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THE INFLUENCE OF CERTAIN ENDOCRINE SECRETIONS  
ON AMINO ACID OXIDASE\*

by

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THE INFLUENCE OF CERTAIN ENDOCRINE SUBSTANCES  
ON ACID-BASE BALANCE

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## ABSTRACT

### THE INFLUENCE OF CERTAIN ENDOCRINE SECRETIONS ON AMINO ACID OXIDASE

#### OBJECT

In the course of studies designed to investigate the effects of stress (hypothermia, shock and irradiation) on the amino acid oxidase system of the liver and kidney, it became necessary to know the influence of the blood amino acid level and of certain endocrine secretions on the activity of this enzyme system. Alterations in the oxygen uptake, as measured by the Warburg manometric technique, were used as indices of the changes in the enzyme activity.

#### RESULTS AND CONCLUSIONS

Intraperitoneal administration of an amino acid mixture (casein hydrolysate) to normal rats produced an enhancement of the amino acid oxidase activity in the liver and kidney. Accelerated amino acid oxidation was simultaneously associated with increased blood urea and frequently increased glucose levels. In adrenalectomized and hypophysectomized animals, similarly treated, the liver amino acid oxidase activity was not augmented. Administration of an adrenal cortical extract to normal and adrenalectomized animals accelerated the activity of the enzyme in the liver and kidney. An accelerating effect of the secretion of the adrenal cortex was also observed in vitro. The increase in enzymic activity of the liver observed in normal animals after amino acid administration is apparently mediated through the pituitary-adrenal cortex system. The nature of the mechanism of the stimulation remains a matter for investigation.

The pituitary may also inhibit liver amino acid oxidase activity by way of its growth hormone, for the liver of hypophysectomized animals showed increased amino acid oxidase activity.

Thyroidectomized animals showed a decreased amino acid oxidase activity of the liver but an increased oxidase activity of the kidney. Administration of amino acids led to a stimulation of the liver oxidase.

Epinephrine was found to inhibit amino acid oxidase activity of the liver. The effect of insulin was doubtful.

The results obtained also indicate that while the activity of the liver amino acid oxidase is mainly under endocrine control, the kidney amino acid oxidase activity, at least partly, can be influenced directly by the amino acid level in the blood.



## RECOMMENDATIONS

Similar experiments should be carried out with castrated animals in order to determine the regulatory effect of gonadal secretions on the amino acid oxidase activity. Additional studies should be carried out with hypophysectomized animals.

It also is suggested that the possibility of an amino acid tolerance test be investigated in cases of either liver or certain endocrine (pituitary-adrenal cortex) insufficiencies. It is postulated that amino acid metabolism in patients with liver disease or certain endocrine (pituitary-adrenal cortex) deficiencies might be sufficiently impaired to decrease significantly the rate of disappearance of the amino acids from the blood. If such proved to be the case, the rate of removal of intravenously administered amino acids may serve as an index of the degree of the liver or pituitary-adrenal cortex disfunction.

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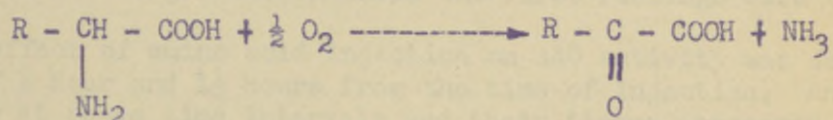
THE INFLUENCE OF CERTAIN ENDOCRINE SECRETIONS  
ON AMINO ACID OXIDASE

I. INTRODUCTION

The problem of intermediate protein metabolism has been studied for many years. Research during these years has answered many of the questions but many still remain unanswered.

It is known that amino acids can either be converted into body protein, "anabolism", or can be deaminated to form energy producing intermediates, "catabolism". The first step in the catabolic process is the deamination of amino acids. This reaction is catalyzed by the enzyme, amino acid oxidase, "AAO". There are two distinct amino acid oxidase systems (1):  
(a) l-amino acid oxidase which specifically deaminates l-amino acids and  
(b) d-amino acid oxidase which specifically deaminates d-amino acids.

