depending on size) were placed on a piece of sterile filter paper supported across the central well and in contact with the medium. The unit was covered with its lid.

The cultures were incubated at 20°C in a gassed incubator (95% O_2/5% CO_2) for 24 hours. Before assaying the liver slices for enzyme activity, they were washed in 0,9% saline and blotted dry.

Each culture was done in duplicate.

2.2.5.2 Preparation of Bovine Serum for Culture Medium

Approximately 10 litres of bovine blood was collected from the abattoir. It was kept at room temperature for approximately one hour and then at 4°C for at least 24 hours. The serum was drained off and centrifuged at 10 000 r.p.m. for 10 minutes in the cold. The

supernatant was filtered through millipore filters using the sequence described above (see 'Organ Cultures'). The sterile filtrate was bottled in 20 ml. quantities in sterile McCartney bottles, and frozen to store.

Before use, the serum received heat treatment, by being kept at 55°C for 30 minutes.

CHAPTER 3 - RESULTS

3.1 The Effect of Diet on the Urea Cycle Enzyme Levels

3.1.1 The Effect of Dietary Protein Contens on the Urea Cycle Enzyme Levels

Schimke (1962a) has shown that the enzymes of the urea cycle in rat liver show a positive correlation to the protein content of the diet. It was initially decided to establish enzyme levels in rats fed on diets of different protein content. While Schimke's rats were fed on 15%, 30% and 60% protein diets, the rats in the present study were maintained on diets as follows.

Batches of six-week old, male, Sprague-Dawley rats were maintained for seven days on three different diets, namely, a control or normal diet of mouse pellets containing approximately 20% protein; a diet containing 10% protein and one containing 75% protein. The 10% and 75% protein diets were prepared as described under 'Methods'.

The different diets had no detrimental effects on the rats. At the end of the seven-day period, the animals were killed and their livers were assayed for enzymes as described under 'Methods'.

Results are shown in Tables 2 and 3 and Figures 5 and 6. Activities are plotted as a percentage of the value on a normal diet. Actual activities of enzymes in rats on the normal diet are also quoted. Each result represents a mean of the number of animals shown above each column in the figure or in brackets in the table. Significance of difference from levels on a normal diet was determined by the Student's t-test

Table 2 - Table of Results for Figure 5 -

Diet Dependence of Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	*Normal (20% Protein)*		*10% Protein*		*75% Protein*	
Enzyme	Activity per unit wet weight + standard error	Activity per unit wet weight + standard error expressed as a percentage of normal	Activity per unit wet weight + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Activity per unit wet weight + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal
C.P.S.	5,83 ± 0,230	100 ± 3,94 (16)	43,3 ± 3,04 (14)	0,001	188 ± 10,4 (12)	0,001
O.T.C.	266 ± 10,3	100 ± 3,87 (16)	63,1 ± 2,84 (11)	0,001	150 ± 3,05 (16)	0,001
A.S.S.	3,02 ± 0,166	100 ± 5,52 (8)	85,0 ± 10,4 (5)	N.S.	198 ± 9,28 (14)	0,001
A.S.L	3,27 ± 0,183	100 ± 5,62 (4)	67,3 ± 3,19 (3)	0,005	200 ± 13,5 (7)	0,001
Arg.	897 ± 27,6	100 ± 3,10 (14)	62,0 ± 2,26 (11)	0,001	107 ± 3,19 (16)	N.S.

Figure 5 : Urea cycle enzyme activities per unit wet weight in the livers of rats fed on diets of different protein content.

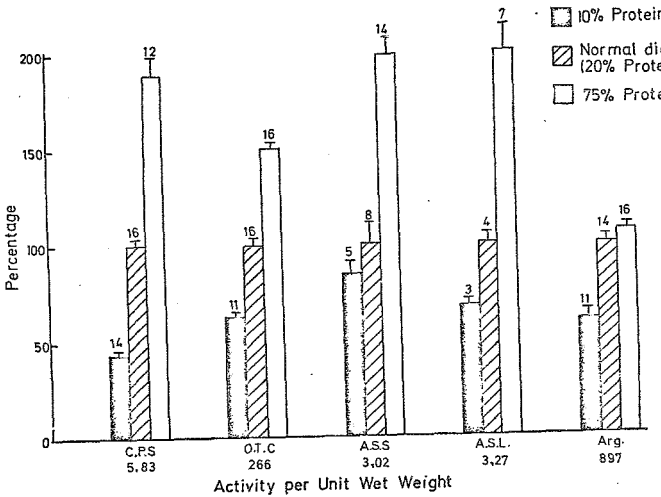


Fig. 5 - Diet Dependence of Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 3 - Table of Results for Figure 6 -

Diet Dependence of Specific Activity of Urea Cycle Enzymes

Diet	Normal (20% Protein)		10% Protein		75% Protein	
Enzyme	Specific activity + standard error $\times 10^2$	Specific activity + standard error expressed as a percentage of normal	Specific activity + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Specific activity + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal
C.P.S.	2,76 ± 0,164	100 ± 5,94 (16)	57,4 ± 4,29 (10)	0,001	186 ± 16,9 (12)	0,001
O.T.C.	126 ± 3,55	100 ± 2,77 (15)	76,5 ± 3,79 (11)	0,001	144 ± 6,36 (16)	0,001
A.S.S.	1,31 ± 0,129	100 ± 9,89 (8)	81,0 ± 6,17 (4)	N.S.	223 ± 20,1 (14)	0,001
A.S.L.	1,39 ± 0,143	100 ± 10,3 (4)	88,9 ± 8,25 (4)	N.S.	229 ± 9,73 (7)	0,001
Arg.	417 ± 26,4	100 ± 6,34 (14)	78,2 ± 6,70 (7)	0,05	106 ± 4,98 (16)	N.S.

Figure 6 : Specific activities of the urea cycle enzymes in the livers of rats fed on diets of different protein content.

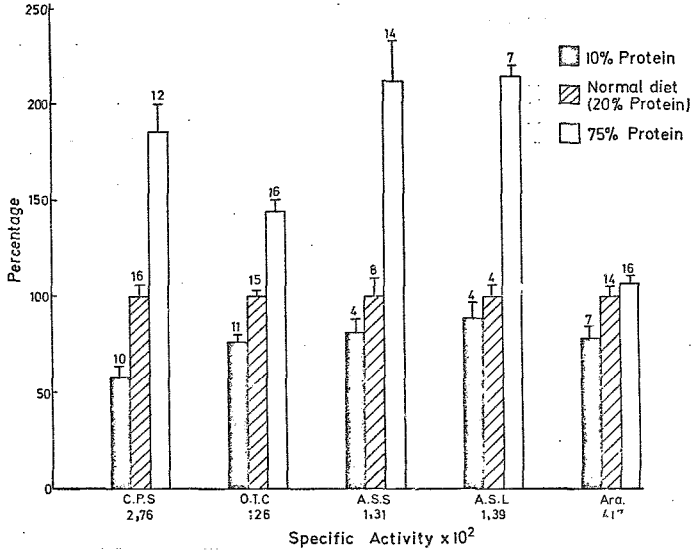


Fig. 6 - Diet Dependence of Specific Activity of Urea Cycle Enzymes

and was accepted as significant at the $2P \leq 0{,}050$ level.

If the activities per unit wet weight are considered, all enzymes showed a decrease in activity (compared with normal levels) in the livers of rats fed on the 10% protein diet, while the 75% protein caused a significant increase of all enzyme levels except arginase (Table 2 and Figure 5).

Specific activity showed the same trend, decreases occurring in all enzymes on the 10% protein diet. Arginase was the only enzume not showing a significant increase on the 75% protein diet after seven days of feeding (Table 3 and Figure 6).

This correlation between protein content of the diet and enzyme levels (both activity per unit wet weight and specific activity) is in partial agreement with Schimke's work (Schimke, 1962a). Schimke showed a definite increase in activity of all urea cycle enzymes on the high protein diet, whereas, in the present study, all enzymes except arginase showed this positive correlation.

3.1.2 . Time Course of Adaptation of the Urea Cycle Enzymes to Dietary Protein

Batches of rats were maintained on the two extreme diets, namely, 10% and 75% protein, for two different time periods : seven days and 21 days. The diets were prepared as described under 'Methods'. The controls were rats fed on the normal diet of mouse pellets (20% protein).

The results of the seven-day feeding period appear in Tables 2 and 3 and Figures 5 and 6, while Tables 4 and 5 and Figures 7 and 8 show the contrast between the seven-day feeding effect and the 21-day feeding effect.

TABLE 4 - TABLE OF RESULTS FOR FIGURE 7

TIME COURSE OF ADAPTATION OF ACTIVITY OF UREA CYCLE ENZYMES PER UNIT WET WEIGHT TO DIETARY PROTEIN

Diet	Normal (20% Protein)		10% Protein					75% Protein				
Time Period			7 days		21 days			7 days		21 days		
Enzyme	Activity per unit wet weight and standard error	Activity per unit wet weight ± standard error expressed as a percentage of normal	Activity per unit wet weight ± standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Activity per unit wet weight ± standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Confidence limit of significance of difference from 7-day level	Activity per unit wet weight ± standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Activity per unit wet weight ± standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Confidence limit of significance of difference from 7-day level
C.P.S	5,83±0,230	100±3,94 (16)	43,3±3,04 (14)	0,001	14,2±1,49 (3)	0,001	0,001	186±10,4 (12)	0,001	237±9,21 (5)	0,001	0,005
O.T.C	266±10,3	100±3,87 (16)	63,1±2,84 (11)	0,001	43,6±0,436 (3)	0,001	0,001	150±3,05 (16)	0,001	63,9±4,22 (5)	0,001	0,001
A.S.S	3,02±0,166	100±5,52 (8)	85,0±10,4 (5)	N.S	49,7±3,64 (3)	0,001	0,020	198±9,28 (14)	0,001	293±9,64 (5)	0,001	0,001
A.S.L	3,27±0,183	100±5,62 (4)	67,3±3,19 (3)	0,005	54,1±8,99 (3)	0,010	N.S	200±13,5 (7)	0,001	327±21,9 (5)	0,001	0,001
Arg.	897±27,6	100±3,10 (14)	62,0±2,26 (11)	0,001	45,7±2,80 (3)	0,001	0,001	107±3,19 (16)	N.S.	133±6,91 (5)	0,001	0,005

Figure 7 : The time course of adaptation of activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on normal, 10% and 75% protein diets for seven days and 21 days.

L - 10% protein diet

N - normal diet (20% protein)

H - 75% protein.

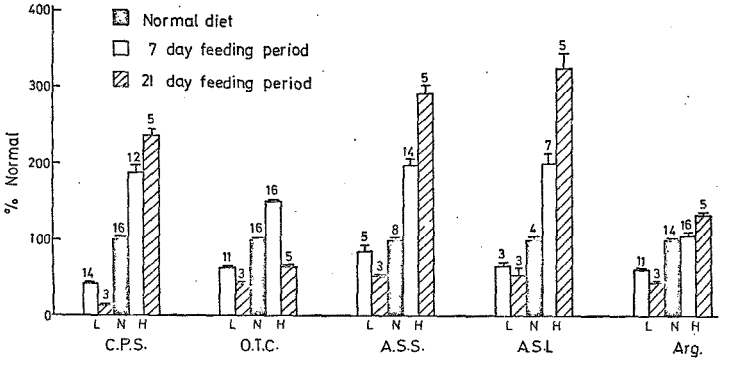


Fig. 7 - Time Course Adaptation of Activity of Urea Cycle Enzymes per Unit Wet Weight to Dietary Protein

TABLE 5 – TABLE OF RESULTS FOR FIGURE 8

TIME COURSE OF ADAPTATION OF SPECIFIC ACTIVITY OF UREA CYCLE ENZYMES TO DIETARY PROTEIN

Diet	Normal (20% Protein)		10% Protein				75% Protein			
Time Period			7 days		21 days			7 days	21 days	
Enzyme	Specific activity + standard error $\times 10^2$	Specific activity + standard error expressed as a percentage of normal	Specific activity + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Specific activity + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Confidence limit of significance of difference from 7-day level	Specific activity + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Specific activity + standard error expressed as a percentage of normal	Confidence limit of significance of difference from normal	Confidence limit of significance of difference from 7-day level
C.P.S	$2,76 \pm 0,164$	$100 \pm 5,94$ (16)	$57,4 \pm 4,29$ (10)	0,	$5 \pm 1,66$ (3)	0,001	0,001	$186 \pm 16,9$ (12)	0,001	$213 \pm 12,54$ (5)	0,001	N.S.
O.T.C.	$126 \pm 3,55$	$100 \pm 2,77$ (15)	$76,5 \pm 3,79$ (11)	0,001	$65,5 \pm 8,65$ (3)	0,005	N.S.	$144 \pm 6,36$ (16)	0,001	$95,2 \pm 6,86$ (5)	N.S.	0,05
A.S.S	$1,31 \pm 0,129$	$100 \pm 9,89$ (8)	$81,0 \pm 6,17$ (4)	N.S.	$73,4 \pm 8,32$ (3)	N.S.	N.S.	$223 \pm 20,1$ (14)	0,001	$287 \pm 7,63$ (5)	0,001	0,01
A.S.L.	$1,39 \pm 0,143$	$100 \pm 10,3$ (4)	$88,9 \pm 8,25$ (4)	N.S.	$83,5 \pm 19,5$ (3)	N.S.	N.S.	$229 \pm 9,73$ (7)	0,001	$323 \pm 30,5$ (5)	0,001	0,02
Arg.	$417 \pm 26,4$	$100 \pm 6,34$ (14)	$78,2 \pm 6,70$ (7)	0,050	$64,3 \pm 9,11$ (3)	0,01	N.S.	$106 \pm 4,98$ (16)	N.S.	$122 \pm$ (5)	N.S.	N.S.

Figure 8 : The time course of adaptation of specific activity of the urea cycle enzymes to dietary protein in the livers of rats fed on normal, 10% and 75% protein diets for seven days and 21 days.

L - 10% protein diet

N - normal diet (20% protein)

H - 75% protein diet

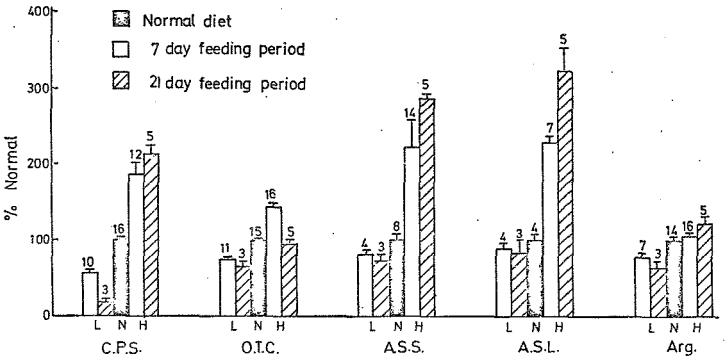


Fig. 8 - Time Course Adaptation of Specific Activity of Urea Cycle Enzymes to Dietary Protein

Results are expressed as a percentage of the value on a normal diet.

Schimke (1962a) showed stabilised levels after eight days, but according to present results no overall stabilised level was reached after seven days of feeding. Table 4 and Figure 7 show that on the 10% protein diet the activity per unit wet weight of C.P.S., O.T.C., A.S.S., arginase and possibly A.S.L. was significantly lower after 21 days than after seven days. Similar behaviour of specific activity was observed (Table 5 and Figure 8).

