REGULATION OF THE UREA CYCLE ENZYME, ARGINASE IN RAT BRAIN

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PREFACE

The research work embodied in this dissertation has been carried out in School of Life Sciences, Jawaharlal Nehru University, New Delhi. The work is in original and has not been submitted so far, in part or full for any other degree or diploma of any university.

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SUMMARY AND FOREWORD

SUMMARY

Hormones control and regulate metabolic processes in mammalian tissues. Thyroid hormones and insulin are the predominant controllers of may pathways such as carbohydrate metabolism and amino acid metabolism. Arginase is one of the key enzymes in the synthesis of urea and its activity has been shown to be modified with different hormones. The activity, regulation and general properties of arginase have been well worked out for liver. The presence of arginase in brain has attracted attention from investigators only recently. The function, regulation and metabolic control of brain arginase has still to be worked out. In the present dissertation such an attempt has been made. Some general properties of arginase like Michaelis-Menten constant, effect of incubation time and effect of enzyme concentration have been studied. The level of arginase in two regions of the rat brain was studied under thyroid conditions and also in diabetic animals. Localization of arginase showed its presence in both particulate and soluble fractions. The function and importance of arginase in brain is discussed.

FOREWORD

Krebs and Henseleit (1932) were the pioneers to deduce the outlines of the urea cycle in liver tissue. Since then, there has been continuous investigation into the steps, mechanism and regulation of urea cycle. In ureotelic animals, urea is formed through urea cycle and is a less toxic excretory product. Subsequent researches of Ratner et al. (1953; 1960) have established the details of enzymatic machinery in urea synthesis. Later researches traced urea formation in extrahepatic tissues, even though its significance and formation is little understood. Kaysen et al. (1973) reported the urea formation in kidney. Researches of Van Elsen et al. (1975) traced the urea forming enzymes in diploid fibroblasts. The specific activity of arginase in liver, kidney, erythrocytes and fibroblasts were of 750/45/45/1 ratios respectively. Researches of Kesava Rao et al. (1976) pointed out different forms of arginase in lung tumors. The enzymic composition of lactating mammary gland is appropriate for the major conversion of arginine into proline which is known to occur in intact gland (Yip et al., 1972). Occurrence of some mental retardations associated with the urea cycle intermediates have invoked the interest of its study in brain tissue also (Buniatian et al., 1966; Sadasivudu et al., 1974).

INTRODUCTION

INTRODUCTION

Arginase (L-arginine amidohydrolase: EC 3.5.3.1) is the last enzyme of the urea cycle. It catalyzes the conversion of arginine into urea and ornithine. Ornithine reenters the cycle and the urea formed is eliminated as an excretory product. All the organisms capable of synthesizing arginine may possess the enzymes of urea cycle but arginase is specific to those capable of synthesizing urea e.g. E. coli (Ratner, 1973).

The essentiality of arginase is considered to be an evolved state of organism which retained arginase and selectively repressed other enzymes as nitrogenous excretory products are eliminated through different metabolic pathways (Watts et al., 1966). Presence of arginase in the organs lacking urea cycle enzymes reflects a selective repression of enzymes during their differentiation and development (Mora et al., 1965a). Inspite of arginine's sole contribution to ornithine formation (Nesheim et al., 1963), it is a nutrient to chicken (Klose et al., 1938) for insects (House, 1965) and for cells in tissue culture (Eagle, 1955). Physiologically arginase can function for the metabolism of arginine to proline or glutamine (Fig. 8). Van Elsen et al. (1975) reported that arginase present in extrahepatic tissues is different from hepatic arginase. They reported that the known arginase isoenzymes separated on DEAE cellulose namely,

by Porembska (1973) A_1, A_3, and A_4 also exist in fibroblast strain, which consistently show either A_1 and A_3 (liver pattern) or A_1 and A_4 (kidney pattern) or mixed pattern of A_1, A_3 and A_4.

DISTRIBUTION OF THE UREA CYCLE ENZYMES:

Reactions that feed aminoacids into the urea cycle particularly, aspartate transaminase, glutamate dehydrogenase, carbamyltransferase are located in the mitochondria. The first two enzymes of the urea cycle namely, carbamyl phosphate synthetase and L-ornithine transcarbamylase are localized in the mitochondria. The last three enzymes of urea cycle arginino-succinate synthetase, argininosuccinase and arginase are localized in cytosol. The overall reaction of urea cycle is as follows:

$$2NH_3 + Co_2 + 3ATP + 3 H_2O \longrightarrow Urea + 2ADP + AMP + 4Pi.$$

The above pattern of distribution of urea cycle enzymes is found in liver. Thus, complex compartmentation of urea cycle reactions, of aminoacid metabolism and urea synthesis appears to be necessary to prevent accumulation of free NH_3 in blood which is highly toxic.

Kidney is known to contain argininosuccinate synthetase and argininosuccinase in sufficient quantities. Rat kidney contains somewhat lower levels than that of ox and pig. All the enzymes of the urea cycle are present in rat kidney but at much lower levels (Ratner, 1973).

Most of the ammonia formed in brain is disposed through glutamine since brain cannot synthesize citrulline. Occurrence of mental retardations associated with urea cycle inter-mediates invoked the interest of studying the enzymes involved in detail. The last part of urea cycle, starting from citrulline to arginine to urea has been reported in rat brain (Buniatian et al., 1966; Sadasivudu, et al., 1974).

The enzymic steps in the urea cycle and participation of various amino acids in urea synthesis of brain were studied by Buniatian et al. (1966). It has been reported that considerable amounts of urea is formed in rat and frog brains (4.36 - 4.52 u moles/g fresh wt. of tissue respectively). None is found in chicken brain. Frog and rat brains showed pronounced arginase activity. In brain tissues of all animals studied, there were no traces of enzymes synthesizing citrulline from ammonia and carbon dioxide (Buniatian,et al.1966).

REGIONAL DISTRIBUTION OF THE UREA CYCLE IN BRAIN:

There is significant difference in the amounts of the urea synthesizing enzymes in the three regions of the brain, namely, cerebellum, cerebral hemispheres and brain stem (Sadasivudu et al., 1974). The activity of arginino-succinate synthetase was almost the same in the three regions. The crebellum showed lower activity of argininosuccinase. Cerebral cortex had highest activity of aspartate

transcarbamylase and arginino succinase. The brain stem
had lowest activity of all the enzymes and cerebellum
appears to have highest arginase activity (Grisolia et al.,
(1972).

ISOENZYMES OF ARGINASE:

Herzfeld and Raper (1976) were of the opinion that the
arginase present in extrahepatic tissues might be different
from liver tissue. The results of Stewart et al. (1977)
indicated that the brain arginase differs from liver arginase
and resembles the arginase of other tissues in some respects.
The lack of the reaction of purified kidney arginase with
antisera prepared against liver arginase indicated that the
two are different (Kaysen et al., 1973).

Porembska (1973) reported four forms of different
arginases, namely, A_1, A_2, A_3 and A_4, according to the order
they emerged on DEAE cellulose column in liver, kidney,
submaxillary gland and brain of rat. Arginase A_1 was
present in all tissues studied, A_2 in submaxillary gland,
A_3 in liver and A_4 in kidney. The arginase from lung tumor
corresponds to A_1 and A_4 of kidney according to the above
classification. In kidney A_4 was the main form and A_1
secondary, which accords with the lung tumor.

The list of other tissues containing the urea cycle
enzymes include spleen, muscle, skin, thyroid, thymus and
lung which have one or two of the urea cycle enzymes. The

significance of these enzymes in these tissues is yet to be understood.

REGULATION OF THE UREA CYCLE:

Meijer et al. (1975) reported that added ornithine increases the rate of the urea production in hepatocytes when ammonia is the major nitrogen donor. Urea production also varies with dietary and hormonal conditions (Schimke, 1962). This shows the dependence of the urea cycle on factors other than the enzymes. The rate-limiting reaction of the cycle is at argininosuccinate synthetase level. Lactate was reported to have a stimulatory effect on urea production (Briggs et al., 1975). Stimulatory effect of lactate may be due to the availability of aspartate for condensation with citrulline. The relationship between lactate concentration and rate of the urea production was hyperbolic. Other precursors of glucose also stimulate urea production but none more than lactate. The major role of lactate lies in providing C_4 intermediates i.e. aspartate.

Accumulation of citrulline and low concentration of succeeding intermediates suggested a limitation at argininosuccinate level. Availability of aspartate to condense with the citrulline may be the cause for its accumulation (Briggs et al., 1976).

Since the urea cycle involves both mitochondrial and cytosol components, transport between these two compartments also contributes to the regulation of urea cycle (Stubbs et al., 1975).

HORMONAL CONTROL:

A. Pancreatic Hormones:

The metabolic patterns of diabetic rats and those treated with glucagon were characterized by a high rate of amino acid metabolism and urea output (McLean et al., 1965).

Randle (1963) pointed out the antagonistic effects of glucagon and insulin on carbohydrate metabolism and nitrogen metabolism.

In diabetic condition, the concentration of aspartic acid declined, whereas glutamate dehydrogenase and glutamate remained unchanged. This together with the increase in the ratio of NADH to NAD suggested that the substrate and co-enzymes changes were in part as a result of, rather than, a causitive effect of the increased urea out put in diabetic condition (Glock et al., 1955).

Factors like ATP and carbon dioxide may also be lowered in diabetic condition (Krahl, 1961).

Alloxan and glucagon have antagonistic actions, therefore, the destruction of B-cells by alloxan treatment leaves the animal subjected only to glucagon. Glucagon secretion may be lowered in diabetic rats than in normal, as the rate of

secretion is partially governed by blood sugars, so a small rate in the absence of insulin can exert dramatic effects (Tyberghein, 1961).