The activity of amino acid oxidase may be considered a measure of amino acid catabolism. The reaction is as follows:



It may be observed that ammonia results from this reaction. In the liver, the ammonia participates in the formation of urea and in the process of transamination. The deamination of amino acids in the liver is accompanied by an increase in the urea of the blood. Changes in the level of blood urea therefore may be used as a partial index of the amino acid oxidase activity.

It was the object of this study to investigate the interrelation of the influence of amino acid concentration in the blood and the secretion of the anterior pituitary, adrenal and thyroid glands on the rate of activity of the liver and kidney amino acid oxidase systems. Alterations in the oxygen uptake, as measured by the Warburg manometric technique, were used as criteria for changes in the enzymic activity.

II. EXPERIMENTAL

White male rats of the Sprague-Dawley strain weighing between 250-300 grams were employed. They were starved for 18 hours before the experiment, but were permitted water. Four groups of animals were employed: normal, adrenalectomized, thyroidectomized and hypophysectomized. Within each group a series of experiments was carried out in each of which two animals were injected intraperitoneally with a 10 per cent solution of an enzymic casein hydrolysate\* while one animal was simply pierced with a needle. For purposes of convenience, the latter procedure will be called "dry needle".

\* Amigen, prepared by Mead Johnson & Company, Evansville, Indiana. We wish to express our appreciation to Dr. Warren M. Cox of that company for generously supplying us with this preparation.





The liver extracts were prepared by cutting out approximately three grams (weighed to the nearest 0.1 of a milligram) of tissue from the animals (killed by decapitation) and by homogenizing the tissue with 6 ml. of phosphate buffer (pH 7.35) for five minutes. The buffer employed was a modified Krebs' buffer containing NaCl (0.95 per cent),  $MgSO_4$  (3.82 per cent), KCl (1.15 per cent) and made to pH of 7.35 with HCl and  $Na_2HPO_4$ . After homogenization, the minced-tissue mixture was centrifuged at high speed for ten minutes. The supernatant was decanted and 2 ml. portions were used for the enzyme activity assay. Kidney was processed in a similar manner with approximately 1.2 grams of tissue being homogenized with 10 ml. of the same buffer for ten minutes. Samples of each tissue were taken to determine the ration of wet to dry weights so that correction could be made for variation in water content of the tissue.

It was necessary to attain a constancy of the tissue processing conditions so that the enzyme activity would vary only with the animal and its experimental environment. Accordingly, a specific number of minutes was allotted from the time the animals were killed until the tissue was homogenized and from homogenization until the first readings were taken.

The effect of amino acid injection on AAO activity was determined after periods of  $\frac{1}{2}$  hour and  $1\frac{1}{2}$  hours from the time of injection. Animals were sacrificed at these time intervals and their tissue processed as related.

In the Warburg manometric technique, employed for the determination of oxygen uptake, the flasks were filled as follows:

- Main Chamber - 2 ml. of buffered supernatant homogenate.
- Side Arm - Experimental flask - 0.2 ml. of a 0.1 molar dl-alanine solution in buffer.  
- Control flask - 0.2 ml. of buffer.
- Central Well - 0.2 ml. of a 10 per cent sodium hydroxide solution.

After replacing the air in the flasks with oxygen, they were placed in the water bath and equilibrated for 10 minutes at  $36.8^{\circ}C$ .

After tipping the substrate into the main chamber, readings were taken every fifteen minutes for an hour with the flasks shaking 80 times per minute. The oxygen uptake is expressed in microliters of  $O_2$  per gram of wet tissue-homogenate extracted.

The in vitro and in vivo experiments have been carried out in a similar manner in order to determine the effects of insulin, adrenal cortical extract and epinephrine on the AAO system.