On the 75% protein diet, the 21-day feeding period resulted in the activity per unit wet weight of C.P.S., A.S.S., A.S.L. and arginase showing a significant increase above levels after the seven-day feeding period (Table 4 and Figure 7). O.T.C. showed anomalous behaviour in that the activity per unit wet weight of liver was significantly decreased after the 21-day feeding period both with respect to normal and seven-day levels.

If specific activity is considered, increases in all enzymes occurred, except O.T.C. which showed a decrease on the 75% protein diet after 21 days, compared with seven days (Table 5 and Figure 8).

Because of the fluctuation of enzyme levels, it was necessary to have controls which were fed on the same diet as the experimental rats for exactly the same time period. All results are thus expressed as a percentage of these control levels. All rats were fed on their specific diets for seven days prior to hormone treatment and other experimentation.

3.2 The Influence in vivo of the Hormones, Cortisol and Thyroxine, on the Urea Cycle Enzyme Levels

3.2.1 The Effect of Cortisol Administration on Enzyme Levels

3.2.1.1 Response of O.T.C. to Different Doses of Cortisol

Five different doses of cortisol, 1, 5, 10, 20 and 40 mg. per 100 g. body weight were administered to batches of rats which had been fed on the normal mouse pellet diet (20% protein) since weaning. Each dose was injected intraperitoneally.

Control rats received no cortisol treatment.

Six hours after cortisol administration, all rats were killed and their livers were assayed for O.T.C. activity.

Table 6 shows the effect on activity per unit wet weight of liver and specific activity. Each result represents a mean of the number of animals indicated in the table.

A significant effect on the activity per unit wet weight of O.T.C. after six hours was produced by 20 mg. of cortisol per 100 g. body weight and 40 mg. of cortisol per 100 g. body weight. In both cases there was a significant decrease in activity compared with controls. No significant effect on O.T.C. levels was observed with any of the other doses.

It was subsequently decided to administer 20 mg./100 g. body weight in all cortisol experiments.

3.2.1.2 Response of O.T.C. to Cortisol Administration after Different Time Intervals

Batches of rats previously fed on the normal rat pellet diet were injected intraperitoneally with cortisol (20 mg. per 100 g. body weight).

Table 6 - O.T.C. Response to Different Doses of Cortisol

Hydrocortisone administered (mg/100 g body weight)	Activity per unit wet weight of O.T.C. + standard error	No. of animals used for mean result	Confidence limit of significance of difference from control	Specific Activity of O.T.C. + standard error x 10^2	No. of animals used for mean result	Confidence limit of significance of difference from control
0	312 ± 11,1	12	-	143 ± 10,7	12	-
1	287 ± 28,6	8	N.S.	162 ± 19,2	8	N.S.
5	294 ± 18,0	8	N.S.	164 ± 10,5	8	N.S.
10	380 ± 46,6	4	N.S.	184 ± 21,5	4	N.S.
20	249 ± 8,68	8	0,001	128 ± 13,6	8	N.S.
40	255 ± 8,70	4	0,005	141 ± 18,9	4	N.S.

The rats were killed and their livers assayed for O.T.C. activity after the following time periods : six hours, eight hours, 12 hours, 16 hours, 20 hours and 24 hours.

Control rats (0 hours) received no cortisol treatment.

Table 7 shows the effect on activity per unit wet weight of liver and specific activity of O.T.C. after the different time intervals. Each result represents a mean of the number of animals indicated in the table.

Significant effects on O.T.C. activity per unit wet weight were observed after eight hours (an increase in activity) and 16 hours (a decrease in activity). No significant effect on the specific activity of O.T.C. was observed.

3.2.1.3 General Cortisol Effect in Rats fed on Diets of Different Protein Content

Schimke has shown that the administration of large doses of cortisone acetate (25 mg. per 100 g. body weight) to rats resulted in increased levels of activity of all urea cycle enzymes which were proportional to the increased urea excreted (Schimke, 1963). The rats were fed on a 15% protein diet for a total of 13 days; injections were given daily.

In the present study, three types of experiments were carried out :

(i) An investigation of the effect on the urea cycle enzyme levels eight hours after a single dose of cortisol in rats fed on normal, low and high protein diets.

(ii) An investigation of the effect on the urea cycle enzyme levels 24 hours after a single dose of cortisol in rats fed on normal, low and high protein diets.

Table 7 - O.T.C. Response to Cortisol Administration after Different Time Intervals

Time interval between injection and assay	Activity per unit wet weight of O.T.C. + standard error	No. of animals used for mean result	Confidence limit of significance of difference from control	Specific activity of O.T.C. + standard error x 10^2	No. of animals used for mean result	Confidence limit of significance of difference from control
0	261 ± 10,6	4	-	117 ± 18,4	4	N.S.
6	235 ± 11,6	4	N.S.	131 ± 18,1	4	N.S.
8	303 ± 8,74	4	0,025	127 ± 9,22	4	N.S.
12	273 ± 14,7	4	N.S.	142 ± 7,32	4	N.S.
16	211 ± 9,15	4	0,020	-	4	-
20	227 ± 21,9	3	N.S.	114 ± 6,01	3	N.S.
24	236 ± 3,69	4	N.S.	138 ± 3,57	4	N.S.

(iii) An investigation of the effect of daily cortisol administration on the urea cycle enzyme levels in rats fed on the two extreme diets (10% and 75% protein).

3.2.1.3.1 *The Effect on the Urea Cycle Enzyme Levels Eight Hours after a Single Cortisol Injection*

Three groups of rats were fed on three different diets - normal, 10% and 75% protein - for seven days prior to the administration of cortisol. Diets were prepared as discussed under 'Methods'.

The control rats received no hormone treatment.

The experimental rats were given a single intraperitoneal injection of cortisol (20 mg. per 100 g. rat). Eight hours later the animals were killed and their livers immediately assayed for the urea cycle enzymes as described under 'Methods'.

The effects on activity per unit wet weight of liver are shown in Table 8 and Figure 9, while the effects on specific activity are shown in Table 9 and Figure 10. Results are expressed as a percentage of the control.

Cortisol was found to cause a significant increase in the activity per unit wet weight of liver of C.P.S., arginase and possibly A.S.S. and A.S.L. on the 10% protein diet, and of O.T.C., A.S.S. and A.S.L. on the normal diet (20% protein) - see Table 8 and Figure 9. On the 75% protein diet, however, cortisol had no effect on any of the five enzymes with respect to activity per unit wet weight of liver.

If specific activity is considered, a significant increase was observed in arginase, and possibly C.P.S. and A.S.S. levels on the

Table 8 - Table of Results for Figure 9

Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 8 Hours After a Single Dose of Cortisol

Diet	Normal (20% Protein)			10% Protein			75% Protein		
Enzyme	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	$100 \pm 3,90$ (16)	$98,5 \pm 4,4$ (8)	N.S.	$100 \pm 7,00$ (14)	$136 \pm 12,3$ (7)	0,02	$100 \pm 5,50$ (12)	$105 \pm 5,40$ (8)	N.S.
O.T.C.	$100 \pm 3,90$ (16)	$114 \pm 3,00$ (8)	0,01	$100 \pm 5,10$ (8)	$105 \pm 4,60$ (7)	N.S.	$100 \pm 3,10$ (16)	$99,2 \pm 3,10$ (8)	N.S.
A.S.S.	$100 \pm 4,90$ (7)	$122 \pm 6,60$ (5)	0,025	$100 \pm 12,3$ (5)	$125 \pm 11,9$ (4)	N.S.	$100 \pm 4,30$ (6)	$93,3 \pm 2,60$ (7)	N.S.
A.S.L.	$100 \pm 4,73$ (12)	$127 \pm 8,89$ (8)	0,020	$100 \pm 4,70$ (3)	$115 \pm 4,30$ (4)	N.S.	$100 \pm 4,28$ (7)	$88,5 \pm 5,36$ (8)	N.S.
Arg.	$100 \pm 3,10$ (14)	$95,4 \pm 5,65$ (7)	N.S.	$100 \pm 3,65$ (11)	$143 \pm 7,23$ (4)	0.001	$100 \pm 2,98$ (16)	$93,6 \pm 2,56$ (6)	N.S.

Figure 9 : *The effect on the activity of the urea cycle enzymes per unit wet weight eight hours after a single cortisol injection in rats fed on normal, low and high protein diets.*

L - 10% protein diet

N - normal diet (20% protein)

H - 75% protein diet.

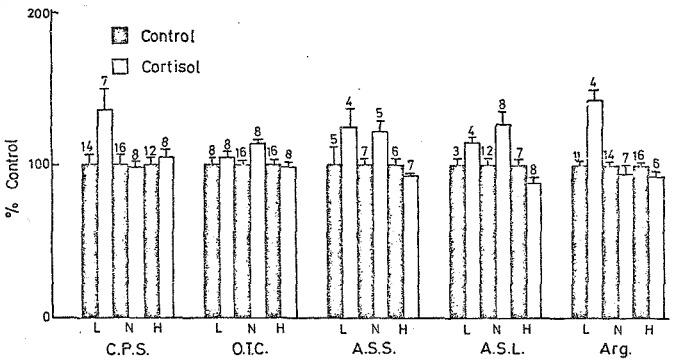


Fig. 9 - Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 8 Hours after a Single Dose of Cortisol

Table 9 - Table of Results for Figure 10

Effect on Specific Activity of Urea Cycle Enzymes 8 Hours After a Single Dose of Cortisol

Diet	Normal (20% Protein)			10% Protein			75% Protein		
Enzyme	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	$100^{\pm}5,94$ (16)	$101^{\pm}7,17$ (8)	N.S.	$100^{\pm}7,47$ (10)	$118^{\pm}10,6$ (7)	N.S.	$100^{\pm}9,08$ (12)	$124^{\pm}8,27$ (8)	N.S.
O.T.C.	$100^{\pm}2,77$ (15)	$116^{\pm}7,41$ (8)	N.S.	$100^{\pm}5,70$ (8)	$90,0^{\pm}3,02$ (7)	N.S.	$100^{\pm}6,85$ (8)	$110^{\pm}4,98$ (8)	N.S.
A.S.S.	$100^{\pm}6,81$ (7)	$136^{\pm}15,8$ (5)	N.S.	$100^{\pm}11,9$ (5)	$116^{\pm}13,9$ (4)	N.S.	$100^{\pm}14,3$ (6)	$96,8^{\pm}6,42$ (7)	N.S.
A.S.L.	$100^{\pm}3,89$ (12)	$126^{\pm}11,3$ (8)	0,05	$100^{\pm}4,56$ (3)	$105^{\pm}4,65$ (4)	N.S.	$100^{\pm}4,39$ (7)	$102^{\pm}5,72$ (8)	N.S.
Arg.	$100^{\pm}6,34$ (14)	$102^{\pm}12,0$ (7)	N.S.	$100^{\pm}8,57$ (7)	$140^{\pm}10,3$ (4)	0,02	$100^{\pm}4,71$ (16)	$114^{\pm}5,8$ (6)	N.S.

Figure 10 : The effect on the specific activity of the urea cycle enzymes eight hours after a single cortisol injection in rats fed on normal, low and high protein diets.

L - 10% protein diet

N - normal diet (20% protein)

H - 75%

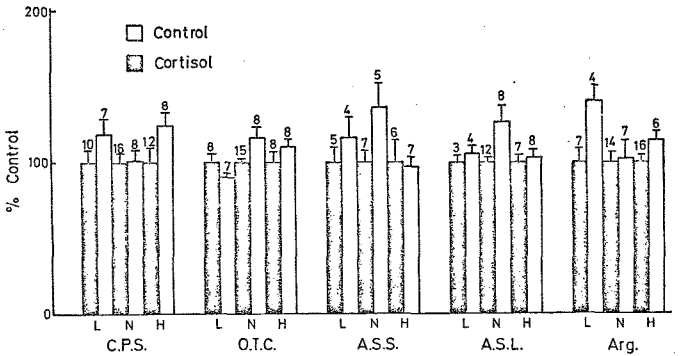


Fig. 10 - Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 8 Hours after a Single Dose of Cortisol

10% protein diet, and in A.S.L. and possibly O.T.C. and A.S.S. levels on the normal (20% protein) diet. On the 75% protein diet, cortisol had no significant effect. (Table 9 and Figure 10).

3.2.1.3.2 The Effect on the Urea Cycle Enzyme Levels 24 Hours after a Single Cortisol Injection

Two groups of rats were fed on two different diets - 10% protein and 75% protein - for seven days prior to, and during the experiment.

The control rats received no hormone treatment.

The experimental rats were given a single intraperitoneal injection of cortisol (20 mg. per 100 g. body weight). Twenty-four hours later the animals were killed and their livers immediately assayed for urea cycle enzymes.

The effects on activity per unit wet weight of liver are shown in Table 10 and Figure 11, while the effects on specific activity are shown in Table 11 and Figure 12. Results are expressed as a percentage of the control.

Cortisol was found to cause a significant decrease in the activity of O.T.C. per unit wet weight in rats fed on the 10% protein diet. (No result was obtained for A.S.S. and A.S.L. due to experimental error.) O.T.C. showed the same decrease in activity on the 75% protein diet, while C.P.S. underwent a significant increase on the 75% protein diet (Table 10 and Figure 11).

If specific activity is considered, O.T.C. showed a significant decrease on cortisol treatment in rats fed on the low protein diet. On the 75% protein diet, cortisol treatment caused significant increases in C.P.S. and arginase after 24 hours (Table 11 and Figure 12).

Table 10 - Table of Results for Figure 11

Effect on Activity of Urea Cycle Enzymes per Unit Weight 24 Hours After a Single Dose of Cortisol

Diet	*10% Protein*			*75% Protein*		
Enzymes	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental	
C.P.S.	100 ± 7,00 (14)	119 ± 18,0 (4)	N.S.	100 ± 5,50 (12)	115 ± 3,50 (4)	0,05
O.T.C.	100 ± 5,10 (8)	74,8 ± 2,90 (4)	0,005	100 ± 3,10 (16)	84,9 ± 2,0 (8)	0,001
A.S.S.	100 ± 12,3 (5)	-	-	100 ± 4,30 (6)	93,3 ± 4,80 (7)	N.S.
A.S.L.	100 ± 4,70 (3)	-	-	100 ± 4,28 (7)	88,0 ± 2,24 (7)	0,05
Arg.	100 ± 3,65 (11)	108 ± 11,3 (4)	N.S.	100 ± 2,98 (16)	102 ± 3,26 (7)	N.S.