B. Effect of Adrenalectomy and Growth Hormone:

Adrenalectomy caused a reduction in the activities of the urea cycle enzymes. Liver arginase activity was lowered by 40% in pair fed adrenalectomised rats (McLean et al. 1963).

Marked decrease of nitrogen metabolism was observed during adrenalectomy and growth hormone treatment (Long et al. 1940). This decrease in nitrogen metabolism was reflected in arginase activity (Folley et al., 1948b). Changes in the general metabolic pattern produced by the two treatments were different, although decline in urea synthesis was a common feature. Gluconeogenesis from proteins was effected in adrenalectomy and there was increased deposition of amino acids into proteins in the case of animals treated with growth hormone (Long et al., 1940; Kretter et al., 1957).

Treatment with corticosterone acetate increased the activities of soluble fraction enzymes like argininosuccinate synthetase, argininosuccinase and arginase in normal rats and restored normal values in adrenalectomised rats. Profound response was shown by arginase.

The effect of growth hormone on arginase activity was not significant.

C. Effect of Thyroid Hormones:

The effect of hyperthyroidism on some enzymes of the urea cycle in liver have been reported (Grillo, 1964) and also the effect of thyroid hormones on the induction of urea cycle enzymes in amphibia (Ratner, 1973; Winom et al., 1972). Menahan et al. (1969) have reported an increased rate of urea formation and decreased rate of gluconeogenesis by isolated perfused liver from hypothyroid rats.

The cyclic necleotides have been linked to the regulation of citrulline and urea formation (Yamazaki et al., 1977; Bryla et al., 1977; Edkins et al., 1976). Thyroidectomy causes a marked increase in cyclic nucleotide phosphodiesterase activity of liver (Gumma et al., 1977) and adipose tissue (Amstrong et al., 1974; Van Inwegen et al., 1975; Correze et al., 1976). c

EFFECT OF CARBON TETRACHLORIDE:

Carbon tetrachloride poisoning of rats showed marked influence over the urea cycle enzymes localized in mitochondria (McLean et al., 1964). Administration of carbon tetrachloride led to liver damage causing a number of metabolic changes of which nitrogen metabolism is of profound significance. This change is associated with a decline in the rate of amino acid incorporation into proteins (Smuckler et al., 1962), a decrease in amino acid activation (Rossi et al.. 1963a) and increase in concentration of ammonia

in the liver (Gordon, 1959) and an increase in ammonia
and amino acid in blood. These changes are reflected
in urea synthesizing enzymes of urea cycle.

The other mechanism might be due to some early
changes in the binding of these enzymes to mitochondria
followed by their leakage from damaged cells. But intra-
cellular distribution of these enzymes in homogenates
prepared in iso-osmotic sucrose does not support this
view.

Acute poisoining with carbon tetrachloride might lead
to release of some hormones like adrenaline (Selye, 1950)
which will activate these enzymes.

Mitochondrial linked enzyme activity depends on metal
ions like Mg^{2+}, Zn^{2+}, Na^+ and K^+ (Cohen et al., 1961). But
information regarding the effect of carbon tetrachloride
treatment on metal ions is very scanty.

EFFECT OF AZO-DYE CARCINOGENS:

There is a marked decrease in the activities of all
the urea cycle enzymes in primary liver tumors induced
with 3'-methyl aminoazobenzene (McLean et al., 1964).

The activities of the urea cycle enzymes were very
low in primary liver tumors. During early period of
treatment, there was a transient change in carbamyl
synthetase activity. After two weeks of treatment,

arginase activity was also found to increase. Longer periods of treatment led to decrease of all urea cycle enzymes.

Direct counts of mitochondria were low in comparison with normal rats. This explains about the decline of mitochondrial enzymes.

Urea cycle enzymes alter in an adaptive manner to the levels of amino acid catabolism, and hormonal conditions leading to breakdown of amino acids. It is possible that the changes in the urea cycle enzymes might be due to secondary alterations in other enzymes controlling the amino acid catabolism. The first change induced by azo-dye is at endoplasmic reticulum, a site associated with protein synthesis, provides further evidence that alteration in the urea cycle enzymes may be secondary to early changes.

The activities of the urea cycle enzymes of soluble fraction retained their activities during precancerous stage to a greater extent than the mitochondrial enzymes.

UREA CYCLE ENZYMES DURING DEVELOPMENT:

All the urea cycle enzymes showed low activities prenatally but there was a rapid enhancement postnatally (Kennan et al., 1959). The capacity of liver slices to synthesize urea was almost absent during development of rats and guinea pigs (Raiha et al., 1965).

Quantitative measurement of arginase in various tissues of the rat during fetal development showed that it occurs in liver first and later develops to higher concentration in adult liver and to significant levels in other tissues (Greengard, 1970).

Argininosuccinate was reported to be the rate limiting reaction and its development was found to correspond best with development of overall-urea producing capacity (Brown et al., 1959). Puromycine injections and adrenalectomy at birth reduced or inhibited the enzyme activities.

The immediate postnatal increase of these enzymes concerned with nitrogen metabolism represent a primary increase brought about by some unknown factor and the postnatal increase an adaptive change in the enzyme activity due to shift in the balance between intake of nitrogenous compounds and their subsequent utilisation for growth.

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental animals:

Adult female rats of Holtsman Strain weighing between 200-225 g were used for experimental purposes. The rats were fed ad lib with the laboratory diet. For the estimation of arginase (L-arginine amidohydrolase: EC 3.5.3.1) activity in brain, cerebral hemispheres and cerebellum were used separately.

Starvation:

The rats were starved for 48 hrs. Water was provided during starvation. Normal rats were maintained along with them which were given laboratory diet ad lib.

Thyroidectomy:

Rats weighing 200-225 g were operated, using anaesthetic ether. Thyroidectomised and control rats were pair fed for 25-30 days after operation. One per cent calcium lactate was given in drinking water. 25 days after thyroidectomy, T_3 (triiodothyronine, 15 µg/ 100 g body wt.) injections were given intraperitoneally to the rats for three days before sacrifice.

Hyperthyroidism:

The normal rats were given T_3 (15 µg/100 g body wt.) injections for three consecutive days to make them

hyperthyroid. They were pair fed with control rats and both group of animals were sacrificed on the fourth day.

Alloxan diabetes:

Adult female rats weighing about 200 g were starved for 48 hrs. before injection of the alloxan. Alloxan (20 mg/100 g body wt.) was injected to each rat subcutaneously, it was prepared the same day by dissolving 1 g in 10 ml of 0.154 M sodium acetate buffer, pH 5.4. The treated rats were injected two units of protamine-zinc-insulin daily for the first seven days. This treatment reduces the mortality of diabetic rats. The animals were sacrificed three weeks after insulin withdrawl and arginase activity was assayed in the cerebral hemispheres and cerebellum as described.

Estimation of blood sugars:

50 μl of blood was taken from the heart into 0.2 ml of distilled water. The samples were frozen overnight and thawed the next day. The precipitate was spun down. In the supernatants glucose was estimated spectrophotometrically by the hexokinase reaction (Bergmeyer, 1974).

The assay system for glucose determination contained the following: 0.25 ml of glycylglycine/Mg^{++}, 0.25 M/0.1 M pH 7.6; 0.1 ml of NADP (2 mg/ml), 0.05 ml of ATP/Mg^{++}, 0.2 M/0.05 M pH 7.3; 0.01 ml of extract (1:5); 0.01 ml of

glucose-6-phosphate dehydrogenase (1:5) and 1 unit of
hexokinase in a final volume of 1.25 ml. The increase
in O.D. was measured after addition of hexokinase and
the change in O.D. was taken as a measure of the glucose
in the extract. The blood glucose value was calculated
from the formula:

$$\frac{\text{Change in O.D. x Dilution}}{\text{amount extract x factor}} = \text{u moles of glucose}$$

The molar extinction coefficient of NADPH was taken
as 6.22 (Bergmeyer et al., 1974). The blood sugar is
expressed in mg%.

Preparation of homogenate:

Rats were killed by cervical dislocation and brains
were excised and chilled immediately. Cerebral hemispheres
and cerebellum were weighed promptly. The homogenising
medium was 0.1M manganese-saline (1.69 g of manganese
sulphate in 0.9% saline). Tissue homogenates were prepared
(1:10) by using a Potter Elvehjem type homogeniser fitted
with a teflon plunger. Whole homogenates were used
initially for arginase assay. In later experiments, where
distribution studies were performed, whole homogenates were
centrifuged at 10,000 rpm for 10 minutes, in a K 24 (MLW)
refrigerated centrifuge. The homogenate was separated into
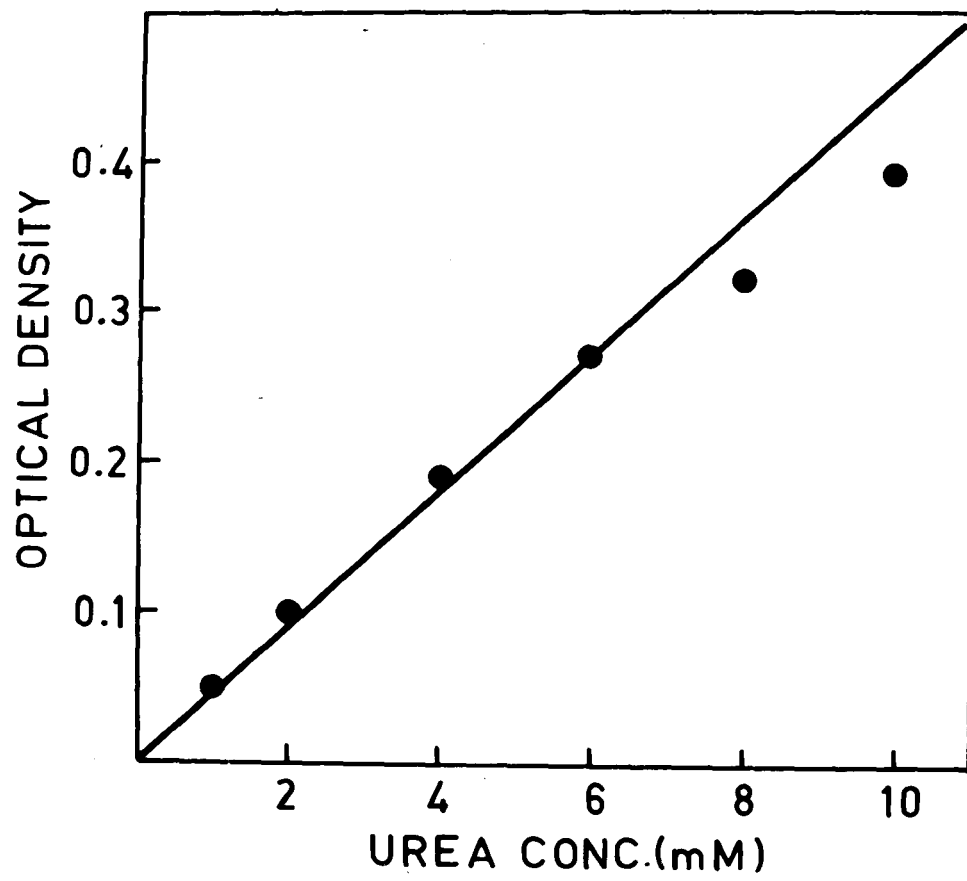soluble and total particulate fraction (TPF) containing

FIG.1

nuclei, mitochondria and synaptosomes. The soluble
and total particulate fractions were of 1:5 dilution.

Determination of Michaelis constant and estimation
of some general properties of arginase were performed
with whole homogenates.

Activation of homogenate for arginase assay:

The whole homogenate and separated fractions were
incubated at $37^{o}C$ for thirty minutes to achieve complete
activation of arginase as described by Folley et al.
(1948a) and modified for brain by Stewart et al. (1977).

Estimation of arginase:

Arginase was assayed as described by Schimke (1970)
with some modifications. The assay mixture contained in
a total volume of 2.35 ml othe following: 1.25 ml of 0.46 M
arginine pH 9.5 and 0.6 ml of 1.25 M glycine buffer pH 9.5.
The reaction was started by the addition of 0.5 ml of the
activated enzymes (whole homogenate or separated fractions).
Incubations were carried out at $37^{o}C$ for 15 minutes. The
reaction was stopped by addition of 2 ml of 1 N perchloric
acid. The samples were then centrifuged to remove
precipitate. Urea was estimated in the supernatant by
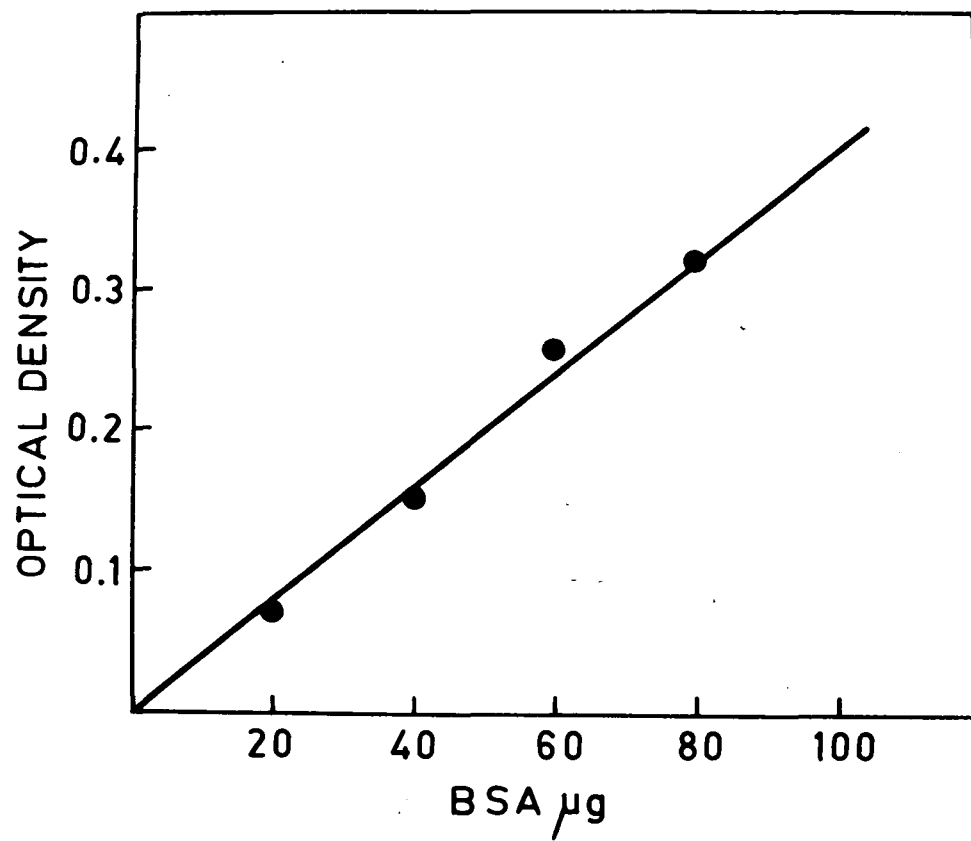the method of Schimke (1970).

FIG·2

Estimation of urea:

1.5 ml of the incubated supernatant was taken and
to it added 0.5 ml of isonitrosopropiophenone and 10 ml
of H_2SO_4/H_3PO_4 mixture (1:3) were added. The samples were
heated in a boiling water bath for one hour in dark. The
samples were cooled to room temperature and the optical
density was read at 540nm using an appropriate blank in
an ELICO (CL20A) Colorimeter.

A standard curve of urea was run containing urea in
the range of 1-10 umoles (Fig. 1).

Protein estimation:

Since manganese interfered in the protein estimations
a modified procedure as used by Kaysen and Strecker (1973)
was applied in the present series of experiments. The
method is as follows: to 0.1 ml of the extracts, 0.9 ml of
0.01N NaOH was added. The samples were allowed to stand at
room temperature for 15 to 30 minutes. The manganese
hydroxide separated as precipitate was removed by centrifu-
gation. The supernatants (0.1 ml) were used for protein
estimation as described by Lowry et al. (1951).

Standard curve of bovine serum albumin dissolved in
manganese saline was run along with the samples (Fig. 2).

Units of activity:

A unit of arginase activity was defined as one μmole
of urea formed at $37^{o}C$ in 60 minutes per g fresh wt. of
tissue.

RESULTS

RESULTS

The present series of experiments reveal some of the
characteristics of arginase enzyme in rat brain and the
influence of some hormones like, thyroid and insulin on
the activity of arginase. The activity of arginase in
liver is about thousand times more than in brain. The
arginases of mouse brain have been purified by Stewart
et al. (1977) and the properties of purified enzyme have
been compared with liver enzyme; significant differences
were observed in some properties of arginase from the two
tissues like, time of activation and isoenzymic pattern.
In the present series of experiments, some general
properties of arginase were determined in brain homogenates,
like time of incubation, effect of enzyme concentration and
the effect of substrate concentration i.e. Michaelis-Menten
constant. Two different regions of the brain, namely,
cerebral hemispheres and cerebellum were used to study the
effect of some hormones and for subcellular distribution
studies.

Various reports have indicated the presence of urea
cycle enzymes in brain (Buniatian et al., 1966; Sadasividu
et al., 1974, 1976). Of all the urea cycle enzymes present
in brain, arginase is of unique importance, since it has
been attributed other functions, than just forming urea like,
formation of glutamate semialdehyde and proline. Buniatian
et al. (1966) reported the presence of arginase from fishes

TABLE 1   ARGINASE ACTIVITY IN NORMAL RATS

	Cerebral hemispheres	Cerebellum
μ moles/g/hr	49.87 ± 2.04	44.47 ± 2.76
μ moles/mg. pr.	0.53 ± 0.13	0.81 ± 0.07
Protein/mg/g	89.87 ± 6.6	61.75 ± 6.7

to mammalian brain and arginase activity was found to be about 42.3 μ moles/g/hr. Sadasivudu et al. (1976) reported differential distribution of arginase in different regions of brain, with the cerebellum having highest arginase activity.

The results of the present experiments are presented in Table 1. The data indicates that the arginase activity in cerebellum and cerebral hemispheres is not different significantly. The protein content of the regions, namely, cerebral hemispheres and cerebellum is presented in the same table.

The kinetic properties of arginase in brain has not been reported earlier; therefore, in the present series of experiments Michaelis-Menten constant of arginase was determined in brain homogenates, together with some other properties of arginase.