For the in vivo experiments: (a) 0.5 I.U. of crystalline insulin (Squibb) was injected intramuscularly, (b) 1 ml. of aqueous adrenal cortical extract (Upjohn) was injected hourly, intramuscularly, into the respective animal during a four hour period prior to sacrificing, or (c) 0.3 ml. of a 1:1000 adrenalin hydrochloride solution (Parke-Davis) was injected in the same manner as b, depending on the experiment. For the corresponding in vitro experiments, 0.05 I.U. of insulin, 0.1 ml. of adrenal cortical extract (Upjohn) or 0.1 ml. of

The liver extracts were prepared by cutting out approximately three grams (weighed to the nearest 0.1 of a milligram) of tissue from the animals (killed by decapitation) and by homogenizing the tissue with 8 ml. of phosphate buffer (pH 7.35) for five minutes. The buffer employed was a modified Krebs' buffer containing NaCl (0.92 per cent), K<sub>2</sub>SO<sub>4</sub> (0.32 per cent), KCl (1.12 per cent) and made to pH of 7.35 with HCl and Na<sub>2</sub>HPO<sub>4</sub>. After homogenization, the mixed-tissue mixture was centrifuged at high speed for ten minutes. The supernatant was decanted and 5 ml. portions were used for the enzyme activity assay. Kidney was processed in a similar manner with approximately 1.5 grams of tissue being homogenized with 10 ml. of the same buffer for ten minutes. Samples of each tissue were taken to determine the ratio of wet to dry weights so that correction could be made for variation in water content of the tissues.

It was necessary to obtain a consistency of the tissue processing conditions so that the enzyme activity would vary only with the animal and the experimental environment. Accordingly, a specific number of minutes was allotted from the time the animals were killed until the tissue was homogenized and from homogenization until the first readings were taken.

The effect of saline acid injection on AAO activity was determined after periods of 1/2 hour and 1 1/2 hours from the time of injection. Animals were sacrificed at these time intervals and their tissues processed as related.

In the Warburg manometric technique, employed for the determination of oxygen uptake, the flasks were filled as follows:

- Main Chamber - 5 ml. of buffered phosphate buffer.
- Side arm - Experimental flask - 0.5 ml. of a 0.1 molar di-amine solution in buffer.
- Control flask - 0.5 ml. of buffer.
- Control Well - 0.5 ml. of a 10 per cent sodium pyruvate solution.

After replacing the air in the flasks with oxygen, they were placed in the water bath and equilibrated for 10 minutes at 37°C.

After timing the substrate into the main chamber, readings were taken every fifteen minutes for an hour with the flask shaking 30 times per minute. The oxygen uptake is expressed in microliters of O<sub>2</sub> per gram of wet tissue-homogenate extracted.

The *in vitro* and *in vivo* experiments have been carried out in a similar manner in order to determine the effects of insulin, adrenal cortical extract and epinephrine on the AAO system.

For the *in vivo* experiments: (a) 0.5 I.U. of crystalline insulin (Boehr) was injected intramuscularly, (b) 1 ml. of aqueous adrenal cortical extract (Upjohn) was injected hourly intramuscularly into the respective animal during a four hour period prior to sacrifice, or (c) 0.3 ml. of a 1:1000 adrenalin hydrochloride solution (Parke-Davis) was injected in the same manner as b, depending on the experiment. For the corresponding *in vitro* experiments, 0.05 I.U. of insulin, 0.1 ml. of adrenal cortical extract (Upjohn) or 0.1 ml. of

1:25,000 commercial adrenalin solution was used in each of the control and the experimental flasks which were prepared otherwise as described previously. Control determinations were run for each group of the in vivo and in vitro experiments.

Blood glucose (2), urea (3), amino acids (4) and hematocrit determinations were carried out simultaneously on blood obtained at the time the animals were decapitated.

Animals were adrenalectomized from a single horizontal incision, posteriorly in the lumbar region. For four days, these animals were kept on a normal diet but given 1 per cent saline as drinking water. For the subsequent three days, they were taken off saline and given plain water and for the last eighteen hours, they were starved. These animals averaged a 25 gram weight loss from operation to day of experiment.

Hypophysectomized animals were operated via a para-tracheal approach and were kept 14 to 21 days on a milk, chopped meat and bread diet before the experiment. This group had its own control group fed on a similar diet. The hypophysectomized animals averaged 50 grams less in weight than their litter mate controls at the time of sacrifice.

Animals were thyroidectomized and maintained for at least 21 days before being subjected to experimental procedures. Only those animals that evinced a minimum of 25 per cent decrease in B.M.R. were used. The average lowering of B.M.R. among the twenty-four animals used was 35.2 per cent (see Table 1).