Figure 11 : The effect on the activity of the urea cycle enzymes per unit wet weight 24 hours after a single cortisol injection in rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

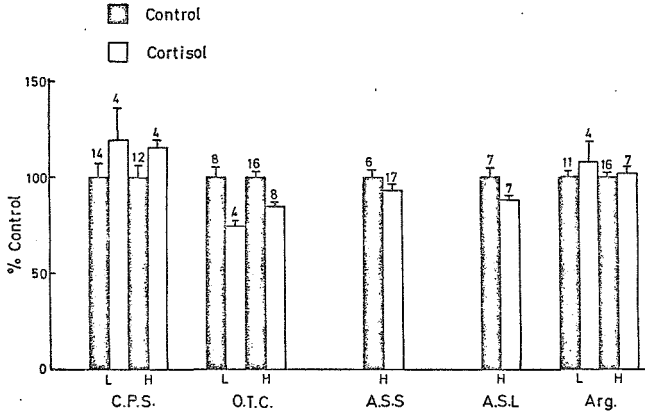


Fig. 11 - Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 24 Hours after a Single Dose of Cortisol

Table 11 - Table of Results for Figure 12

Effect on Specific Activity of Urea Cycle Enzymes 24 Hours After a Single Dose of Cortisol

Diet	10% Protein			75% Protein		
Enzyme	Specific Activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental	
C.P.S.	100 ± 7,47 (10)	114 ± 14,7 (4)	N.S.	100 ± 9,08 (2)	136 ± 6,05 (4)	0,05
O.T.C.	100 ± 5,70 (8)	72,0 ± 4,70 (4)	0,005	100 ± 6,85 (8)	88,8 ± 2,92 (8)	N.S.
A.S.S.	-	-	-	100 ± 14,3 (6)	101 ± 11,3 (7)	N.S.
A.S.L.	-	-	-	100 ± 4,39 (7)	97,1 ± 2,59 (7)	N.S.
Arg.	100 ± 8,57 (7)	106 ± 15,2 (4)	N.S.	100 ± 4,71 (16)	117 ± 4,72 (7)	0,025

Figure 12 : The effect on the specific activity of the urea cycle enzymes 24 hours after a single cortisol injection in rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

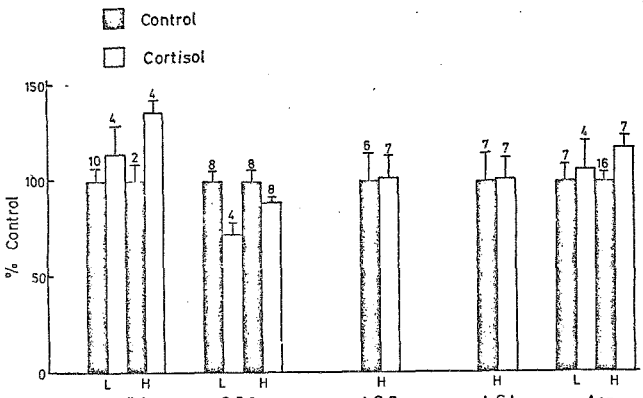


Fig. 12 - Effect on Specific Activity of Urea Cycle Enzymes 24 Hours after a Single Dose of Cortisol

3.2.1.3.3 The Effect of Urea Cycle Enzyme Levels of Daily Cortisol Administration

Batches of rats were fed on 10% protein and 75% protein diets, respectively, for seven days prior to cortisol treatment. During cortisol administration, they were maintained on the same respective diets.

The control rats received no hormone treatment.

The experimental rats were given daily injections of cortisol (20 mg. per 100 g. body weight) for four consecutive days after the initial seven-day feeding period. Eight hours after the fourth injection, the animals were killed and the livers of control and experimental rats were assayed for enzymes.

Tables 12 and 13 show the effect of daily cortisol administration on the five enzymes. The effect is also depicted in Figures 13 and 14. Results are expressed as a percentage of the control. Each result represents a mean of the number of animals indicated above the standard error.

On the low protein diet (10% protein), cortisol administration caused significant increases in the activity per unit wet weight of liver and specific activity of C.P.S., A.S.S. and arginase (Tables 12 and 13 and Figures 13 and 14). The most marked effect was on arginase where a 2-fold increase was observed. A.S.S. showed an increase slightly less than 2-fold, while C.P.S. increased 1,5-fold. The increases in O.T.C. and A.S.L. levels which Schimke has reported (Schimke, 1963) in rats maintained on a similar diet (15% protein) and receiving similar hormonal doses (25 mg. cortisol per 100 g. body weight), were not observed.

Table 12 - Table of Results for Figure 13

The Effect of Daily Cortisol Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10% Protein			75% Protein		
Enzyme	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental	
C.P.S.	100 ± 6,27 (10)	144 ± 2,42 (3)	0,001	100 ± 4,92 (3)	129 ± 7,01 (4)	0,025
O.T.C.	100 ± 6,80 (10)	107 ± 2,70 (3)	N.S.	100 ± 1,40 (4)	75,5 ± 1,90 (4)	0,001
A.S.S.	100 ± 4,88 (10)	177 ± 9,11 (4)	0,001	100 ± 4,29 (4)	101 ± 4,05 (4)	N.S.
A.S.L.	100 ± 3,69 (9)	90,2 ± 4,24 (3)	N.S.	100 ± 3,94 (4)	65,6 ± 3,69 (4)	0,001
Arg.	100 ± 8,09 (7)	207 ± 6,88 (3)	0,001	100 ± 7,47 (4)	123 ± 1,31 (3)	0,05

Figure 13 : The effect of daily cortisol administration on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fet on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

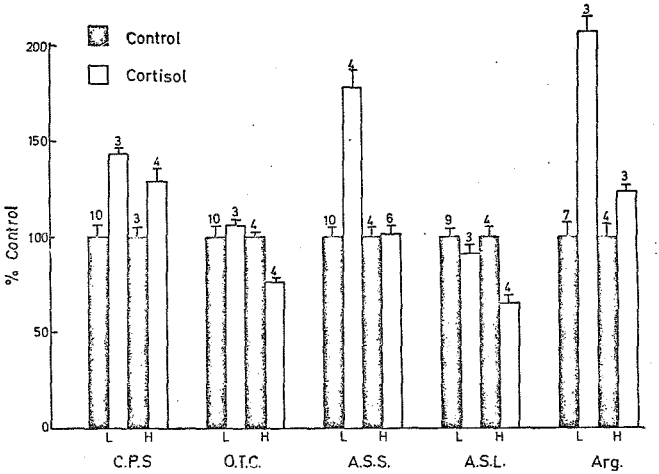


Fig. 13 - *Effect of Daily Cortisol Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight*

Table 13- Table of Results for Figure 14

The Effect of Daily Cortisol Administration on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein			75% Protein		
Enzyme	Specific Activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental	
C.P.S.	100 ± 5,90 (10)	148 ± 6,60 (3)	0,001	100 ± 6,10 (3)	141 ± 5,10 (4)	0,005
O.T.C.	100 ± 4,97 (10)	111 ± 5,58 (3)	N.S.	100 ± 3,86 (4)	89,0 ± 3,70 (4)	N.S.
A.S.S.	100 ± 5,16 (10)	184 ± 10,8 (4)	0,001	100 ± 4,18 (4)	119 ± 2,14 (4)	0,010
A.S.L.	100 ± 5,00 (9)	89,8 ± 0,50 (3)	N.S.	100 ± 4,90 (4)	77,2 ± 4,20 (4)	0,020
Arg.	100 ± 7,41 (7)	197 ± 3,45 (3)	0,001	100 ± 4,88 (4)	142 ± 0,78 (3)	0,001

Figure 14 : The effect of daily cortisol administration on the specific activity of the urea cycle enzymes in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

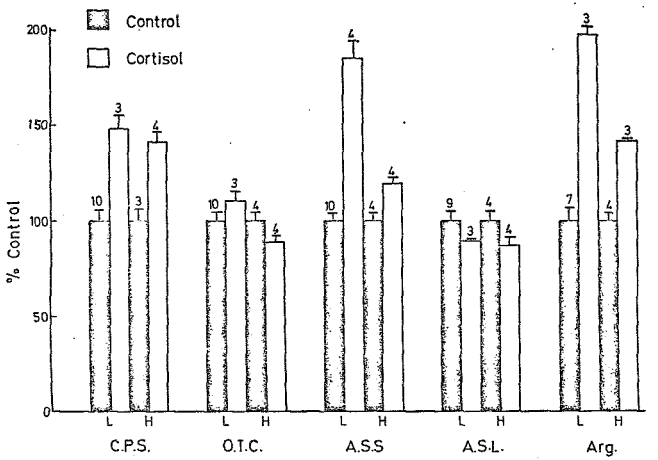


Fig. 14 - Effect of Daily Cortisol Administration on Specific Activity of Urea Cycle Enzymes

Cortisol administration to rats on the high protein diet (75% protein) did, however, not show such marked effects as occurred in rats maintained on the low protein diet. If activity per unit wet weight of liver is considered, C.P.S. and arginase again showed significant increases. A.S.S. was not affected, while O.T.C. and A.S.L. showed a significant decrease on the high protein diet (in contrast to little response on the low protein diet) - see Table 12 and Figure 13.

Significant increases in specific activity were found for C.P.S., A.S.S. and arginase on the 75% protein diet. O.T.C. and A.S.L. showed a decrease (Table 13 and Figure 14).

3.2.2 The Effect of Adrenalectomy on Enzyme Levels

Batches of rats were fed on three different diets, normal (containing 20% protein), 10% protein and 75% protein, respectively, for seven days prior to adrenalectomy. Post-operatively, all rats were given 5% glucose and 0,9% sodium chloride in their drinking water, and were maintained on their respective diets until killed for enzyme assays. Ample sodium chloride was freely available as a lick.

Sham operations were carried out on control rats. Enzyme levels in sham-operated animals showed no significant change with respect to normal rats.

Experimental rats were adrenalectomised after the initial seven-day feeding period. Three days after the operations, all animals were killed and their livers were assayed for the urea cycle enzymes.

Results appear in Tables 14 and 15, and Figures 15 and 16. All results are expressed as a percentage of the control. Each result

Table 14 - Table of Results for Figure 15

Effect of Adrenalectomy on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10% Protein			75% Protein		
Enzyme	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Adrenalectomised		Control	Adrenalectomised	
C.P.S.	$100 \pm 6,60$ (5)	$64,3 \pm 3,40$ (5)	0,001	$100 \pm 6,44$ (2)	$83,4 \pm 3,39$ (3)	N.S.
O.T.C.	$100 \pm 7,00$ (5)	$70,1 \pm 4,20$ (5)	0,005	$100 \pm 2,40$ (2)	$121 \pm 5,80$ (3)	0,025
A.S.S.	$100 \pm 11,3$ (4)	$63,7 \pm 5,90$ (3)	0,025	$100 \pm 0,390$ (2)	$83,5 \pm 0,930$ (3)	0,001
A.S.L.	$100 \pm 6,56$ (4)	$140 \pm 7,52$ (3)	0,010	$100 \pm 8,73$ (2)	$104 \pm 1,70$ (3)	N.S.
Arg.	$100 \pm 7,10$ (3)	$75,6 \pm 4,70$ (2)	0,050	$100 \pm 15,6$ (2)	$53,7 \pm 5,50$ (3)	0,05

Figure 15 : The effect of adrenalectomy on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on normal, low and high protein diets.

L - 10% protein diet
N - normal diet (20% protein)
H - 75% protein diet

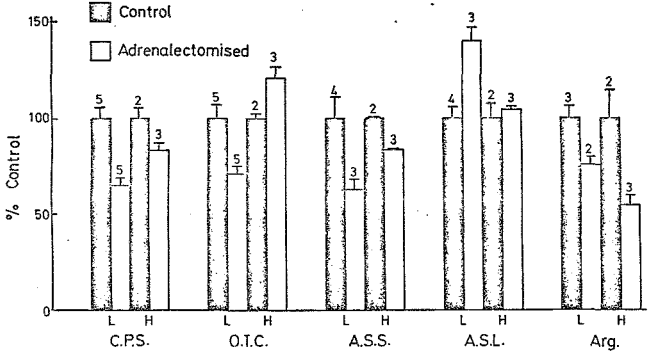


Fig. 15 - Effect of Adrenalectomy on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 15 - Table of Results for Figure 16

Effect of Adrenalectomy on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein				75% Protein			
Enzyme	Specific activity + standard error of control X 10^2	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity + standard error of control X 10^2	Specific activity + standard error expressed as a percentage of control		Confidence limit of significance of difference from control
		Control	Adrenalectomised			Control	Adrenalectomised	
C.P.S.	0,840±0,0678	100±8,10 (5)	75,0±3,30 (5)	0,025	4,92±0,0333	100±0,700 (2)	88,6±4,80 (3)	N.S.
O.T.C.	112±2,43	100±2,17 (5)	93,1±9,80 (5)	N.S.	113±8,17	100±7,21 (2)	125±8,15 (3)	N.S.
A.S.S.	1,06 ±0,0807	100±7,62 (4)	74,0±11,6 (3)	N.S.	3,23±0,250	100±7,73 (2)	88,1±3,24 (3)	N.S.
A.S.L.	1,24±0,115	100±9,3 (4)	157±7,10 (3)	0,005	3,25±0,517	100±15,9 (2)	109±2,4 (3)	N.S.
Arg.	299±14,7	100±4,91 (3)	87,6±9,04 (2)	N.S.	609±51,9	100±8,53 (2)	57,5±6,72 (3)	0,05

Figure 16 : The effect of adrenalectomy on the specific activity of the urea cycle enzymes in the livers of rats fed on normal, low and high protein diets.

L - 10% protein diet

N - normal diet (20% protein)

H - 75% protein diet

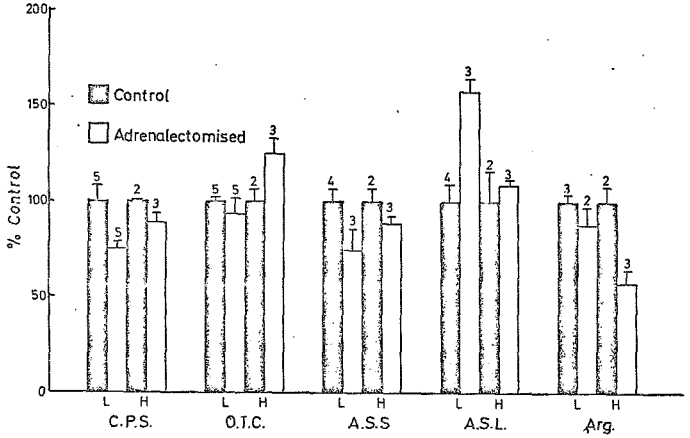


Fig. 16 - Effect of Adrenalectomy on Specific Activity of Urea Cycle Enzymes

represents a mean of the number of animals indicated above the standard error.

Adrenalectomy caused a significant decrease in the activity per unit wet weight of liver of C.P.S., O.T.C., A.S.S. and arginase on the 10% protein diet; A.S.L. showed a significant increase (Table 14 and Figure 15). The latter result differs from Schimke's work which showed, on adrenalectomy, a decrease in all enzyme levels, including A.S.L. in rats fed on a low protein diet (Schimke, 1963). If specific activity is considered, on the 10% protein diet, only C.P.S. and possibly A.S.S. and arginase showed a decrease in levels, while A.S.L. levels again increased significantly (Table 15 and Figure 16).

On the 75% protein diet, adrenalectomy caused a significant decrease in activity per unit wet weight of A.S.S., arginase and possibly C.P.S. O.T.C. now showed an increase in activity as did A.S.L. (Table 14 and Figure 15).

A similar pattern of behaviour of specific activity was observed. C.P.S., A.S.S. and arginase underwent a decrease in levels; the arginase result being significantly different from the sham operated rats. An increase in O.T.C. and A.S.L. also occurred (Table 15 and Figure 16).