TIME OF INCUBATION:

The time of incubation for arginase in crude extracts of different tissues show a wide range of variation. Stewart et al. (1977) have shown that the urea formation by homogenates of mouse brain was linear upto 70 minutes. Sadasivudu et al. (1976) have not mentioned the time of incubation. In kidney arginase, the incubation time was ten minutes (Kaysen et al., 1973), during which the pH was kept at 9.5. The enzyme concentration was so chosen that not more than 5% of the
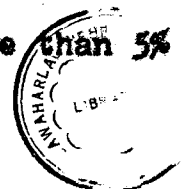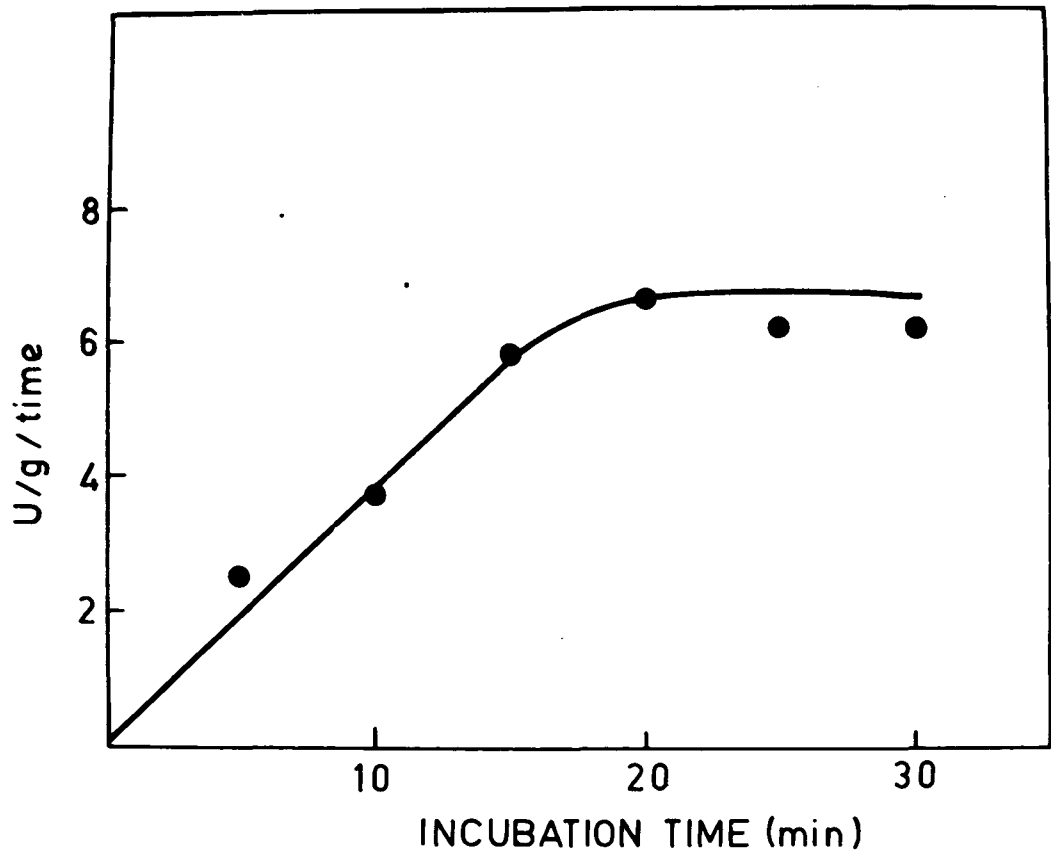
FIG.3

substrate was hydrolysed in 10 minutes. The incubation
time for liver arginase was five minutes (McLean et al.,
1963).

Considering the results discussed above, it was
decided that a time curve for the activity of arginase
is needed to find out the optimal time of activity in
the case of crude brain homogenates. The results are
shown in Fig. 3. The data show that the urea formation
is linear upto fifteen minutes and plateaus to a contant
value at thirty minutes maintaining the activity constant
after that time. Therefore, in all future experiments
fifteen minutes was taken as the time of incubation.
At this time the percentage of substrate converted to
product was 1.3%.

EFFECT OF ENZYME CONCENTRATION:

Brain contains much lower arginase activity compared
with liver and kidney. In the present experiments 1:10
diluted homogenates were used for determination of arginase
activity in brain, unlike in liver where they are further
diluted. Various enzyme concentrations ranging from 0.1 ml
(0.9 mg protein) to 0.8 ml (7.2 mg protein) were used for
obtaining optimal enzyme activity. The results are given
in Fig. 4 and show that the enzyme activity increased
proportionately from 0.1 ml to 0.4 ml reaching to an
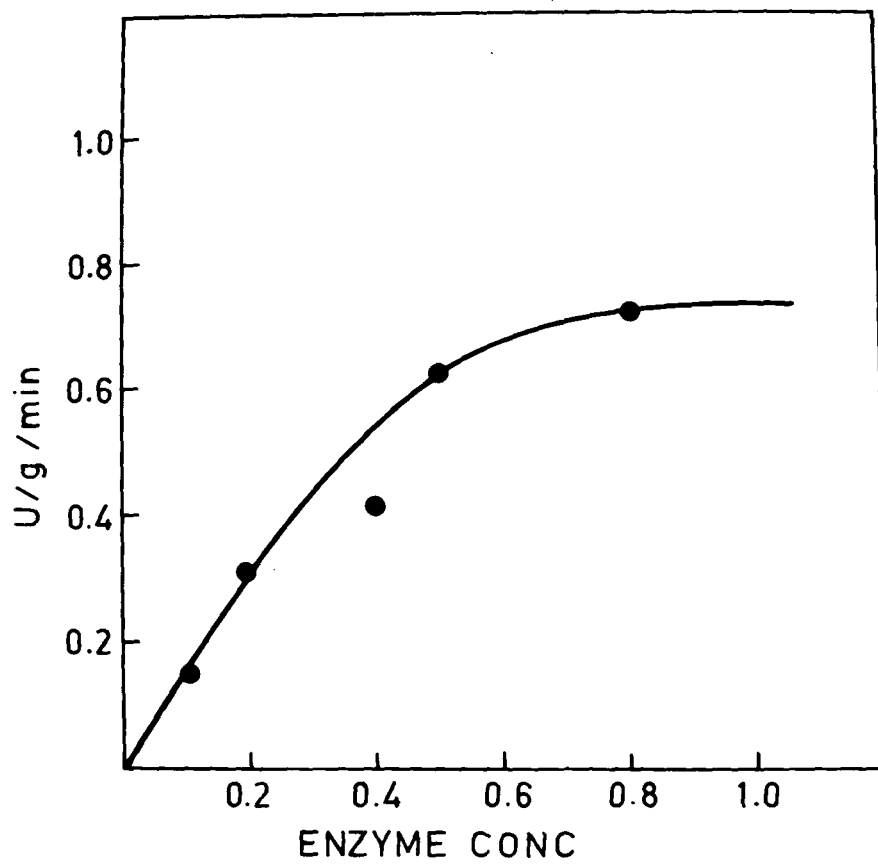optimal activity with 0.5 ml and obtaining a plateau with

FIG.4

Fig. 5 : The values on the x and y axes have been
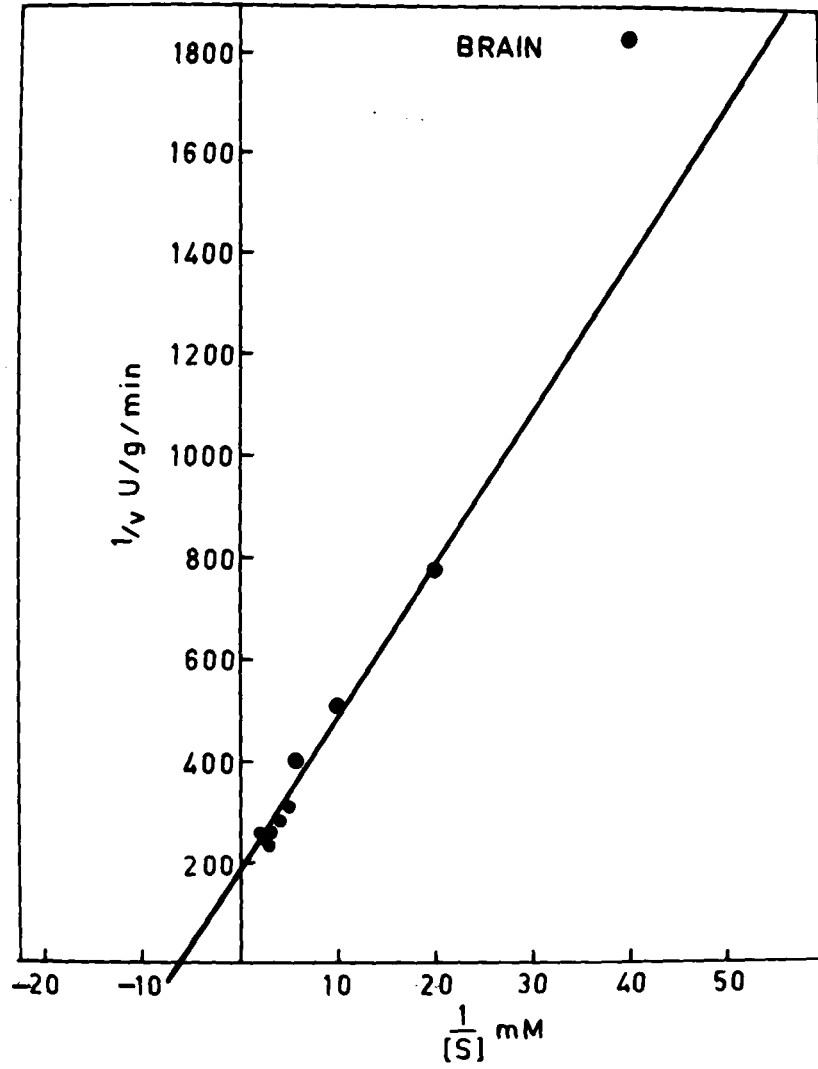multiplied by 1000.

Lineweaver-Burk Plot

FIG. 5

0.8 ml extract. Since optimal activity was obtained with 0.5 ml (4.5 mg protein) of extract, it was routinely used for all experiments.