TABLE 1

BASAL METABOLIC RATE IN THYROIDECTOMIZED AND NORMAL ANIMALS

	Calories Per Square Meter Per Hour	Number of Animals	% Reduction
Normals	38.1 ± 3.0	9	
Thyroidectomized	24.7 ± 1.83	24	35.2%

Another group of animals was operated (neck dissection) in a sham fashion to determine whether any effect on the amino acid oxidase resulted from the operation alone. No changes were found when the results were compared with those of unoperated control animals.

III. RESULTS AND DISCUSSION

A. Liver Amino Acid Oxidase

From Table 2, it is obvious that administration of amino acids to normal animals caused a pronounced increase in the liver amino acid oxidase activity. However, neither adrenalectomized nor hypophysectomized animals similarly treated, showed this increase. The effect of the increased blood

1:25,000 commercial phenolphthalein solution was used in each of the control and experimental flasks which were prepared otherwise as described previously. Control determinations were run for each group of the 12 days and in three experiments.

Blood glucose (2), urea (3), amino acids (4) and respiratory determinations were carried out simultaneously or blood obtained at the time the animals were sacrificed.

Animals were administered from a sterile horizontal incision, post-ly only in the lower region. For four days, these animals were kept on a normal diet but given 1 per cent saline as drinking water. For the subsequent three days, they were taken off saline and given plain water and for the last eighteen hours, they were starved. These animals averaged a 25 gram weight loss from operation to day of experiment.

Hypophysectomized animals were operated via a para-thoracic approach and were kept in a cage on a milk, chopped meat and bread diet before the experiment. This group had the same control group fed on a similar diet. The hypophysectomized animals averaged 30 grams less in weight than their litter mate controls at the time of sacrifice.

Animals were thyroidectomized and maintained for at least 21 days before being subjected to experimental procedure. Only those animals that gained a minimum of 25 per cent decrease in S.M.R. were used. The average lowering of S.M.R. among the twenty-four animals used was 35.2 per cent (see Table I).

TABLE I  
BASEL METABOLIC RATE IN THYROIDECTOMIZED AND NORMAL ANIMALS

Reaction	Number of Animals	Calories Per Square Meter Per Hour
Normal	9	38.1 ± 3.0
Hypothyroid	24	24.7 ± 1.8

Another group of animals was operated (neck dissection) in a similar fashion to determine whether any effect on the amino acid oxides resulted from the operation alone. No changes were found when the results were compared with those of unoperated control animals.

### III. RESULTS AND DISCUSSION

#### A. Liver Amino Acid Oxidase

From Table I, it is obvious that administration of amino acids to normal animals caused a pronounced increase in the liver amino acid oxidase activity. However, neither thyroidectomized nor hypophysectomized animals similarly treated, showed this increase. The effect of the increased blood

amino acid level on the liver amino acid oxidase is probably mediated through the pituitary-adrenal system. The nature of the "priming" mechanism for the pituitary-adrenal cortex stimulation after amino acid administration still must be investigated. The question whether the elevated blood amino acid level causes a direct or indirect stimulation of the anterior pituitary cannot as yet be answered. Paschke and Schwoner (5) postulated that the pituitary produces a "protein metabolism hormone", which is released on stimulation by a protein meal and may be found in the urine. Thus, they conclude that protein itself furnishes its own trigger mechanism for its metabolism.

The effect of the adrenal-cortical secretion on the oxidase activity of the liver was also observed by in vitro and in vivo experiments. Table 3 illustrates that normal as well as adrenalectomized animals, given adrenal cortical extracts, showed an increased oxidase activity of the liver while untreated adrenalectomized animals have a decreased AAO activity (Table 4). In vitro experiments also showed a similar accelerating effect of cortical extracts upon amino acid oxidase activity of the liver (Table 3).