3.2.3 The Effect of Adrenalectomy Followed by Cortisol Administration on the Urea Cycle Enzyme Levels

3.2.3.1 Effect after Eight Hours

Batches of rats were fed on three different diets, normal (containing 20% protein), 10% protein and 75% protein, respectively, for seven days prior to surgery. Post-operatively, all rats were given 5% glucose and 0,9% sodium chloride in their drinking water, and were maintained on their

respective diets until used for enzyme assays. Ample sodium chloride was freely available as a lick.

Sham operations were done on control rats.

The remaining rats were adrenalectomised. Three days after adrenalectomy, a portion of the rats received a single intraperitoneal injection of 20 mg. of cortisol per 100 g. body weight. The remainder received no hormone treatment. All rats were killed and their livers assayed for the urea cycle enzymes eight hours after cortisol administration.

The effects of adrenalectomy, and adrenalectomy plus cortisol administration on the five enzyme levels, in rats fed on the three different diets, are shown in Tables 16 and 17 and Figures 17 and 18.

The effects of adrenalectomy have been discussed (see "Results : 3.2.2")

On the 10% protein diet, the activity per unit wet weight of liver of C.P.S., A.S.S. and arginase in adrenalectomised rats treated with cortisol were still significantly lower than sham-operated rats. A.S.L. levels were still significantly higher than control levels. A similar response of specific activity was observed.

If one compares enzyme levels in adrenalectomised rats with those in adrenalectomised rats treated with cortisol, one sees that on the 10% protein diet, no significant effect occurred with respect to activity per unit wet weight. The specific activity of C.P.S. was significantly increased at eight hours (Tables 16 and 17 and Figures 17 and 18).

On the 75% protein diet, cortisol administration to adrenalectomised

Table 16 - Table of Results for Figure 17

Effect of Adrenalectomy and Adrenalectomy plus a Single Dose of Cortisol (6 Hours after Administration) on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10 % Protein						75 % Protein					
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of B from A	Activity per unit wet weight and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of D from C
		Adrenalectomised						Adrenalectomised				
	Control	No Cortisol Treatment (A)	Plus Cortisol Treatment (B)	No Cortisol Treatment	Plus Cortisol Treatment		Control	No Cortisol Treatment (C)	Plus Cortisol Treatment (D)	No Cortisol Treatment	Plus Cortisol Treatment	
C.P.S.	100 ± 6,60 (5)	64,3 ± 3,40 (5)	70,5 ± 2.52 (5)	0,001	0,005	N.S.	100 ± 6,44 (2)	83,4 ± 3,39 (3)	79,0 ± 3,05 (2)	N.S.	0,050	N.S.
O.T.C.	100 ± 7,00 (5)	70,1 ± 4,20 (5)	75,3 ± 9.80 (5)	0,005	N.S.	N.S.	100 ± 2,40 (2)	121 ± 5,80 (3)	105 ± 5,50 (3)	0,025	N.S.	N.S.
A.S.S.	100 ± 11,3 (4)	63,7 ± 5,90 (3)	61,0 ± 4,39 (2)	0,025	0,020	N.S.	100 ± 0,390 (2)	83,5 ± 0,930 (3)	72,8 ± 0,380 (3)	0,001	0,001	0,001
A.S.L.	109 ± 6,56 (4)	140 ±7,52 (3)	154 ±4,29 (2)	0,010	0,001	N.S.	100 ± 8,73 (2)	104 ± 1,70 (3)	94,2 ± 5,66 (3)	N.S.	N.S.	N.S.
Arg.	100 ± 7,10 (3)	75,6 ± 4,70 (2)	71,1 ± 5,20 (2)	0,050	0,025	N.S.	100 ± 15,6 (2)	53,7 ± 5,50 (3)	55,9 ± 1,61 (3)	0,050	0,050	N.S.

Figure 17 : The effect of adrenalectomy and adrenalectomy *plus* a single dose of cortisol eight hours after administration on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

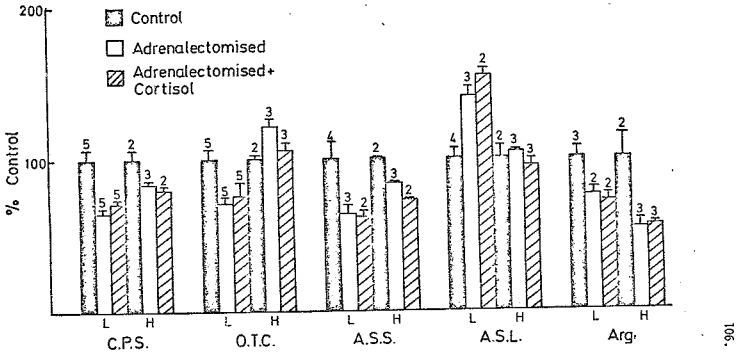


Fig. 17 - Effect of Adrenalectomy and Adrenalectomy plus a Single Dose of Cortisol (8 Hours after Administration) on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 17 - Table of Results for Figure 18

Effect of Adrenalectomy and Adrenalectomy plus a Single Dose of Cortisol (8 Hours after Administration) on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein						75% Protein					
Enzyme	Specific activity and standard error expressed as a percentage of control			Confidence limit of significance of difference from *control*		Confidence limit of significance of *differen* difference of B from A	Specific activity and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of D from C
	Control	Adrenalectomised					Control	Adrenalectomised				
		No Cortisol Treatment (A)	Plus Cortisol Treatment (B)	No Cortisol Treatment	Plus Cortisol Treatment			No Cortisol Treatment (C)	Plus Cortisol Treatment (D)	No Cortisol Treatment	Plus Cortisol Treatment	
C.P.S.	100 ± 8,10 (5)	75,0 ± 3,30 (5)	87,7 ± 2,50 (5)	0,020	N.S.	0,010	100 ± 0,700 (2)	88,6 ± 4,80 (3)	84,1 ± 0,700 (2)	N.S.	0,001	N.S.
O.T.C.	100 ± 2,17 (5)	93,1 ± 9,80 (5)	99,6 ± 12,8 (5)	N.S.	N.S.	N.S.	100 ± 7,20 (2)	123 ± 8,15 (3)	109 ± 4,64 (3)	N.S.	N.S.	N.S.
A.S.S.	100 ± 7,62 (4)	74,0 ± 11,6 (3)	73,2 ± 0,79 (2)	N.S.	0,020	N.S.	100 ± 7,73 (2)	88,1 ± 3,24 (3)	77,1 ± 2,84 (2)	N.S.	0,050	N.S.
A.S.L.	100 ± 9,30 (4)	157 ± 7,10 (3)	183 ± 15,5 (2)	0,005	0,005	N.S.	100 ± 15,9 (2)	109 ± 2,40 (3)	99,1 ± 6,00 (3)	N.S.	N.S.	N.S.
Arg.	100 ± 4,91 (3)	87,6 ± 9,54 (2)	81,0 ± 1,37 (2)	N.S.	0,020	N.S.	100 ± 8,53 (2)	57,5 ± 6,72 (3)	59,9 ± 0,710 (3)	0,020	0,010	N.S.

Figure 18 : The effect of adrenalectomy and adrenalectomy plus a single dose of cortisol eight hours after administration on the specific activity of the urea cycle enzymes in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

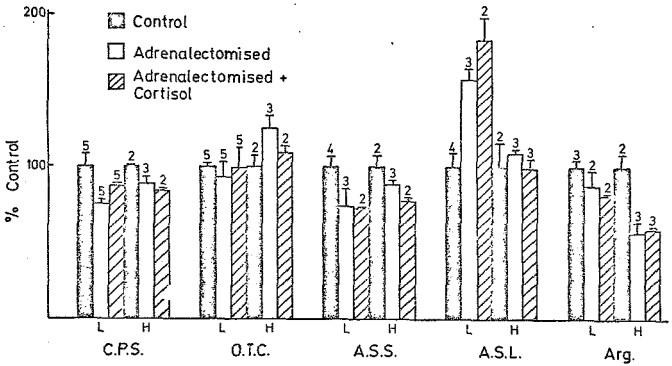


Fig. 18 - Effect of Adrenalectomy and Adrenalectomy plus a Single Dose of Cortisol (8 Hours after Administration) on Specific Activity of Urea Cycle Enzymes

rats resulted in a significant decrease in activity per unit wet weight of A.S.S. with respect to adrenalectomised levels. After cortisol treatment the activity per unit wet weight of C.P.S., A.S.S. and arginase at eight hours was still significantly lower than levels in sham-operated rats, as was the specific activity of these enzymes (Tables 16 and 17 and Figures 17 and 18). No significant change, compared with adrenalectomised levels, was observed in specific activity on the high protein diet, eight hours after cortisol administration.

3.2.3.2 Effect of Daily Cortisol Administration

Batches of rats were fed on the two extreme diets, 10% protein and 75% protein, respectively, for seven days prior to adrenalectomy. Post-operatively, all rats were given 5% glucose plus 0,9% sodium chloride in their drinking water, and were maintained on their respective diets until their enzymes were assayed. Ample sodium chloride was freely available.

Sham operations were done on all control rats.

The remaining rats were adrenalectomised. A portion of the adrenalectomised rats received the following hormone treatment. A day before, and immediately after adrenalectomy, each received an intraperitoneal cortisol injection (20 mg. per 100 g. body weight). Each received daily cortisol injections (20 mg. per 100 g. body weight) for three consecutive days thereafter (a total of five cortisol injections). Eight hours after the last cortisol treatment, assays were carried out on all rats.

Results appear in Tables 18 and 19 and Figures 19 and 20. All results are expressed as a percentage of the control. Each result represents a mean of the number of animals indicated above the standard error.

Table 18 - Table of Results for Figure 19

Effect of Adrenalectomy and Adrenalectomy Plus Daily Cortisol Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10 % Protein						75 % Protein					
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control			Confidence limit of significance of difference from control)		Confidence limit of significance of difference of B from A	Activity per unit wet weight and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of D from C
	Control	Adrenalectomised					Control	Adrenalectomised				
		No Cortisol Treatment (A)	Plus Cortisol Treatment (B)	No Cortisol Treatment	Plus Cortisol Treatment			No Cortisol Treatment(C)	Plus Cortisol Treatment (D)	No Cortisol Treatment	Plus Cortisol Treatment	
C.P.S.	100 ± 6,27 (10)	74,7 ± 6,21 (5)	172 ± 9,50 (4)	0,020	0,001	0,001	100 ± 15,0 (2)	103 ± 5,90 (6)	119 ± 11,5 (4)	N.S.	N.S.	N.S.
O.T.C.	100 ± 6,80 (10)	93,4 ± 4,50 (5)	114 ± 2,20 (4)	N.S.	N.S.	0,001	100 ± 2,50 ((2)	93,0 ± 3,60 (6)	98,9 ± 1,40 (3)	N.S.	N.S.	N.S.
A.S.S.	100 ± 4,88 (10)	80,8 ± 3,51 (5)	150 ± 8,25 (4)	0,010	0,001	0,001	100 ± 5,67 (2)	96,4 ± 3,23 (6)	90,9 ± 3,79 (4)	N.S.	N.S.	N.S.
A.S.L.	100 ± 3,69 (9)	121 ±9,42 (5)	98,0 ± 5,51 (4)	N.S.	N.S.	N.S.	100 ± 0,73 (2)	166 ± 6,84 (6)	121 ± 0,71 (3)	0,001	0,001	0.001
Arg.	100 ± 8,09 (7)	83,5 ± 13,8 (5)	213 ± 10,0 (4)	N.S.	0,001	0,001	100 ± 2,20 (2)	78,7 ± 4,35 (6)	147 ± 7,08 (3)	0,005	0,001	0,001

Figure 19 : The effect of adrenalectomy and adrenalectomy plus daily cortisol administration of the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

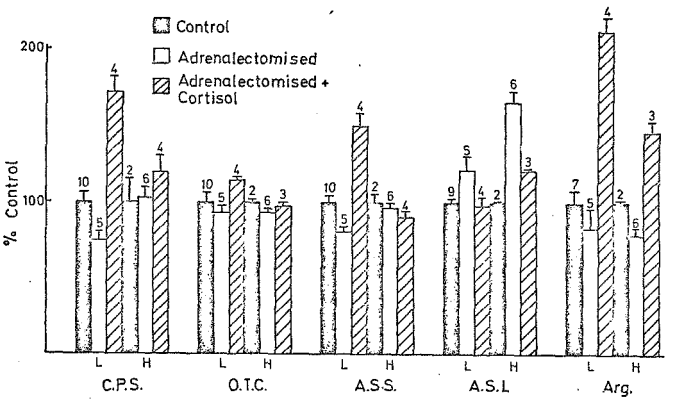


Fig. 19 - Effect of Adrenalectomy and Adrenalectomy plus Daily Cortisol Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 19 - Table of Results for Figure 20

Effect of Adrenalectomy and Adrenalectomy Plus Daily Cortisol Administration on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein						75% Protein					
Enzyme	Specific activity and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of differen difference of B from A	Specific activity and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of D from C
	Control	Adrenalectomised					Control	Adrenalectomised				
		No Cortisol Treatment (A)	Plus Cortisol Treatment (B)	No Cortisol Treatment	Plus Cortisol Treatment			No Cortisol Treatment (C)	Plus Cortisol Treatment (D)	No Cortisol Treatment	Plus Cortisol Treatments	
C.P.S.	100 ± 5,90 (10)	57,6 ± 3,60 (5)	128 ± 5,70 (4)	0,001	0,005	0,001	100 ± 13,1 (2)	99,4 ± 6,80 (6)	129 ± 15,8 (4)	N.S.	N.S.	N.S.
O.T.C.	100 ± 4,97 (10)	72,6 ± 1,73 (5)	85,7 ± 2,16 (4)	0,001	0,020	0,001	100 ± 4,50 (2)	89,6 ± 4,50 (6)	103 ± 2,72 (3)	N.S.	N.S.	0,050
A.S.S.	100 ± 5,16 (10)	52,4 ± 3,12 (5)	111 ± 5,29 (4)	0,001	N.S.	0,001	100 ± 3,65 (2)	93,0 ± 4,06 (6)	98,0 ± 6,55 (4)	N.S.	N.S.	N.S.
A.S.L.	100 ± 5,00 (9)	90,7 ± 5,50 (5)	70,4 ± 3,20 (4)	N.S.	0,001	0,010	100 ± 1,60 (2)	161 ± 9,80 (6)	148 ± 15,7 (3)	0,001	0,020	N.S.
Arg.	100 ± 7,41 (7)	59,5 ± 9,27 (5)	144 ± 5,27 (4)	0,005	0,001	0,001	100 ± 0,17 (2)	75,9 ± 5,08 (6)	156 ± 13,4 (3)	0,005	0,005	0,001

Figure 20 : The effect of adrenalectomy and adrenalectomy plus daily cortisol administration on the specific activity of the urea cycle enzymes in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

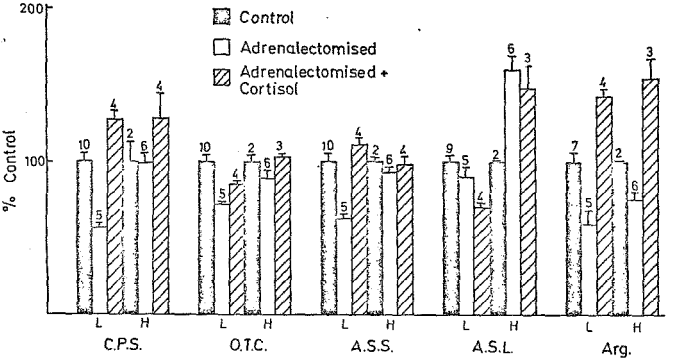


Fig. 20 - Effect of Adrenalectomy and Adrenalectomy plus Daily Cortisol Administration on Specific Activity of Urea Cycle Enzymes

Adrenalectomy again caused a significant decrease in the activity per unit wet weight of liver of C.P.S., A.S.S. and possibly arginase on the 10% protein diet, while A.S.L. showed an increase. O.T.C. was little affected (Table 18 and Figure 19). Under the same conditions, C.P.S., O.T.C., A.S.S. and arginase showed a decrease in specific activity (Table 19 and Figure 20).