EFFECT OF SUBSTRATE CONCENTRATION:

The effect of substrate (arginine) concentration on arginase activity was determined. Herzfeld et al. (1976) reported that the arginases present in the brain were different from liver tissue. Stewart et al. (1977) reported two different froms of arginase in mouse brain with Km values of 32 mM and 38 mM in purified brain extracts. In the present experiments, tissue homogenates were used to determine the affinity of the enzyme with respect to the substrate. Arginase activity showed a gradual increase from 25 mM concentration upto 300 mM arginine. After 300 mM there was slight decrease in the activity reaching a plateau at 400 mM.

The results are plotted as Lineweaver-Burk graph and are shown in Fig. 5. The Michaelis-Menten constant of brain arginase as calculated from the plot to be 154 mM.

Diluted samples of liver homogenates with the same range of arginine concentration were also used for determination of km value to compare with brain values. The Lineweaver-Burk plot of the liver is shown in Fig. 6, and gave a K_m value of 200 mM.

Fig. 6 : The values on x and y axes have been
multiplied by 10,000.

Lineweaver-Burk Plot

LIVER

$\frac{1}{[V]}$ U/g/min
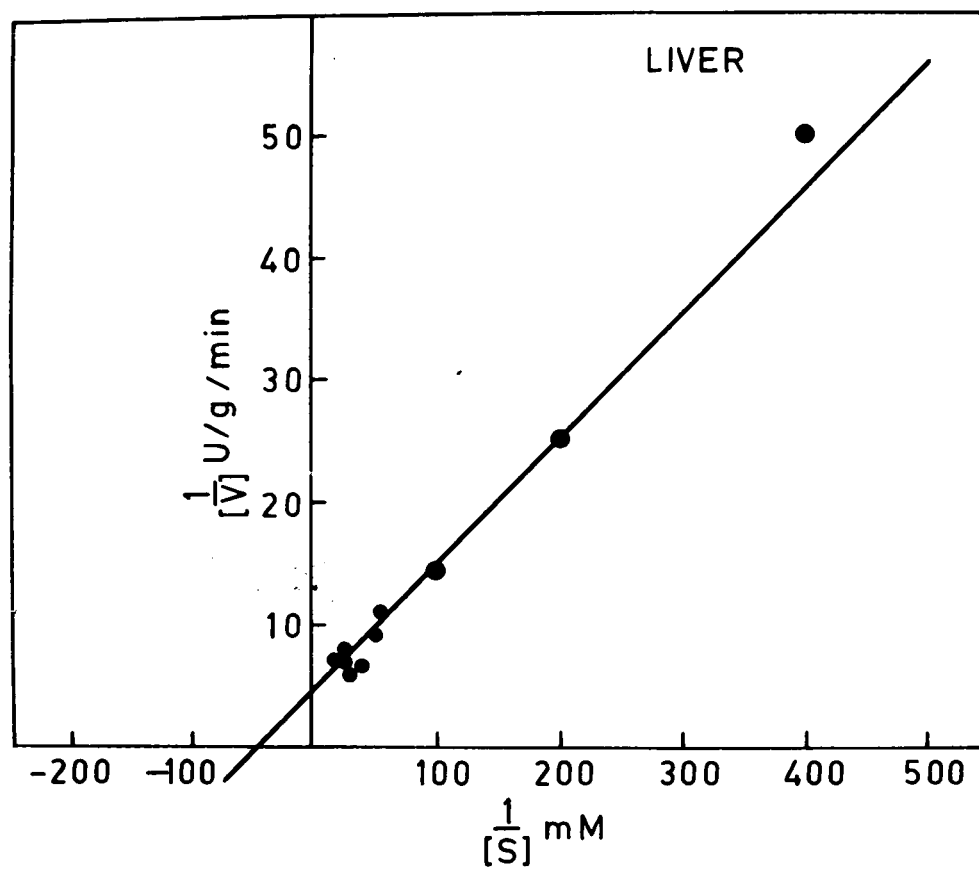
$\frac{1}{[S]}$ mM

FIG 6

The K_m values of arginase of both brain and liver are higher than those reported in literature, probably due to the use of crude homogenates in the present series of experiments.

DISTRIBUTION OF ARGINASE:

The urea cycle enzymes present in the liver show a different pattern of distribution compared with extra-hepatic tissues like brain, kidney and lung. In liver, the first two enzymes of cycle, namely, carbamyl phosphate synthetase and ornithine transcarbamylase are present in the mitochondria and the last three enzymes are localized in the cytosolic fraction. Various reports on the activity of urea cycle enzymes from extraheptic tissues indicated, the presence of arginase in the mitochondria as well as in cytosol, which indicates some significance for this localization and attributes a different function to the arginase (Kaysen et al., 1973). The present study was carried out using whole homogenate and total particulate fractions (TPF) of brain samples prepared as described in the methods section. The results are shown in Table 2. Both cerebellum and cerebral hemispheres were used for this study.

The results show that 48% of the total arginase activity of whole homogenate in the cerebral hemispheres

TABLE 2   DISTRIBUTION OF ARGINASE IN RAT BRAIN

Region	Whole homogenate μ moles/g/hr	Soluble μ moles/g/hr	Pellet μ moles/g/hr
Cerebral hemispheres	47.5 ± 2.54	22.64 ± 4.19	17.5 ± 1.13
Cerebellum	40.13± 2.66	24 ± 1.53	10.10± 0.62
Protein mg/g			
Cerebral hemispheres	84.1 ± 8.3	43.66 ± 7.03	47.41± 2.7
Cerebellum	56.6 ± 8.9	32.66 ± 5.2	23.33± 3.68

Fig. 7 : Distribution of arginase in subcellular fractions.
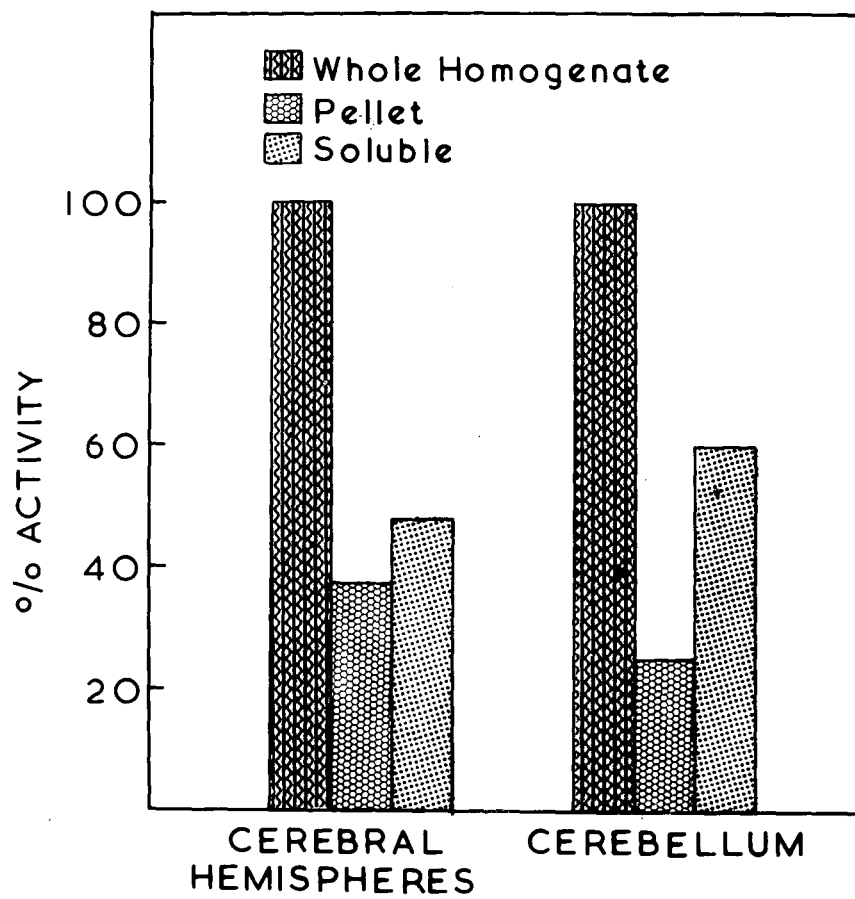
FIG. 7

was localized in the cytosol, whereas cerebellum showed 60% of total activity in the soluble fraction.

The total particulate fraction (TPF) of cerebral hemispheres contained 37% of arginase activity and 25% in case of the cerebellum. The present results show about 84% of recovery of arginase in the soluble and total particulate fractions. This differential distribution of arginase in brain indicates some significance of arginase in relation to brain function.

EFFECT OF STARVATION:

Dietary and nutritional status of animals influence a number of metabolic pathways. In starvation, utilisation of internal energy sources takes place leading to breakdown of glycoogen, protein and the formation of ketone bodies etc. As a result, free fatty acid and amino acid concentrations in the body fluids increases. In order to maintain physiological condition and homeostasis in the body, a number of enzymes will be activated to deal with the physiological imbalances. Our experiments are designed to observe the effect of starvation on arginase activity. Brain is an active tissue and eliminatesammonia and carbon dioxide as soon as they are formed in excess. In the present experiments, we have studied the effect of 48 hrs starvation on brain arginase levels. The arginase

TABLE 3 EFFECT OF STARVATION ON RAT BRAIN ARGINASE

Region	Control μ moles/g/hr	Starvation μ moles/g/hr
Cerebral hemispheres	47.5 ± 2.54	99.4 ± 7.07
Cerebellum	40.13± 2.66	114.0 ± 5.4
Protein mg/g		
Cerebral hemispheres	110.0 ± 1.0	108.0 ± 4.2
Cerebellum	75.0 ± 3.5	82.0 ± 0.9

activity was markedly increased. The results are shown
in Table 3. The effect was more pronounced on cerebellum
compared to cerebral hemispheres. There was about 2.1
fold increase in cerebral hemispheres and 2.85 fold in
cerebellum compared to normal values. The increase in
the arginase activity may be due to increase in ammonia,
amino acid and free fatty acids concentrations.