Cortisol, administered daily, to adrenalectomised rats on the 10% protein diet, produced marked increases in the activity per unit weight and specific activity of all enzymes except A.S.L. The level of A.S.L. showed no change per unit wet weight and a significant drop in specific activity due to daily cortisol administration (Tables 18 and 19 and Figures 19 and 20).

On the 75% protein diet, adrenalectomy caused a significant drop in activity per unit wet weight of liver and specific activity of arginase, and an increase in that of A.S.L. (Tables 18 and 19 and Figures 19 and 20).

Daily cortisol administration to adrenalectomised rats on the high protein diet, caused significant increases in the activity per unit wet weight and specific activity of arginase and possibly C.P.S. compared with both adrenalectomised and sham-operated values. A significant decrease in activity per unit wet weight and a decrease in specific activity was observed in the case of A.S.L. The specific activity of O.T.C. was significantly increased by daily cortisol administration (Tables 18 and 19 and Figures 19 and 20).

3.2.4 The Effect of Thyroxine Administration on Enzyme Levels

3.2.4.1 Response of Enzyme Levels to two Different Doses of Thyroxine

Batches of rats maintained on the normal diet of mouse pellets (20%

protein) since weaning were divided into two experimental groups. The one group received an intraperitoneal thyroxine injection of 1 mg. per 100 g. body weight; the other an injection of 2 mg. per 100 g. body weight.

Control rats received no hormone treatment.

Eight hours after thyroxine administration, all rats were killed and their livers immediately assayed for the urea cycle enzymes.

The effect of the two different doses of thyroxine on activity per unit wet weight is shown in Table 20 and Figure 21. The effect on specific activity is shown in Table 21 and Figure 22. Results are expressed as a percentage of the control. Each result represents a mean of the number of animals shown above the standard error.

If activity per unit wet weight is considered, the smaller dose of thyroxine (1 mg. per 100 g. body weight) caused a possible increase in C.P.S., while the larger dose (2 mg. per 100 g. body weight) caused a significant decrease in C.P.S., A.S.L. and arginase levels. In addition, O.T.C. and A.S.L. activity per unit wet weight were significantly decreased by the 1 mg. per 100 g. body weight dose of thyroxine (Table 20 and Figure 21).

No significant effects on specific activity were observed (Table 21 and Figure 22).

3.2.4.2 *The Response of O.T.C. to Thyroxine Administration after Different Time Intervals*

Rats which had previously been fed on a normal diet of rat pellets (20% protein) were injected intraperitoneally with thyroxine (1 mg. per 100 g. body weight). At different time periods thereafter, namely four, 12 and 24 hours, batches of rats were killed and their livers immediately assayed for O.T.C. activity.

Table 20 - Table of Results for Figure 21

Response of Activity of Urea Cycle Enzymes per unit Wet Weight to Two Different Doses of Thyroxine on Normal Diet (20 % Protein)

Dose	1 mg/100 g. body weight			2 mg./100 g. body weight		
Enzyme	*Activity per unit wet weight and standard error expressed as a percentage of control*		*Confidence limit of significance of difference from control*	*Activity per unit wet weight and standard error expressed as a percentage of control*		*Confidence limit of significance of difference from control*
	Control	Experimental		Control	Experimental	
C.P.S.	100 ± 3,03 (3)	110 ± 1,26 (2)	N.S.	100 ± 3,94 (16)	83,4 ± 5,50 (4)	0,025
O.T.C.	100 ± 2,77 (3)	80,0 ± 2,00 (3)	0,001	100 ± 2,37 (4)	85,0 ± 3,00 (4)	N.S.
A.S.S.	100 ± 5,75 (7)	92,5 ± 7,24 (3)	N.S.	100 ± 5,75 (7)	94,1 ± 8,58 (4)	N.S.
A.S.L.	100 ± 5,60 (4)	65,3 ± 8,72 (3)	0,025	100 ± 5,60 (4)	74,2 ± 7,71 (4)	0,05
Arg.	100 ± 2,71 (3)	98,6 ± 8,09 (3)	N.S.	100 ± 4,42 (4)	75,7 ± 1,37 (4)	0,005

Figure 21 : Response of activity of urea cycle enzymes per unit wet weight to two different doses of thyroxine, administered to rats fed on a normal diet (20% protein).

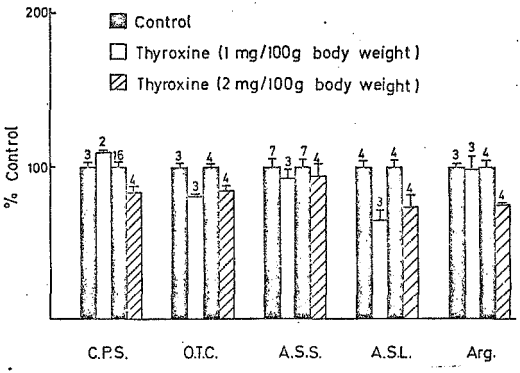


Fig. 21 - Response of Activity of Urea Cycle Enzymes per Unit Wet Weight to Two Different Doses of Thyroxine

Table 21 - Table of Results for Figure 22

Response of Specific Activity of Urea Cycle Enzymes to Two Different Doses of Thyroxine on Normal Diet (20% Protein)

Dose	1 mg./100 g. body weight			2 mg./100 g. body weight		
Enzyme	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental	
C.P.S.	100 ± 2,08 (3)	104 ± 2,40 (2)	N.S.	100 ± 5,94 (16)	102 ± 5,92 (4)	N.S.
O.T.C.	100 ± 2,37 (3)	80,4 ± 12,5 (3)	N.S.	100 ± 3,96 (5)	94,7 ± 3,58 (4)	N.S.
A.S.S.	100 ± 9,73 (7)	97,1 ± 3,97 (3)	N.S.	100 ± 9,73 (7)	96,0 ± 10,2 (4)	N.S.
A.S.L.	100 ± 10,3 (4)	117 ± 11,0 (3)	N.S.	100 ± 10,3 (4)	126 ±13,3 (4)	N.S.
Arg.	100 ± 3,54 (3)	103 ± 1,34 (3)	N.S.	100 ± 13,5 (4)	99,0 ± 1,27 (4)	N.S.

Figure 22 : The response of specific activity of the urea cycle enzymes to two different doses of thyroxine, administered to rats fed on a normal diet (20% protein).

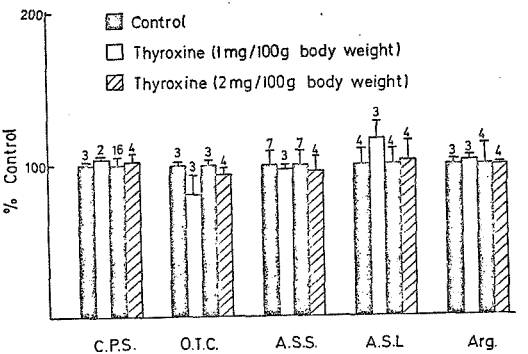


Fig. 22 - Response of Specific Activity of Urea Cycle Enzymes to two Different Doses of Thyroxine

Control rats (0 hours) received no hormone treatment.

Table 22 shows the effect of thyroxine administration on O.T.C. activity per unit wet weight and specific activity after the different time intervals. Each result represents a mean of the number of animals indicated in the table.

No significant effects were observed.

3.2.4.3 General Thyroxine Effect in Rats fed on Diets of Different Protein Content

3.2.4.3.1 Effect on Urea Cycle Enzyme Levels Eight Hours after a Single Dose of Thyroxine

Batches of rats were fed on normal, 10% protein and 75% protein diets, respectively, for seven days prior to thyroxine administration.

The control rats received no hormone treatment.

The experimental rats were given a single intraperitoneal thyroxine injection (1 mg. per 100 g. body weight). Eight hours later all animals were killed and their livers immediately assayed for the urea cycle enzymes.

The effects on activity per unit wet weight of liver are shown in Table 23 and Figure 23, while the effects on specific activity are shown in Table 24 and Figure 24. Results are expressed as a percentage of the control.

If the 10% protein diet is considered, thyroxine was found to significantly increase the activity per unit wet weight of arginase, and decrease the specific activity of C.P.S. (Tables 23 and 24 and Figures 23 and 24). No result was obtained for the specific activity of A.S.S. due to experimental error.

Table 22 : O.T.C. Response after Different Time Intervals to Thyroxine Administration

Time interval between injection and assay	Activity per unit wet weight weight of O.T.C. and standard error	No. of animals used for mean result	Confidence limit of significance of difference from control	Specific activity of O.T.C. and standard error x 10^2	No. of animals used for mean result	Confidence limit of significance of difference from control
0	261 ± 10,6	4	-	117 ± 18,4	4	-
4	260 ± 14,8	4	N.S.	121 ± 6,39	4	N.S.
12	248 ± 15,1	4	N.S.	106 ± 7,05	4	N.S.
24	251 ± 6,34	4	N.S.	105 ± 3,25	4	N.S.

Table 23 -Table of Results for Figure 23

Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 8 Hours after a Single Dose of Thyroxine

Diet	10% Protein			Normal (20% Protein)			75% Protein		
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 7,01 (14)	124 ± 12,1 (4)	N.S.	100 ± 3,03 (3)	110 ± 1,26 (2)	N.S.	100 ± 4,17 (16)	111 ± 3,17 (7)	0,05
O.T.C.	100 ± 3,59 (7)	98,0 ± 5,67 (8)	N.S.	100 ± 2,41 (7)	79,6 ± 1,96 (3)	0,001	100 ± 2,69 (8)	118 ± 2,51 (8)	0,001
A.S.S.	100 ± 5,35 (4)	106 ± 4,26 (2)	N.S.	100 ± 5,75 (7)	92,5 ± 7,24 (2)	N.S.	100 ± 7,78 (8)	94,4 ± 2,67 (4)	N.S.
A.S.L.	100 ± 13,2 (2)	129 ± 11,7 (4)	N.S.	100 ± 5,60 (4)	65,3 ± 8,72 (3)	0,025	100 ± 5,81 (8)	96,0 ± 3,71 (8)	N.S.
Arg.	100 ± 5,32 (8)	128 ± 10,7 (8)	0,05	100 ± 2,58 (7)	98,6 ± 8,09 (3)	N.S.	100 ± 4,87 (8)	125 ± 5,62 (8)	0,005

Figure 23 : The effect on the activity of the urea cycle enzymes per unit wet weight, eight hours after a single thyroxine injection, in the livers of rats fed on normal, low and high protein diets.

L - 10% protein diet

N - normal diet (20% protein)

H - 75% protein diet

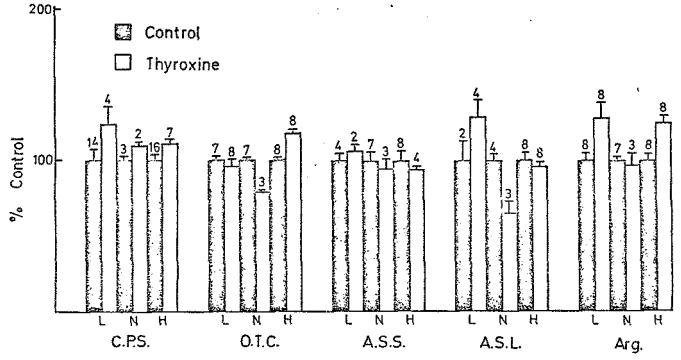


Fig. 23 - Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 8 Hours after a Single Dose of Thyroxine

Table 24 -Table of Results for Figure 24
Effect on Specific Activity of Urea Cycle Enzymes 8 Hours After A Single Dose of Thyroxine

Diet	10% Protein			Normal (20% Protein)			75% Protein		
Enzyme	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 7,47 (10)	59,2 ± 3,16 (4)	0,001	100 ± 2,08 (2)	104 ± 2,40 (2)	N.S.	100 ± 6,74 (16)	117 ± 3,75 (7)	0,05
O.T.C.	100 ± 9,25 (3)	120 ± 4,77 (4)	N.S.	100 ± 2,37 (3)	80,4 ± 12,5 (3)	N.S.	100 ± 5,00 (8)	134 ± 6,52 (8)	0,005
A.S.S.	-	-	-	100 ± 12,9 (3)	97,1 ± 3,79 (3)	N.S.	100 ± 11,2 (8)	113 ± 5,98 (4)	N.S.
A.S.L.	100 ± 15,7 (2)	123 ± 10,2 (4)	N.S.	100 ± 0,000 (1)	117 ± 11,0 (3)	N.S.	100 ± 7,18 (8)	110 ± 3,90 (8)	N.S.
Arg.	100 ± 14,1 (3)	133 ± 10,8 (4)	N.S.	100 ± 3,54 (3)	103 ± 1,34 (3)	N.S.	100 ± 7,18 (8)	141 ± 5,12 (8)	0,005

Figure 24 : The effect on the specific activity of the urea cycle enzymes, eight hours after a single thyroxine injection, in the livers of rats fed on normal, low and high protein diets.

L - 10% protein diet

N - normal diet (20% protein)

H - 75% protein diet

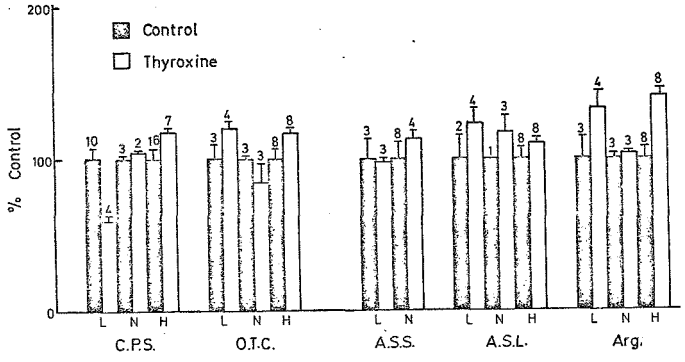


Fig. 24 - Effect on Specific Activity of Urea Cycle Enzymes 8 Hours after a Single Dose of Thyroxine

On the normal diet (20% protein), thyroxine caused a significant drop in the activity per unit wet weight of O.T.C. and A.S.L. (Table 23 and Figure 23). No significant effect on specific activity was observed (Table 24 and Figure 24).

On the 75% protein diet, both the activity per unit wet weight and specific activity of C.P.S., O.T.C. and arginase were increased by thyroxine treatment (Tables 23 and 24 and Figures 23 and 24).

3.2.4.3.2 *Effect on Urea Cycle Enzyme* Levels 24 Hours after after Thyroxine Administration

Batches of rats were fed on normal, 10% and 75% protein diets, respectively, for seven days prior to thyroxine administration.

The control rats received no hormone treatment.

The experimental rats were given a single intraperitoneal thyroxine injection (1 mg. per 100 g. body weight). Twenty-four hours later all animals were killed and their livers immediately assayed for the urea cycle enzymes.

The effects on activity per unit wet weight of liver are shown in Table 25 and Figure 25, while the effects on specific activity are shown in Table 26 and Figure 26. Results are expressed as a percentage of the control.

If the 10% protein diet is considered, thyroxine administration significantly increased the activity per unit wet weight and specific activity of arginase. The activity per unit wet weight of C.P.S. was significantly increased, while the specific activity was decreased (Tables 25 and 26 and Figures 25 and 26). No result for the specific activity of A.S.S. was obtained due to experimental error.

Table 25 - Table of Results for Figure 25

Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 24 Hours After a Single Dose of Thyroxine

Diet	10% Protein			Normal (20% Protein)			75% Protein		
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 7,01 (14)	143 ± 7,67 (4)	0,001	100 ± 3,03 (3)	129 ± 15 (2)	N.S.	100 ± 4,17 (16)	127 ± 3,64 (8)	0,001
O.T.C.	100 ± 3,59 (7)	84,0 ± 2,21 (8)	0,005	100 ± 2,41 (7)	83,9 ± 2,33 (3)	0,005	100 ± 2,69 (8)	123 ± 4,34 (8)	0,001
A.S.S.	100 ± 5,35 (4)	106 ± 12,5 (2)	N.S.	-	-	-	100 ± 7,78 (8)	98,5 ± 3,83 (8)	N.S.
A.S.L.	100 ± 13,2 (2)	98,6 ± 7,94 (4)	N.S.	100 ± 5,60 (4)	75,2 ± 3,36 (3)	0,020	100 ± 5,81 (8)	96,8 ± 4,32 (4)	N.S.
Arg.	100 ± 5,32 (8)	155 ± 14,4 (7)	0,005	100 ± 2,58	99,0 ± 4,80	N.S.	100 ± 4,87 (8)	116 ± 4,78 (12)	0,05

Figure 25 : The effect on the activity of the urea cycle enzymes per unit wet weight, twenty-four hours after a single thyroxine injection, in the livers of rats fed on normal, low and high protein diets.

L - 10% protein diet

N - normal diet (20% proetin)

H - 75% protein

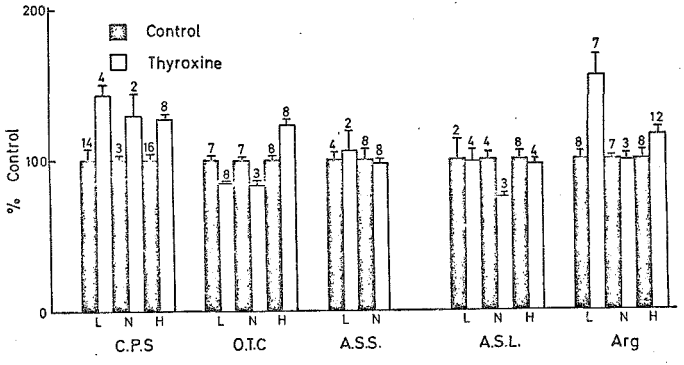


Fig. 25 - Effect on Activity of Urea Cycle Enzymes per Unit Wet Weight 24 Hours after a Single Dose of Thyroxine

Table 26 - Table of Results for Figure 26

Effect on Specific Activity of Urea Cycle Enzymes 24 Hours After a Single Dose of Thyroxine

Diet	10% Protein			Normal (20% Protein)			75% Protein		
Enzyme	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 7,47 (10)	46,8 ± 1,48 (4)	0,001	100 ± 2,08 (3)	129 ± 10,3 (2)	N.S.	100 ± 6,74 (16)	121 ± 4,90 (8)	0,05
O.T.C.	100 ± 9,25 (3)	102 ± 6,78 (4)	N.S.	100 ± 2,37 (3)	82,3 ± 4,93 (3)	0,05	100 ± 5,00 (8)	126 ± 6,04 (8)	0,01
A.S.S.	-	-	-	-	-	-	100 ± 11,2 (8)	99,2 ± 5,92 (8)	N.S.
A.S.L.	100 ± 15,7 (2)	93,7 ± 6,31 (4)	N.S.	100 ± 0,000 (1)	127 ± 4,68 (3)	0,05	100 ± 7,18 (8)	99,1 ± 5,50 (8)	N.S.
Arg.	100 ± 14,1 (3)	171 ± 10,9 (4)	0,02	100 ± 3,54 (3)	97,2 ± 7,68 (3)	N.S.	100 ± 7,18 (8)	117 ± 5,04 (12)	N.S.

Figure 26 : The effect on the specific activity of the urea cycle enzymes, twenty-four hours after a single thyroxine injection, in the livers of rats fed on normal, low and high protein diets.

L - 10% protein diet
N - normal diet (20% protein)
H - 75% protein diet

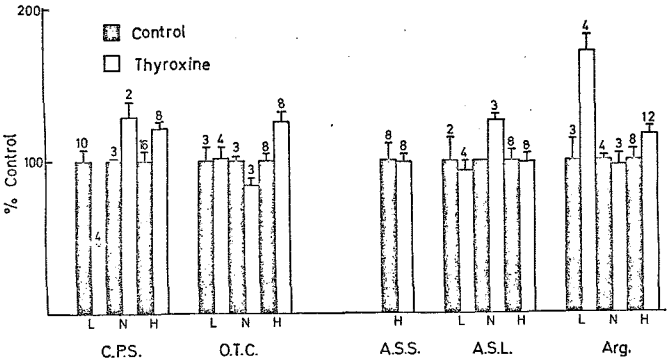


Fig. 26 - Effect on Specific Activity of Urea Cycle Enzymes 24 Hours after a Single Dose of Thyroxine

On the normal diet, the activity per unit weight and specific activity of O.T.C. were both significantly decreased. The activity per unit wet weight of A.S.L. was decreased, while its specific activity was increased (Tables 25 and 26 and Figures 25 and 26). No result was obtained for A.S.S. levels due to experimental error.

On the 75% protein diet, the activity per unit wet weight and specific activity of both C.P.S. and O.T.C. were significantly increased 24 hours after thyroxine treatment. Arginase also showed a significant increase in activity per unit wet weight and possibly specific activity (Tables 25 and 26 and Figures 25 and 26).

3.2.5 The Effect of Thyroidectomy on the Urea Cycle Enzyme Levels

Batches of rats were fed on 10% protein and 75% protein diets, respectively, seven days prior to surgery. Post-operatively, all rats were given 1% calcium gluconate in their drinking water and were maintained on their respective diets until used for enzyme assays (a further two weeks).

Sham operations were done on control rats.

The experimental rats were thyroidectomised after the initial seven-day feeding period. Basal metabolic rates were determined two weeks post-operatively. Assays were then carried out on controls and on rats which had been successfully thyroidectomised.

Results appear in Tables 27 and 28 and Figures 27 and 28. All results are expressed as a percentage of the control values. Each result represents a mean of the number of animals indicated above the standard error.

Thyroidectomy, performed on rats fed on the 10% protein diet, caused

Table 27 - Table of Results for Figure 27

Effect of Thyroidectomy on the Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10% Protein			75% Protein		
Enzyme	Activity per unit wet weight and standard error expressed as a *percentage of control*		Confidence limit of significance *of difference* from control	Activity per unit wet weight and standard error expressed as a *percentage of control*		Confidence limit of significance *of difference* from control
	Control	Thyroidectomised		Control	Thyroidectomised	
C.P.S.	100 ± 10,5 (3)	156 ± 18,0 (4)	0,025	100 ± 4,47 (5)	88,0 ± 1,52 (6)	0,050
O.T.C.	100 ± 0,995 (3)	123 ± 18,6 (4)	N.S.	100 ± 6,95 (3)	118 ± 10,2 (2)	N.S.
A.S.S.	100 ± 7,32 (3)	114 ± 9,00 (4)	N.S.	100 ± 3,65 (5)	91,4 ± 2,37 (6)	N.S.
A.S.L.	100 ± 16,6 (3)	145 ± 15,6 (4)	N.S.	100 ± 9,74 (5)	94,5 ± 6,47 (6)	N.S.
Arg.	100 ± 6,13 (3)	151 ± 14,2 (4)	0,010	100 ± 6,09 (5)	87,1 ± 3,47 (6)	N.S.

Figure 27 : The effect of thyroidectomy on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

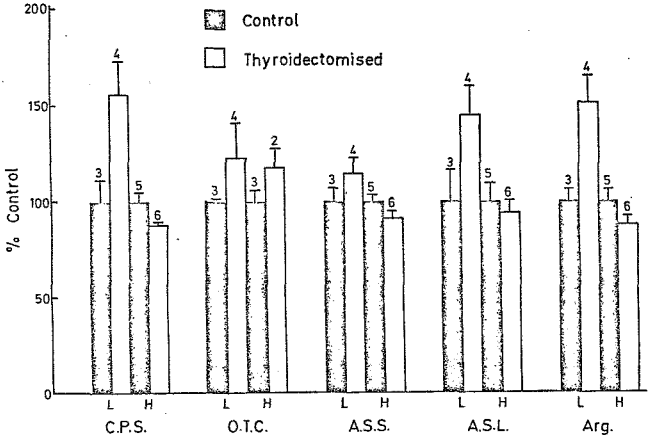


Fig. 27 - Effect of Thyroidectomy on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 28 - Table of Results for Figure 28

Effect of Thyroidectomy on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein			75% Protein		
Enzyme	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Thyroidectomised		Control	Thyroidectomised	
C.P.S.	100 ± 18,8 (3)	150 ± 9,99 (4)	0,05	100 ± 6,63 (5)	94,0 ± 2,65 (6)	N.S.
O.T.C.	100 ± 13,2 (3)	116 ± 9,88 (4)	N.S.	100 ± 11,7 (2)	113 ± 0,185 (2)	N.S.
A.S.S.	100 ± 11,4 (3)	121 ± 4,44 (4)	N.S.	100 ± 3,68 (5)	97,9 ± 2,49 (6)	N.S.
A.S.L.	100 ± 23,4 (3)	149 ± 7,82 (4)	N.S.	100 ± 12,6 (5)	99,8 ± 5,50 (6)	N.S.
Arg.	100 ± 14,2 (3)	158 ± 11,5 (4)	0,02	100 ± 8,26 (5)	86,7 ± 4,56 (6)	N.S.

Figure 28 : The effect of thyroidectomy on the specific activity of the urea cycle enzymes in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

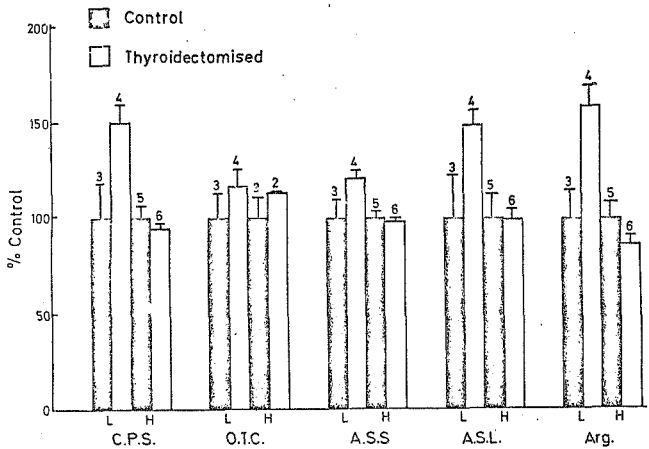


Fig. 28 - Effect of Thyroidectomy on Specific Activity of Urea Cycle Enzymes

a significant increase in the activity per unit wet weight of liver and specific activity of C.P.S. and arginase, and possibly O.T.C., A.S.S. and A.S.L. (Table 27 and 28 and Figures 27 and 28).

On the 75% protein diet, a significant decrease was observed in the activity per unit wet weight of C.P.S. only (Table 27 and Figure 27 No significant effect on specific activity was observed (Table 28 and Figure 28).

3.2.6 The Effect of Thyroidectomy Followed by Thyroxine Administration on the Urea Cycle Enzyme Levels

3.2.6.1 Effect Eight Hours after Thyroxine Administration

Batches of rats were fed on 10% protein and 75% protein diets, respectively, seven days prior to surgery. Post-operatively, all rats were given 1% calcium gluconate (W./V.) in their drinking water, and were maintained on their respective diets until killed for enzyme assays.

Sham operations were done on control rats. (Enzyme levels showed no change with respect to normal rats.)

The remaining rats were thyroidectomised after the initial seven-day feeding period. Basal metabolic rates were determined two weeks post-operatively. A portion of the rats which had been successfully thyroidectomised received a single thyroxine injection (1 mg. per 100 g. body weight). Eight hours later all rats were killed and their livers were assayed for enzymes.

The effects on activity per unit wet weight of liver are shown in Table 29 and Figure 29, and on specific activity in Table 30 and Figure 30. Results are expressed as a percentage of the control. Each result represents a mean of the number of animals indicated above the standard error.

The effects of thyroidectomy have been discussed (see "Results :3.2.5")

On the 10% protein diet, thyroxine administration to thyroidectomised rats caused increases in the activity per unit wet weight of O.T.C. and A.S.S. which were significantly different from the levels in control rats as well as levels in thyroidectomised rats. Possible increases in activity per unit wet weight of C.P.S, A.S.L. and arginase also occurred. These, however, showed significance only with respect to control values (Table 29 and Figure 29).

If specific activity is considered, increases which were significant with respect to control values were observed for all enzymes in rats fed on the 10% protein diet. Only A.S.S. showed an increase in specific activity which was significantly different from levels in thyroidectomised rats (Table 30 and Figure 30).

In animals fed on the 75% protein diet, the effects were not as marked as on the 10% protein diet. Thyroxine treatment caused increases in the activity per unit wet weight of C.P.S. and arginase which were significant with respect to thyroidectomised levels. A.S.S. showed a significant decrease in both activity per unit wet weight and specific activity with respect to control levels. The specific activity of A.S.S. was also significantly different from the values in thyroidectomised rats (Tables 29 and 30 and Figures 29 and 30).