EFFECT OF THYROIDECTOMY:

One of the group of hormones which influences the
protein catabolism are thyroid hormones. Changes in the
protein metabolism can be reflected in urea synthesizing
enzymes. In the present experiments, thryroidectomised
rats were used to observe the influence of hypothyroidism
and hyperthyroidism on brain arginase. Sochor et al.,
(1977) have reported coordinated increase of urea cycle
enzymes under hypothyroid condition with the exception
of arginase in liver tissue. In our experiments, arginase
activity was found to remain unchanged during hypothyroidism
in brain tissue also. Both in cerebellum and cerebral
hemispheres the activity was not significantly altered
from normal values. The results are shown in Table 4. The
observation is in accord with the result obtained in case
of liver tissue.

TABLE 4   EFFECT OF THYROIDECTOMY ON RAT BRAIN ARGINASE

Region	Control μ mole/g/hr	T_x^* μ moles/g/hr	$T_x + T_3^*$ μ moles/g/hr	Normal + T_3 μ moles/g/hr
Cerebral hemispheres	45.2 ± 1.32	43.8 ± 1.5	45.95 ± 5.2	15.13 ± 2.12
Cerebellum	46.3 ± 2.1	45.9 ± 3.93	41.8 ± 1.58	27. 5 ± 1.78
Protein mg/g				
Cerebral hemispheres	93.0 ± 7.31	85.3 ± 3.75	84.0 ± 6.83	93.0 ± 3.45
Cerebellum	68.0 ± 0.98	74.0 ± 2.35	68.5 ± 2.36	71.5 ± 0.13

$^*T_x$ = Thyroidectomised.

$^*T_x + T_3$ = Thyroidectomised + Triidothyronine.

Injections of T_3 (triiodothyronine ; 15 μg/100 g body wt.) to thyroidectomised rats also failed to show any influence on the arginase activity in both cerebral hemispheres and cerebellum. The values were almost equal to the control and thyroidectomised values. Results are shown in the Table 4.

Hyperthyroidism induced by injections of T_3 to normal rats showed a marked influence on the activity of arginase from rat brain. The effect seems to be more pronounced in cerebral hemispheres compared to the cerebellum. In cerebral hemispheres, the activity was decreased three folds compared with control values.

In cerebellum, the decrease in the activity was about 1.8 fold of the control. As T_3 injections to control rats are characterised by increase in amino acid incorporation into proteins of mitochondria free supernatants in liver; it might influence the formation and overall urea synthesizing capacity of the tissue (Tata et al., 1963). Therefore, the present observation of a decreased arginase activity in the brain might be due to the above effect of T_3, enhancing the amino acid incorporation into proteins which may cause a decrease in the substrate for arginase in brain. The results along with protein contents are shown in Table 4.

TABLE 5   EFFECT OF ALLOXAN DIABETES ON RAT BRAIN
           ARGINASE ACTIVITY

	Control μ moles/g/hr	Diabetic μ moles/g/hr
Cerebrall hemispheres	46.72 ± 3.66	72.14 ± 5.3
Cerebellum	39.75 ± 0.005	55.25 ± 1.97
Body weight	201.5 ± 17.25	164.0 ± 9.15
Protein mg/g		
Cerebral hemispheres	81.25 ± 12.35	87.0 ± 6.7
Cerebellum	61.5 ± 5.0	67.28 ± 4.5
Blood sugar mg%	80.22 ± 12.4	226.0 ± 30

EFFECT OF ALLOXAN TREATMENT:

Alloxan treatment of rats leads to destruction of B-cells of pancreas and renders the rats diabetic. Insulin levels in blood declines thereby affecting many metabolic pathways. Diabetic state is associated with an increased nitrogen excretion, which is reflected in urea synthesizing machinery of liver. Arginase is also one of the enzymes which is affected by changes in insulin levels. McLean et al. (1965) have reported that the arginase activity of liver was not significantly altered during diabetes. Recently, however, it has been shown by Sochor et al.(1977) that a marked increase is arginase activity of liver occurs in alloxan treated animals.

In the present experiments with alloxan treated animals, brain homogenates showed a marked increase of arginase activity as compared to control values. The effect was more pronounced in cerebral hemispheres compared to cerebellum. The results are shown in Table 5.

The activity of arginase in homogenates of cerebral hemispheres and cerebellum was increased by 1.5 and 1.38 folds respectively, compared with control values. The blood sugar values for control animals were 80 mg% and that of diabetics were 226 mg%. The present observation of increased arginase activity may be due to some of the changes that are brought by decreased levels of insulin in diabetes.

TABLE 6   UREA CYCLE ENZYMES IN BRAIN (PUBLISHED DATA)

Region	Carbamyl phosphate synthetase	Ornithine carbamyl transferase	Arginino succinic synthetase	Arginino succinic	Arginase	Reference
Whole brain	4.0	0	3.0	6.9	105	Ratner et al. (1960)
	0.05	0	3.2	-	42.3	Buniatian et al.(1966)
Cerebral hemispheres	-	-	8.59	5.62	16.04	Sadasivudu. et al.(1974,76)
Cerebellum	-	-	7.78	3.27	33.33	-do-
Brain stem	-	-	8.013	4.87	12.48	-do-

TABLE 7   RELATED ENZYMES OF THE UREA CYCLE (PUBLISHED DATA)

Reference : Sadasivudu et al. (1976)

Enzyme	Cerebral cortex	Cerebellum	Brain stem
Carbamyl phosphatase*	2458	325.2	244.8
Aspartate trans-carbamylase	192.9	175.1	146.8
Glutamine synthetase**	23.2	25.8	11.5
Glutaminase**	438.0	291.2	15.2
Arginine glycine transaminidase[+]	0.70	0.60	0.72
Ornithine keto-transaminase[++]	0.24	0.40	0.27
Ornithine glyoxglate aminotransferase[++]	0.32	0.14	0.20
Creatinene phosphokinase[#]	0.40	0.40	0.40
Arginine GABA trans-aminase[+]	0.80	0.48	0.68

*u moles of citrulline/g/hr.

**u moles of product/g/hr.

[+] u moles of ornithine/g/hr.

[++]u moles of -pyrroline-5-carboxylic acid/g/min.

[#] u moles of phosphorus liberated/mg/hr.

Fig. 8 : Interrelationships of urea cycle intermediates
with other compounds.

UREA CYCLE

Mitochondria

$CO_2 + H_2O + $ Ammonia

① 2 ATP → ADP + Pi

Carbamyl-Phosphate + ②

Pi → CITRULLINE

Glutamate

NH₃ ⑦ NAD / NADH

Glu.S.A. ✱

⑧ α.K.G. ✱ Proline ⑨

ORNITHINE

✱ **Glutamate Semialdehyde**
✱ **α-Keto Glutarate**

ORNITHINE

CITRULLINE

Aspartate + ATP ③ AMP + Pi

Ammonia + CARBON DIOXIDE ⑥

⑤ UREA

⑩ ARGININE — ⑪ — Guanidoacetate

Glycine

Ornithine

⑫

CREATININE

④ → Fumarate → T.C.A.
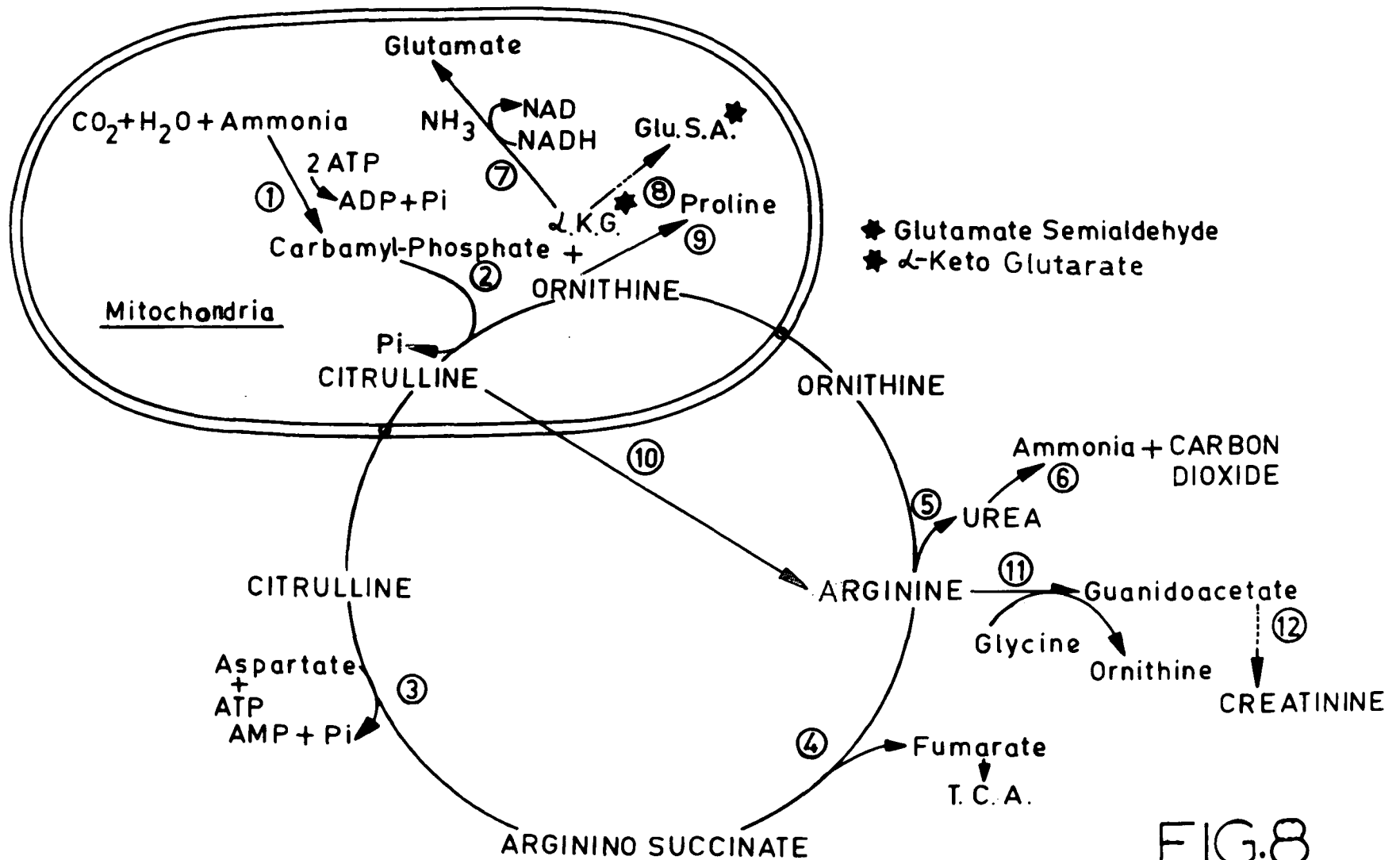
ARGININO SUCCINATE