3.3 Effects of the Hormones, Cortisol and Thyroxine, on Urea Cycle Enzyme Levels in vitro

3.3.1 The Effect of "in vitro" Cortisol Administration on Enzyme Levels

Four groups of rats were maintained on four different diets - normal,

Table 29 - Table of Results for Figure 29

Effect of Thyroidectomy and Thyroidectomy Plus a Single Dose of Thyroxine (8 Hours after Administration) on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10 % Protein						75 % Protein					
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of differende of B from A	Activity per unit wet weight and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of D from C
	Control	Thyroidectomised		Thyroidectomised			Control	Thyroidectomised		Thyroidectomised		
		No Thyroxine Treatment (A)	Plus Thyroxine Treatment (B)	No Thyroxine Treatment	Plus Thyroxine Treatment			No Thyroxine Treatment (C)	Plus Thyroxine Treatment (D)	No Thyroxine Treatment	Plus Thyroxine Treatment	
C.P.S.	100 ± 10,5 (3)	156 ± 18,0 (4)	199 ± 12,0 (4)	0,025	0,001	N.S.	100 ± 4,47 (5)	88,0 ± 1,52 (6)	107 ± 3,58 (3)	0,050	N.S.	0,001
O.T.C.	100 ± 0,995 (3)	123 ± 18,6 (4)	171 ± 7,81 (4)	N.S.	0,001	0,050	100 ± 6,95 (3)	118 ± 10,2 (2)	114 ± 7,03 (3)	N.S.	N.S.	N.S.
A.S.S.	100 ± 7,32 (3)	114 ± 9,00 (4)	171 ± 4,89 (4)	N.S.	0,001	0,001	100 ± 3,55 (5)	91,4 ± 2,37 (6)	82,9 ± 4,05 (3)	N.S.	0,010	N.S.
A.S.L.	100 ± 16,6 (3)	145 ± 15,6 (4)	176 ± 6,51 (4)	N.S.	0,005	N.S.	100 ± 9,74 (5)	94,5 ± 6,47 (6)	138 ± 25,1 (3)	N.S.	N.S.	N.S.
Arg.	100 ± 6,13 (3)	151 ± 14,2 (4)	189 ± 20,8 (4)	0,010	0,005	N.S.	100 ± 6,09 (5)	87,1 ± 3,47 (6)	117 ± 8,68 (3)	N.S.	N.S.	0,010

Figure 29 : The effect of thyroidectomy and thyroidectomy plus a single dose of thyroxine eight hours after administration on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

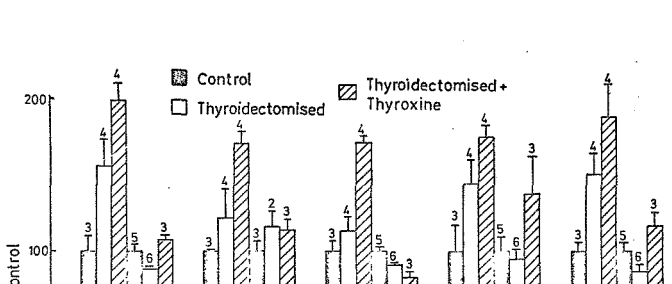


Fig. 29 - Effect of Thyroidectomy and Thyroidectomy plus a Single Dose of Thyroxine (8 Hours after Administration) on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 30 - Table of Results for Figure 30

Effect of Thyroidectomy and Thyroidectomy plus a Single Dose of Thyroxine (8 Hours after Administration) on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein						75% Protein					
Enzyme	Specific activity and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of differen difference of B from A	Specific activity and standard error expressed as a percentage of control			Confidence limit of significance of difference from control		Confidence limit of significance of difference of D from C
	Control	Thyroidectomised					Control	Thyroidectomised				
		No Thyroxine Treatment (A)	Plus Thyroxine Treatment (B)	No Thyroxine Treatment	Plus Thyroxine Treatment			No Thyroxine Treatment (C)	Plus Thyroxine Treatment (D)	No Thyroxine Treatment	Plus Thyroxine Treatment	
C.P.S.	100 ± 16,8 (3)	150 ± 9,99 (4)	187 ± 12,2 (4)	0,050	0,010	N.S.	100 ± 6,63 (5)	94,0 ± 2,65 (6)	106 ± 12,1 (3)	N.S.	N.S.	N.S.
O.T.C.	100 ± 13,2 (3)	16 ± 9,88 (4)	143 ± 7,32 (4)	N.S.	0,020	N.S.	100 ± 11,7 (3)	113 ± 0,185 (2)	111 ± 3,34 (3)	N.S.	N.S.	N.S.
A.S.S.	100 ± 11,4 (3)	121 ± 4,44 (4)	159 ± 10,3 (4)	N.S.	0,005	0,010	100 ± 3,68 (5)	97,9 ± 2,49 (6)	81,3 ± 3,25 (3)	N.S.	0,005	0,005
A.S.L.	100 ± 23,4 (3)	149 ± 7,82 (4)	159 ± 4,76 (4)	N.S.	0,050	N.S.	100 ± 12,6 (5)	93,8 ±5,50 (6)	130 ± 13,2 (3)	N.S.	N.S.	N.S.
Arg.	100 ± 14,2 (3)	158 ± 11,5 (4)	170 ± 11,2 (4)	0,020	0,005	N.S.	100 ± 8,26 (5)	86,7 ± 4,56 (6)	97,3 ± 14,0 (3)	N.S.	N.S.	N.S.

Figure 30 : The effect of thyroidectomy and thyroidectomy plus a single dose of thyroxine eight hours after administration on the specific activity of the urea cycle enzymes in the livers of rats fed on low and high protein diets.

L - 10% protein diet

H - 75% protein diet

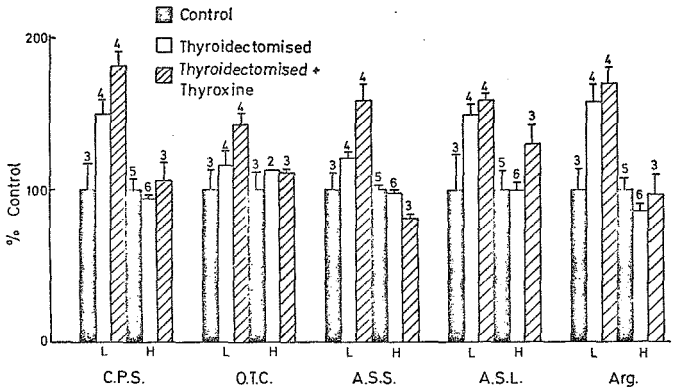


Fig. 30 - Effect of Thyroidectomy and Thyroidectomy plus a Single Dose of Thyroxine (8 Hours after Administration) on Specific Activity of Urea Cycle Enzymes

10%, 34% and 75% protein, respectively, for seven days prior to being killed.

After the seven-day feeding period, the livers of experimental rats were removed and incubated under gas (95% O_2/5% CO_2) at 20°C in a culture medium consisting of Medium 199, Hanks' balanced salt solution, 20% bovine serum, 10% whole egg ultrafiltrate and cortisol (20 mg. per 100 ml. of culture medium) for 24 hours (see "Methods" for details of organ culturing).

The livers of control rats were cultured in a similar manner but were not subjected to cortisol treatment.

The effects on activity per unit wet weight are shown in Table 31 and Figure 31, while the effects on specific activity are shown in *Table 32 and Figure 32.* Results are expressed as a percentage of the control. Each result represents a mean of the number of animals show above the standard error. All cultures were done in duplicate.

No significant effect on enzyme levels was observed with *in vitro* cortisol treatment in rats fed on the above four diets.

3.3.2 The Effect of "in vitro" Thyroxine Administration on Enzyme Levels

Four groups of rats were maintained on four different diets - normal, 10%, 34% and 75% protein, respectively, for seven days prior to being sacrificed.

After the seven-day feeding period, the livers of experimental rats were removed and incubated under gas (95% O_2/5% CO_2) at 20°C in a culture medium consisting of Medium 199, Hanks' balanced salt solution, 20% bovine serum, 10% whole egg ultrafiltrate and thyroxine (1 mg. per 100 ml. of culture medium) for 24 hours (see "Methods" for details of organ culturing).

Table 31 - Table of Results for Figure 31

Effect after 24 Hours in vitro Cortisol Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10 % Protein			Normal (20% Protein)			34 % Protein			75% Protein		
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of diff difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 19,4 (5)	98,1 ± 12,5 (5)	N.S.	100 ± 12,8 (5)	76,1 ± 19,3 (5)	N.S.	100 ± 4,94 (4)	111 ± 8,77 (4)	N.S.	100 ± 7,79 (6)	92,1 ± 4,02 (6)	N.S.
O.T.C.	100 ± 15,3 (6)	103 ± 9,87 (6)	N.S.	100 ± 27,4 (5)	100 ± 6,30 (5)	N.S.	100 ± 14,5 (4)	145 ± 18,9 (4)	N.S.	100 ± 10,5 (6)	99,7 ± 7,92 (6)	N.S.
A.S.S.	100 ± 11,6 (6)	95,2 ± 11,9 (6)	N.S.	100 ± 22,6 (4)	99,9 ± 8,82 (5)	N.S.	100 ± 38,2 (3)	96,0 ± 12,8 (3)	N.S.	100 ± 23,1 (6)	87,0 ± 4,47 (6)	N.S.
A.S.L.	100 ± 18,4 (6)	106 ± 6,23 (6)	N.S.	100 ± 24,9 (5)	108 ± 14,7 (5)	N.S.	100 ± 24,9 (3)	83,9 ± 17,3 (3)	N.S.	100 ± 16,5 (6)	84,4 ± 8,67 (5)	N.S.
Arg.	100 ± 15,5 (6)	99,1 ± 6,40 (6)	N.S.	100 ± 17,3 (5)	104 ± 3,55 (5)	N.S.	100 ± 14,4 (4)	118 ± 14,7 (4)	N.S.	100 ± 10,9 (6)	101 ± 4,74 (6)	N.S.

Figure 31 : The effect, after twenty-four hours *in vitro* cortisol administration, on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on normal, 10%, 34% and 75% protein diets.

10% - 10% protein diet
N - normal diet (20% protein)
34% - 34% protein diet
75% - 75% protein diet

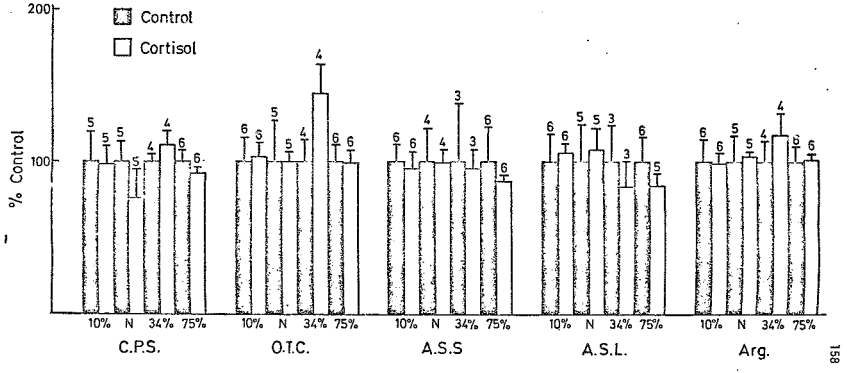


Fig. 31 - Effect after 24 Hours in vitro Cortisol Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 32 - Table of Results for Figure 32

Effect After 24 Hours in vitro Cortisol Administration on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein			Normal (20 % Protein)			34% Protein			75% Protein		
Enzyme	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 14,1 (6)	102 ± 12,3 (6)	N.S.	100 ± 19,6 (5)	94,1 ± 5,64 (5)	N.S.	100 ± 3,31 (4)	94,9 ± 4,90 (4)	N.S.	100 ± 6,46 (4)	95,3 ± 3,69 (6)	N.S.
O.T.C.	100 ± 12,7 (6)	105 ± 9,64 (6)	N.S.	100 ± 10,7 (5)	100 ± 7,32 (5)	N.S.	100 ± 15,7 (4)	125 ± 24,3 (4)	N.S.	100 ± 11,6 (6)	112 ± 11,1 (5)	N.S.
A.S.S.	100 ± 18,4 (6)	99,7 ± 9,84 (6)	N.S.	100 ± 27,4 (4)	98,8 ± 14,5 (4)	N.S.	100 ± 48,5 (3)	86,8 ± 10,1 (3)	N.S.	100 ± 24,9 (5)	88,8 ± 7,39 (5)	N.S.
A.S.L.	100 ± 17,7 (6)	103 ± 7,14 (6)	N.S.	100 ± 26,5 (5)	106 ± 9,66 (5)	N.S.	100 ± 23,9 (4)	83,8 ± 23,8 (4)	N.S.	100 ± 17,0 (6)	93,8 ± 7,03 (5)	N.S.
Arg.	100 ± 11,1 (6)	101 ± 8,56 (6)	N.S.	100 ± 17,1 (5)	105 ± 6,77 (5)	N.S.	100 ± 16,4 (4)	102 ± 7,93 (4)	N.S.	100 ± 10,6 (6)	114 ± 5,41 (6)	N.S.

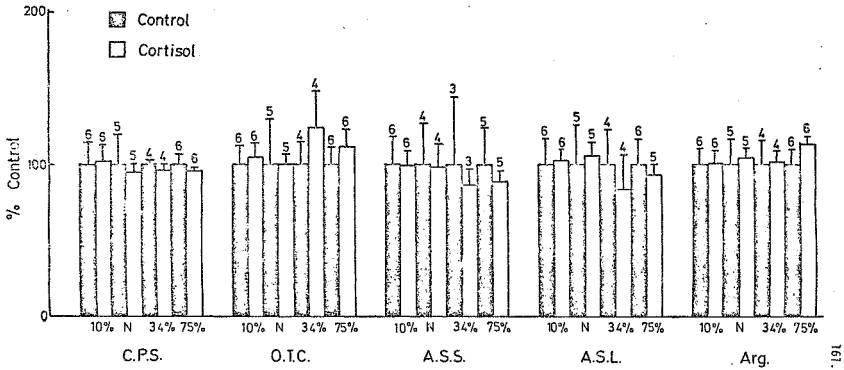


Fig. 32 - Effect after 24 Hours in vitro Cortisol Administration on Specific Activity of Urea Cycle Enzymes

The livers of control rats were cultured in a similar manner but were not subjected to thyroxine treatment.

The effects on activity per unit wet weight are shown in Table 33 and Figure 33, while the effects on specific activity are shown in Table 34 and Figure 34. Results are expressed as a percentage of the control. Each result represents a mean of the number of animals shown above the standard error. All cultures were done in duplicate.

No significant effect on enzyme levels was observed with in vitro thyroxine treatment in rats fed on the above four diets.

Table 33 - Table of Results for Figure 33

Effect After 24 Hours in vitro Thyroxine Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Diet	10 % Protein			Normal (20% Protein)			34 % Protein			75% Protein		
Enzyme	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Activity per unit wet weight and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 19,4 (5)	104 ± 20,7 (5)	N.S.	100 ± 12,8 (5)	101 ± 4,47 (5)	N.S.	100 ± 4,94 (4)	102 ± 2,48 (4)	N.S.	100 ± 7,79 (6)	91,1 ± 5,21 (6)	N.S.
O.T.C.	100 ± 15,3 (6)	109 ± 7,22 (6)	N.S.	100 ± 27,4 (5)	105 ± 9,48 (5)	N.S.	100 ± 14,5 (4)	111 ± 2,98 (4)	N.S.	100 ± 10,5 (6)	105 ± 7,20 (6)	N.S.
A.S.S.	100 ± 11,5 (6)	99,5 ± 13,2 (6)	N.S.	100 ± 22,6 (4)	112 ± 18,2 (4)	N.S.	100 ± 38,2 (3)	99,0 ± 4,30 (2)	N.S.	100 ± 23,1 (6)	82,9 ± 8,48 (6)	N.S.
A.S.L.	100 ± 16,4 (6)	94,5 ± 10,5 (6)	N.S.	100 ± 24,9 (5)	108 ± 12,8 (5)	N.S.	100 ± 24,9 (3)	65,9 ± 18,8 (3)	N.S.	100 ± 16,5 (6)	83,1 ± 8,24 (6)	N.S.
Arg.	100 ± 15,5 (6)	99,9 ± 8,40 (5)	N.S.	100 ± 17,3 (5)	106 ± 15,0 (5)	N.S.	100 ± 14,4 (4)	105 ± 8,68 (4)	N.S.	100 ± 10,9 (6)	100 ± 11,1 (6)	N.S.