FIG.8

REACTIONS OF FIG. 8   UREA CYCLE AND RELATED ENZYME REACTIONS

Enzyme	Reactions
Carbamyl phosphate synthetase	$2 ATP + CO_2 + H_2O \longrightarrow$ Carbamyl phosphate $+ 2 ADP + Pi$
Ornithine carbamyl transferase	Carbamyl phosphate $+$ Ornithine $\longrightarrow$ Citrulline $+ Pi$
Arginino succinate synthetase	Citrulline $+$ Aspartate $+ATP \rightleftharpoons$ Arginino succinate $+$ AMP $+ PPi$
Arginino succinase	Arginino succinate $\longrightarrow$ Arginine $+$ Fumarate
Arginase	Arginine $+ H_2O \rightleftharpoons$ Urea $+$ Ornithine
Urease	Urea $\longrightarrow$ Ammonia $+ CO_2$
Glutamate dehydrogenase	2-Oxo-glutarate $+ NH_3 \longrightarrow$ Glutamate
Ornithine ketotransaminase	Ornithine$+$2-Oxo-glutarate $\longrightarrow$ Glutamate-4-Semialdehyde
Amino transferase	Ornithine $\longrightarrow$ Glutamate semialdehyde $\longrightarrow$ Pyroline-5-Carboxylate $\longrightarrow$ Proline
Arginine deiminase	Arginine $\longrightarrow$ Citrulline
Glycine amido transferase	Arginine $\longrightarrow$ Guanidoacetate
Creatine kinase	Guanidoacetate $\longrightarrow$ Methyl guanidoacetate $\xrightarrow{\text{Creatine kinase}}$ Creatine phosphate $\xrightarrow{Pi}$ Creatinine

DISCUSSION

DISCUSSION

Similar to other extrahepatic tissues the presence, function and regulation of arginase and other enzymes of urea cycle in brain have been subjected to various discussions in recent years (Sadasivudu et al., 1974,1976). Brain is a heterogenous tissue having different cell types. Neuronal function is greatly affected by the presence of ammonia and therefore, its elimination is of importance in brain function. Researches by various scientists have explored the latter half of the urea cycle enzymes in brain (Ratner et al., 1953, 1960; Buniatian et al., 1966). In brain ornithine which is the end product of the urea cycle does not reenter the cycle, unlike the route of hepatic tissue. The ornithine formed in the end may be converted to glutamic acid with 2-oxoglutarate and glutamate is of great importance in neuronal function. The other route of ornithine may towards formation of proline, physiologically an important amino acid. There are some reports on the formation of creatinine through guanido-acetate compounds which are energy source for metabolism, (Blass, 1960). The interrelationships of urea cycle intermediates with other compounds is shown in Fig. 8.

Buniatian et al. (1966) reported the presence of urea cycle enzymes in mammalian brain. Subsequent re-searches of Sadasivudu et al., (1974, 1976) showed the

differential distribution of urea cycle enzymes in brain.
Eventhough, the latter half of the urea cycle enzymes are
present in brain, ammonia formed during neuronal activity
is disposed in the form of glutamine (Richter et al., 1948).
The significance of the urea cycle in brain is yet to be
understood; the formation of citrulline may be ruled out
due to the absence of the first two enzymes, namely,
carbamyl phosphate synthetase and ornithine transcarbamylase.
Small amounts of citrulline present in brain might be derived
from blood (Shaw et al., 1965).

Sadasivudu et al. (1974) reported higher arginase activity
in cerebellum compared with cerebral hemispheres, though
cerebellum has little capacity to produce arginine. Our
present experimental results are slightly different from the
results of Sadasivudu et al. (1974) and showed same amount
of arginase activity in both the regions under normal
conditions. The arginase activity, however, showed a
differential effect under hormonal conditions.

The published activities of urea cycle enzymes in brain
are shown in Table 6 and 7.

The incubation time of the assay of arginase from all
the reports showed wide range of variations. Therefore, a
time curve, the assay of arginase activity was performed.
The results showed that the arginase enzyme activity was

linear upto fifteen minutes and then plateaus to a constant value at thirty minutes. In case of liver, the incubation time was five minutes. The activity of liver arginase is thousand times higher compared with brain. Stewart et al. (1977) reported that urea formation in mouse brain was linear upto 70 minutes concluding that the concentration of mouse brain arginase was lower compared with that of rat brain arginase thereby needing a longer time of incubation. The optimal activity in our experiments was obtained in fifteen minutes. Five minutes of incubation for liver diluted (1:1000) converted 4% of arginine, whereas homogenates diluted (1:10) of rat brain converted only 1.3% arginine in specified time.

Different enzyme concentrations were used in our experiments to determine the optimal concentration of enzyme for maximal activity. Arginase activity with 1:10 diluted brain homogenates increased from 0.1 ml to 0.4 ml enzyme concentrations proportionally; later a plateau was reached at 0.8 ml concentration of enzyme. In case of liver, homogenates of 1:1000 dilution, 0.2 ml enzyme concentration gave optimal activity. It is obvious from these enzyme concentrations used to obtain optimal activity that the arginase concentration in brain was much lower compared with liver.

EFFECT OF SUBSTRATE CONCENTRATION:

Higher concentration of substrates have shown inhibitory effect on enzyme activity (McLean et al., 1965). In our experiments, with different substrate concentrations, the activity was linear upto 300 mM concentration and reaching a plateau finally. The plateau shows a saturation point at 400 mM concentration of substrate.

There are a few reports on the value of Michaelis-Menten constant of arginase from various tissues. Stewart et al. (1977) reported two isoenzymic forms of arginase with K_m of 32 mM and 38 mM in purified fractions of mouse brain. Schimke (1970) reported a K_m value of 3-5 mM for liver arginase. In the present set of experiments with crude homogenates, higher than the above K_m values were obtained for liver and brain tissues. The brain K_m was 154 mM and that of liver was 200 mM Fig. 5 and 6. The higher K_m values could be possibly due to the use of crude fractions in the present experiments. Higher K_m value of brain arginase indicates lower affinity towards the substrate.

Various workers have shown the isoenzymic forms of arginase with different K_m values (Herzfeld et al., 1976; Stewart et al., 1977). Porembska (1973) classified different forms of isoenzymes into A_1, A_2, A_3 and A_4 according to the order they emerged on DEAE cellulose column in different

tissues like kidney, brain, fibroblasts and liver.
Herzfeld et al. (1976) reported that 96% of liver arginase
is of Type III form. Stewart et al. (1977) reported I and
II forms of isoenzymes in mouse brain.

The role of these different isoenzymes specific for
various tissues still remain to be worked out. Their
kinetic properties, importance of different K_m values might
be of physiological significance in the in vivo system.

The above general properties of arginase in brain show
that the arginase concentration of brain is much less than
that of liver, and that it also differs in isoenzymic forms.
These preliminary experiments were undertaken to standardize
and understand the method for determining optimal activity
of arginase in brain homogenates.

DISTRIBUTION OF ARGINASE:

Eventhough, the presence of urea cycle enzymes is
reported in brain and other extrahepatic tissues like
kidney and lungs, its presence and physiological signi-
ficance has been subjected to various discussions.
Kaysen et al. (1973) reported, the presence of arginase
in kidney, both in soluble and particulate fractions.
In liver, arginase is localized only in the soluble fraction
where its function is well defined. Presence of arginase

both in soluble and particulate fractions has lead to the attributions of different physiological functions in two different loci.

In our experiments with brain homogenates, presence of arginase was observed both in soluble and total particulate fractions (TPF). The results showed that 48% of whole homogenate activity in cerebral hemispheres was localized in the soluble fraction and 37% in particulate fraction. In cerebellum, 60% of the activity was in cytosolic fraction and 25% in particulate fractions. The present results indicate that arginase, besides forming urea, may also participate in some other metabolic pathways.

The metabolic role which can be attributed to arginase is towards formation of ornithine which is an important precursor for compounds of great importance to brain like glutamic acid and proline and polyamines through ornithine decarboxylase (Baquer et al., 1976). Sadasivudu et al. (1976) reported that in brain, arginine can a good source for glutamic acid, therefore, the presence of arginase in the particulate fractions could be contributing towards the formation of glutamic acid, as the enzymes concerned with conversion of ornithine to glutamic acid are localized in particulate fraction. This function was also attributed to kidney arginase by Kaysen et al.(1973).