Figure 33 : The effect, after twenty-four hours *in vitro* thyroxine administration, on the activity of the urea cycle enzymes per unit wet weight in the livers of rats fed on normal, 10%, 34% and 75% protein diets.

10% - 10% protein diet
N - normal diet (20% protein)
34% - 34% protein diet
75% - 75% protein diet

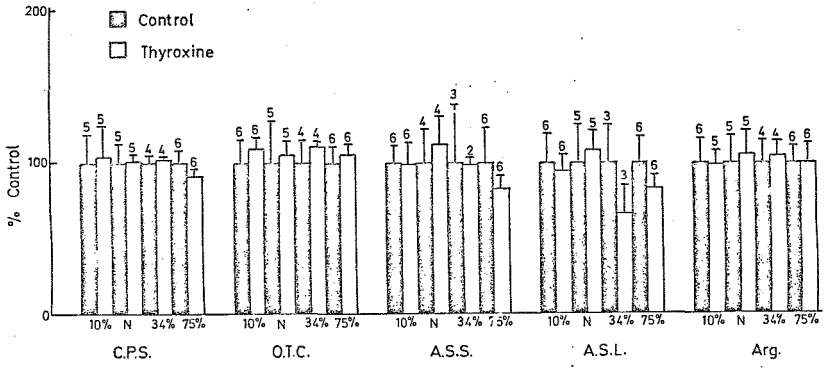


Fig. 33 - Effect after 24 Hours in vitro Thyroxine Administration on Activity of Urea Cycle Enzymes per Unit Wet Weight

Table 34 - Table of Results for Figure 34

Effect After 24 Hours in vitro Thyroxine Administration on Specific Activity of Urea Cycle Enzymes

Diet	10% Protein			Normal (20 % rotein)			34% Protein			75% Protein		
Enzyme	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control	Specific activity and standard error expressed as a percentage of control		Confidence limit of significance of difference from control
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
C.P.S.	100 ± 14,1 (6)	112 ± 17,1 (6)	N.S.	100 ± 19,6 (5)	116 ± 11,9 (4)	N.S.	100 ± 3,31 (4)	96 ± 7,14 (4)	N.S.	100 ± 6,46 (6)	82,5 ± 6,96 (6)	N.S.
O.T.C.	100 ± 12,7 (6)	118 ± 7,72 (6)	N.S.	100 ± 30,7 (5)	113 ± 15,2 (5)	N.S.	100 ± 15,7 (4)	102 ± 6,39 (4)	N.S.	100 ± 11,6 (6)	102 ± 8,83 (6)	N.S.
A.S.S.	100 ± 18,4 (6)	114 ± 14,3 (6)	N.S.	100 ± 27,4 (4)	119 ± 16,4 (4)	N.S.	100 ± 44,6 (3)	95,2 ± 5,74 (2)	N.S.	100 ± 24,9 (5)	75,0 ± 15,1 (5)	N.S.
A.S.L.	100 ± 17,7 (6)	99,1 ± 10,4 (6)	N.S.	100 ± 26,5 (5)	104 ± 13,2 (4)	N.S.	100 ± 23,9 (4)	83,3 ± 17,2 (3)	N.S.	100 ± 17,0 (6)	76,4 ± 9,81 (6)	N.S.
Arg.	100 ± 11,1 (6)	102 ± 7,14 (5)	N.S.	100 ± 17,1 (5)	96,9 ± 6,39 (4)	N.S.	100 ± 16,4 (4)	101 ± 9,40 (4)	N.S.	100 ± 10,6 (6)	88,9 ± 3,97 (6)	N.S.

Figure 34 : The effect, after twenty-four hours *in vitro* thyroxine administration, on the specific activity of the urea cycle enzymes in the livers of rats fed on normal, 10%, 34% and 75% protein diets.

10% - 10% protein diet
N - normal diet (20% protein)
34% - 34 % protein diet
75% - 75% protein diet

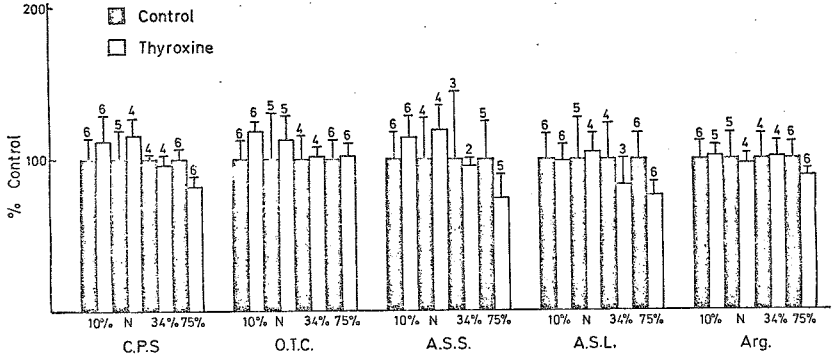


Fig. 34 Effect after 24 Hours in vitro Thyroxine Administration on Specific Activity of Urea Cycle Enzymes

CHAPTER 4 - DISCUSSION

4.1 The Effect of Diet on the Urea Cycle Enzyme Levels

With the exception of arginase in rats fed on a high protein diet, Schimke's results (Schimke, 1962a) were confirmed. The activity per unit wet weight of liver of all five enzymes (except arginase on the 75% protein diet) showed a positive correlation with the protein content of the diet after seven days of feeding on the specific diet (Table 2 and Figure 5). This correlation was also found to be true for specific activity (Table 3 and Figure 6). The effect on specific activity is more significant as it shows that the dietary effect on the urea cycle enzymes is specific and not merely a general effect on all liver proteins.

Arginase levels were decreased on the low protein diet compared with normal food, but did not rise much above the high activity in rats fed on the normal diet of approximately 20% protein. There are two possible explanations for this observation. Either arginase is already fully induced on the normal diet, or the time period required for adaptation to the high protein diet is longer than seven days. That the latter explanation is at least partly true is shown by the slight increase in level of arginase after 21 days on a 75% protein diet.

A study of the time course of adaptation of the urea cycle enzyme levels to dietary protein content indicates that, contrary to Schimke's results (1962a), adaptation is not complete at eight days. Some further adaptation to both low and high protein diets occurs in all five enzymes including arginase. Again the effect is true for both activity per unit wet weight of liver and specific activity. At 21 days, enzyme levels on the low protein diet (10% protein) were lower than those after seven

days of feeding, while the levels of all enzymes, except O.T.C., at 21 days on the high protein diet (75% protein) were higher than those after seven days of feeding on the same diet (Tables 4 and 5, and Figures 7 and 8). Adaptation to dietary protein thus appears to be a slower process than previously thought.

4.2 *In vivo* Effect of Hormones on the Urea Cycle Enzyme Levels in Rats maintained on Diets of Different Protein Content

In assessing hormonal effects on enzyme levels in the liver, it is important to bear in mind that activity per unit wet weight could be altered by changes in non-protein constituents of the liver. Specific activity, on the other hand, could not be altered by such changes. In the present investigation, similar results were obtained in general whichever method of expressing enzyme activity was used.

Eight hours after a single cortisol treatment, small increases in specific activity were seen in most of the urea cycle enzymes, especially on the low protein diet (Tables 8 and 9, and Figures 9 and 10). The significance of many of these increases, however, falls short of the 5% confidence limit.

At 24 hours, small increases in C.P.S. were seen, while O.T.C. showed a significant decrease (Tables 10 and 11, and Figures 11 and 12).

Daily administration of cortisol for four days showed a more clear-cut picture (Tables 12 and 13, and Figures 13 and 14). On both low and high protein diets, C.P.S., A.S.S. and arginase showed an increase in specific activity. O.T.C. was little affected, while the specific activity of A.S.L. was decreased.

In contrast to the co-ordinated positive response of enzyme levels

to large daily doses of cortisol found by Schimke (1963), the present work indicates that not all enzymes of the urea cycle are closely correlated. On the 10% protein diet the enzymes which were similarly affected were C.P.S., A.S.S. and arginase, each showing an increase in specific activity after a four-day cortisol treatment, and eight hours after a single cortisol injection. This increase was also seen on the 75% protein diet for C.P.S. and arginase. On the other hand, O.T.C. and A.S.L. displayed anomalous behaviour in that daily cortisol treatment produced no increases on a low protein diet, and actual decreases on a high protein diet. There were also decreases in O.T.C. 24 hours after a single dose of cortisol.

Adrenalectomy caused a decrease in the levels of C.P.S., O.T.C., A.S.S. and arginase in animals fed on the low protein diet. A.S.L. showed a significant increase in specific activity (Tables 14 and 15 and Figures 15 and 16). On a high protein diet, the decreases in C.P.S., A.S.S. and arginase, when expressed as percentages of control values, were smaller than on the low protein diet.

Little change in the levels of urea cycle enzymes was seen eight hours after a single dose of cortisol was administered to adrenalectomised animals. (Tables 16 and 17 and Figures 17 and 18). Daily administration of cortisol to adrenalectomised rats gave more clear-cut results. On the 10% protein diet, there was an increase in the levels of C.P.S., O.T.C., A.S.S. and arginase, compared with adrenalectomised, untreated animals. A.S.L. again showed a decrease in specific activity on cortisol treatment (Tables 18 and 19 and Figures 19 and 20).

The effect of daily cortisol treatment on adrenalectomised rats fed on the 75% protein diet was much less marked. Only arginase showed a

significant increase in these animals (Tables 18 and 19 and Figures 19 and 20).

The results of the present experiments differ in some respects from those of Schimke (1962a, 1963). They do not indicate complete correlation in response of the five urea cycle enzymes. Only C.P.S., A.S.S. and arginase showed fairly consistent increases on cortisol treatment both in short-term and long-term experiments, and decreases on adrenalectomy. O.T.C. did not increase on cortisol treatment of unoperated animals, but, in animals on a 10% protein diet, its level could be lowered by adrenalectomy, and raised by subsequent cortisol treatment. A.S.L., on the other hand, was raised by adrenalectomy and lowered by repeated cortisol treatment of intact or adrenalectomised animals.

The differences of the present results from those of Schimke could be due to a strain difference of the experimental animals used. Nor can the possibility be excluded that O.T.C. and A.S.L. could be induced by cortisol in the animals presently studied, but under different experimental conditions. For example, the doses of cortisol used were similar to those of Schimke; an extensive study of the dose response of O.T.C. and A.S.L. might show positive responses at some other dose of hormone.

Schimke's observations that dietary induction of urea cycle enzymes is not dependent on an intact adrenal gland are confirmed by the present findings. In animals adapted to high protein diet, adrenalectomy actually produced a smaller relative decrease in urea cycle enzymes than in those on low protein diet. The difference in levels between high- and low-protein fed rats was thus not diminished by adrenalectomy.

The response of urea cycle enzymes to cortisol on the other hand, is diet-dependent. Animals fed 10% protein showed much more pronounced responses to cortisol treatment than those on normal diet or 75% protein. Adrenalectomy too, had the greatest effect on a 10% protein diet.

The small effect of cortisol on a high protein diet may imply that the enzyme is already highly induced, and little further increase can be produced by the hormone. Schimke (1963) has suggested that cortisol does not induce urea cycle enzymes directly, but acts through stimulating protein catabolism. In his view, the high level of amino acid breakdown is the common factor between high protein diet and cortisol treatment, and is the primary inducer of urea cycle enzymes. The results of the present experiments are not inconsistent with this view, though only in vitro work can provide a clear test of its validity.

A number of facts are, however, equally consistent with a more direct involvement of cortisol. One is the decrease in urea cycle enzymes which occurs after adrenalectomy even on a 75% protein diet. The possibility cannot be excluded that this decrease is due to lower food intake in operated animals. Food intake was not controlled in the present experiments, but there was no indication that operated animals drastically reduced their food consumption.

More significant is the fact that, even on a high protein diet, some effect of the hormone was seen on C.P.S., A.S.S. and arginase. In the adrenalectomised animals, hormone treatment also produced increases in C.P.S. and arginase which went beyond the levels in the controls. It would appear that the effects of cortisol and diet are, at least to some extent, additive.

Thyroxine treatment of intact animals produced significant increases in arginase in some experiments. Little effect on any of the other enzymes was seen (Tables 25 and 26 and Figures 25 and 26).

Thyroidectomy performed on animals maintained on a low protein diet, resulted in small increases in all urea cycle enzymes (Tables 27 and 28 and Figures 27 and 28). It is interesting that thyroxine treatment of these animals resulted in further increased enzyme levels (Tables 29 and 30 and Figures 29 and 30). It would appear that removal of the animal's thyroid had sensitized it to the hormone, so that induction effects could be seen which do not occur in normal animals. On a high protein diet, there was little effect of thyroidectomy and no induction by thyroxine could be observed (Tables 27 and 28 and Figures 27 and 28).

The dietary regulation of the urea cycle persists in thyroidectomised rats; it is thus not thyroxine-dependent. The effect of thyroidectomy on the other hand, is diet-dependent, being greatest on a low protein diet.

The increase in urea cycle enzymes by thyroidectomy found in the present experiments is in agreement with the results of Grillo and Fossa (1966) on hypothyroid rats.

4.3 The in vitro Hormonal Effect on the Urea Cycle Enzymes in Rats Maintained on Diets of Different Protein Content

Cortisol and thyroxine administration showed no effect on the five enzyme levels in vitro . This was probably due to the low incubation temperature, namely 20°C. It has been found by a co-worker, F. Mattheyse, that although survival at 20°C is good, there are no hormonal effects at this temperature. Induction of C.P.S., A.S.S. and arginase by in vitro cortisol administration is, however, evident at 37°C (Mattheyse - unpublished).

4.4 Conclusion

The results of the present investigation confirm the findings that urea cycle enzymes are controlled by diet. At the same time, they provide evidence of some regulation by cortisol and thyroxine.

Though both hormones are known to stimulate arginase during development (Knox and Greengard, 1965), there is little short-term effect of either on the levels in unoperated young adults. The enzyme levels have reached the adult plateau corresponding to the animals' hormone output. To raise the levels above the plateau requires repeated treatment with large doses of hormone.

The persistence of the hormonal control is also indicated by the effects of surgical interruption of the animals' endogenous hormone output.

Regulation by diet is not dependent on either cortisol or thyroxine; adrenalectomy or thyroidectomy, and normal animals all have enzyme levels which on a 75% protein diet are higher, and on a 10% protein diet are lower than in rats fed the regular diet.

Sensitivity to hormone does, however, depend very greatly on the protein content of the diet.

Finally, the control of the five enzymes of the urea cycle is not always correlated. Some experimental conditions produced opposite effects on different enzymes. These differences may be quantitative rather than qualitative in nature, reflecting a difference in dose-response. They are not inconsistent with the assumption that there are many similarities or perhaps even common steps in the control mechanisms of urea cycle enzymes. At the same time there would appear to be some differences.

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