Defalco et al. (1961) reported that intracisternic
injections of arginine served as a precursor for creatine
in brain. Since guanidobutyric acid, an intermediate
compound of this pathway is reported to have excitatory
role, its disposal can be expected through the above
pathway by converting to creatinine. Particulate
arginase may be contributing to this pathway and thereby
reducing the neurochemical effects of GABA.

Sadasivudu et al. (1976) reported the formation of
proline through an intermediate, $\triangle$-pyrroline-5-carbaxylate
from arginine. The enzymes concerned with formation of
proline are present both in cytosolic and particulate
fractions. Hence, the particulate arginase may also be
contributing to the above conversion.

The cytosolic arginase may be contributing towards
conversion of citrulline derived from blood to arginine
and to final urea.

Cerebral hemispheres are the region for seat of
intelligence, awareness, consciousness, memmory and
interpretation of senstion. The higher localization of
particulate fraction in cerebral hemispheres may be
contributing towards above pathways mentioned, particularly,
glutamic acid and conversion to glutamine to have a balanced
physiological role. Sadasivudu et al. (1976) reported
higher glutaminase activity which is useful for glutamine

conversion to glutamic acid which has an excitatory role.

Compared with cerebral hemisphere the activity present in the cerebellum particulate fraction is lower; this may be because, physiologically and functionally cerebellum differs from the cerebral hemispheres.

The conversion of ornithine to citrulline is yet to be understood. Injections of citrulline leads to excess production of ornithine but injections of ornithine does not lead to formation of citrulline in brain. The above discussion, therefore, show that the localization of arginase both in soluble and particulate fractions in brain may be of importance in carrying out different functions.

EFFECT OF STARVATION:

Dietary and nutritional status of animals play an important role in regulation of metabolic pathways. In starvation, utilisation of internal energy sources takes place, leading to breakdown of glycogen, proteins and fatty acids. This leads to the formation of many intermediary compounds like amino acids, free fatty acids under normal physiological functions. Brain utilises only glucose as a substrate for its energy requirements. In order to maintain homeostasis, a number of other enzymes get activated during starvation.

In our experiments, the effect of starvation on brain arginase, showed a significant increase compared to normal rats (Table 3). The effect was more pronounced in cerebellum compared with cerebral hemispheres. The increase in the activity may be due to the activation of some transaminases in starvation. Briggs et al. (1976) reported stimulatory effect of lactate on urea cycle enzymes in liver. Therefore, in the present experiments, increased arginase activity could also be to increased lactate concentrations during starvation. Formation of amino acids also enhanced the activities of urea cycle enzymes by liberating excess of ammonia. Present observed increase of arginase may be attributed to any of the above changes which may occur during starvation.

The pronounced effect of starvation on cerebellum compared with cerebral hemispheres might be due to differences in this physiological role. Cerebellum is mainly concerned with coordination of body function; the first effect of starvation is expected disturb the coordination of body movements as depletion of energy arises. Hence, the observed higher arginase activity may due to disturbed cerebellum functions.

In comparison, starvation has less effect on cerebral hemispheres as it is mainly concerned with sensory functions of the body.

THYROIDECTOMY:

Thyroid hormones are reported to regulate a number of metabolic processes (Baquer et al., 1976). Change in thyroid functions markedly influences the pattern of carbohydrate and lipid metabolism, (Tata et al., 1963). Hypothyroid condition is usually, associated with reduced rate of amino acid incorporation into proteins. Hyper-thyroid condition induces oxidative enzymes and urea cycle enzymes in amphibian liver (Ratner, 1973; Winom et al., 1972). Sochor et al. (1977) reported increase of all urea cycle enzymes in liver during hypothyroid condition with the exception of arginase.

In our experiments with thyroidectomised rats, the activity of arginase did not change both in cerebellum and cerebral hemispheres compared with normal values. An independent regulation of arginase seems to exist in brain, which is in accord with liver arginase. The brain has two isoenzymic forms of arginase and the localization of arginase in brain is also different from that of liver. Therefore, the regulation of arginase is independent in hypothyroid condition may be contributing towards formation of compounds from ornithine discussed earlier.

Injection of triidothyronine (T_3) to throidectomised rats also failed to show any change in arginase activity

from normal values. This also supports the independent regulation of arginase.

Injections of triiodothyronine to normal rats, however, showed a marked influence on arginase activity. The effect was more pronounced on cerebral hemispheres compared to cerebellum Table 4. Tata et al. (1963) showed that injection of T_3 to normal rats increases the incorporation of amino acids into proteins. The enhanced incorporation was due to increased microsomal activity. Therefore, the increased incorporation of amino acids into proteins during hyperthyroid condition might be reducing the substrate available, thereby causing a decrease in the arginase activity as a subsequent effect.

Secondly, thyroid hormones induce changes in the relative amounts of cellular constituents, such as, pyrimidine nucleotides, ATP, nucleic acids and proteins contents (Pitt-Rivers et al., 1959). Whether or not, they represent the cause or effect of changes in various cellular function or of other constituents which can not be ascertained because of chronic treatment. This may be also the cause for reduced arginase activity during hyperthyroid condition.

Thirdly, thyroxine is acting as an insulinotropic agent, is the well known diabetogenic effect of hyper-

thyroidism (Houssay, 1948). Though the amount of insulin
release is reduced from B-cells in T_3 treated animals,
the arginase activity may decrease because of other factors
like increase of amino acid incorporation into proteins
and reduced ATP levels (Tata et al., 1963).

Thus, thyroidectory appears to differ from many other
hormonal conditions studied, in that there is no decrease
in one pathway associated with a concomitant rise in
opposing routes as in diabetics. The effect of hypo-
thyroidism seems to reduce both synthetic and degradative
routes (Baquer et al., 1976). Hypothyroidism depresses
the response of tissues to rapidly acting hormones.

The present decrease in T_3 treated rats might be due
to factors above discussed. In case of hypothyroidism,
the observed effect of no alterations in activity might
be either due to the independent regulation of arginase
or depressed response of tissues to hormones.

ALLOXAN DIABETES:

Krebs and Henseleit (1932) reported that glucose
did not stimulate urea production but, pyruvate, lactate or
fructose had a marked stimulatory effect on the rate of urea
synthesis. Hypoglyceamia is accompanied by a decrease in
the tissue concentrations of several amino acids, such as
glutamine, GABA (Davis et al., 1970), and leads to concen-

tration of free ammonia. Insulin enhances the transport of glucose into brain cells (Rafaelson, 1961; Gottstein et al., 1967). The results of Lewis (1974) indicated a significant decrease in glycogen glucose-6-phosphate, pyruvate, lactate, 2-oxoglutarate and malate when blood glucose concentration falls below 3 μ moles/g due to a condition of hypoglycemia, caused by an excess of insulin.

The presence experimental results in alloxan diabetic rats showed a marked increase on brain arginase activity. The effect was more pronounced in cerebral hemispheres compared to cerebellum.

The possible explanation for increase of arginase activity might be due to increased nitrogen excretion which is characterstic of diabetic animals (Chaikoff et al., 1950).

In diabetic animals eventhough the blood glucose level increases, it may not be the primary cause for the increase of arginase activity as glucose in vitro failed to show any influence on urea cycle enzymes (Krebs et al., 1932).

The other cause which might have lead to increase of arginase activity may be due to increased concentration of pyruvate and lactate which have been shown to have profound influence on urea cycle enzymes in liver (Lewis et al., 1974).

Insulin enhances the transport of blood glucose to brain. In diabetic condition, the insulin level declines. The decreased level of insulin may deprive the brain from getting glucose. In starvation also, the supply or availability of glucose to brain will be decreased. This leads to utilisation of internal energy sources leading to increased concentrations of free amino acids and fatty acids, thereby indirectly affecting the arginase activity.

The activity of various transaminases is increased in diabetic condition (Baquer et al., 1977). Fitch et al. (1962) have shown an increased fumarase activity in diabetes. They drew attention to the fact that about onethird of amino acids found in proteins pass through fumarate during gluconeogenesis. The increased activity of this enzyme may also be significant in relation to equilibrium position of argininosuccinase, the action of which is to produce arginine and fumarate.

Thurston et al. (1975) have shown that creatinine phosphate accumulates in brain of alloxan diabetic mice, creatinine phosphate has been shown to be an inhibitor of the rate controlling step of glycolysis, namely phospho-fructokinase (Krzanowski et al. 1969). The inhibition or decrease of this pathway may be one of the causes for

the ten-fold increase in brain glucose, as demonstrated by Thurston. Citrate also accumulates, thereby indirectly decreasing the intermediates for the TCA cycle. The increase in the activity of arginase shown in the present work may be, therefore, either due to increased glucose, supplying precursors of the urea cycle through transaminase reactions, which are also elevated during alloxan diabetes, or due to lack of insulin. The effect of insulin injection to the diabetic rats, on the arginase levels should be able to show a change, which is the controlling factor. Further work on these lines is in progress.

Thus, the increase of arginase activity in the present results could be attributed to the above factors.

Therefore, from the above explanation, it can be deduced, that the cyclic pathway operative in liver seems to be lacking in brain as the first two enzymes of the cycle, namely, carbamyl phosphate synthetase and ornithine transcarbamylase are absent and ornithine is not entering the cyclic pathway. Therefore, the presence of arginase is brain may be performing some different physiological function than forming urea alone. It can be concluded, that the retention of arginase by brain signifies selective repression of enzymes.

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FUTURE PLAN OF WORK

Different isoenzymic forms of arginase will be purified and separated from rat brain. Variation in the isoenzymic forms will be studied under hormonal conditions.

Other enzymes of urea cycle e.g. arginosuccinase and arginosuccinate synthetase will also be worked out under similar conditions.