It may be observed in hypophysectomized animals that the amino acid oxidase activity of the liver was increased 100 per cent over that of normal animals (see Table 4). This increase in activity is probably due to the absence of the pituitary growth factor and is in agreement with the concept that the growth hormone may inhibit amino acid catabolism. It has been shown by Szego and White (6) that growth hormone produces increased fatty acid metabolism and fat deposition in the liver when administered to normal starved animals. These investigators suggested that the growth hormone may either inhibit amino acid catabolism or accelerate fat metabolism. Our observations support the former postulate, for in our experiments the hypophysectomized animals showed an increased amino acid oxidase activity in the liver. Russell and Capiello (7) recently reported that when a partially purified preparation of anterior pituitary growth hormone was given to nephrectomized rats, 1 to 2 hours before the periods of observations were begun, the rate of urea formation during the first hour after the administration of a casein hydrolysate was reduced by approximately 40 per cent.

In thyroidectomized animals, administration of amino acids resulted in an increase in amino acid oxidase activity of the liver which, however, was not as pronounced as in normal animals (see Table 2). According to Deane and Greep (8), thyroidectomy leads to an atrophy of the adrenal cortex. This may explain why the increase in liver amino acid oxidase activity in thyroidectomized animals is not as great as in normal animals. Thyroidectomized control animals showed a decreased AAO activity of their liver (Table 4). This finding is in agreement with that of Klein (9) who found that thyroidectomy decreases liver AAO activity.

#### B. Kidney Amino Acid Oxidase

Strictly speaking, comparative results, as found for the liver amino acid oxidase, were not obtained for the kidney amino acid oxidase after amino acid administration (see Table 2). Values were obtained in the normal animal's kidney showing an 89 per cent increase in oxidase activity after  $\frac{1}{2}$  hour but only a negligible increase after  $1\frac{1}{2}$  hours. A similar type of disagreement

amino acid level on the liver amino acid content is probably mediated through the pituitary-thyroid system. The nature of the "training" mechanism for the pituitary-thyroid cortex stimulation after amino acid administration also still must be investigated. The question whether the elevated liver amino acid level causes a direct or indirect stimulation of the anterior pituitary cannot as yet be answered. Feschel and Soloway (2) postulated that the pituitary produces a "protein metabolite hormone", which is released on stimulation by a protein meal and may be found in the urine. Thus, they conclude that protein itself furnishes its own trigger mechanism for the metabolism.

The effect of the adrenal-cortical secretion on the oxalase activity of the liver was also observed by *in vivo* and *in vitro* experiments. Table 3 illustrates that normal as well as adrenalectomized animals, given adrenal cortical extracts, showed an increased oxalase activity of the liver while untreated adrenalectomized animals have a decreased AAO activity (Table 4). *In vivo* experiments also showed a similar accelerating effect of cortical extracts upon amino acid oxalase activity of the liver (Table 3).

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In hypophysectomized animals, administration of amino acids resulted in an increase in amino acid oxalase activity of the liver which, however, was not as pronounced as in normal animals (see Table 2). According to Deane and Grop (6), hypophysectomy leads to an atrophy of the adrenal cortex. This may explain why the increase in liver amino acid oxalase activity in hypophysectomized animals is not as great as in normal animals. Hypophysectomized control animals showed a decreased AAO activity of their liver (Table 4). This finding is in agreement with that of Klotz (8) who found that hypophysectomy decreases liver AAO activity.

### 5. Kidney Amino Acid Oxalase

Strictly speaking, comparative results, as found for the liver amino acid oxalase, were not obtained for the kidney amino acid oxalase after amino acid administration (see Table 2). Values were obtained in the normal animal's kidney showing an 89 per cent increase in oxalase activity after 2 hour fast only a negligible increase after 12 hours. A similar type of disagreement

was also observed in the kidney amino acid oxidase activity of adrenalectomized and hypophysectomized animals. There is no increase after  $\frac{1}{2}$  hour in the adrenalectomized animals but a 59 per cent increase in the amino acid oxidase activity after  $1\frac{1}{2}$  hours. Again, peculiarly, an increase in activity of 29 per cent occurs in the kidney of hypophysectomized animals after  $1\frac{1}{2}$  hours. However, in the thyroidectomized animals, the kidney amino acid oxidase activity was distinctly decreased after amino acid administration. This inhibitory effect has still to be elucidated.

Apparently, there is a distinction between the factors influencing amino acid oxidase activity of the liver and the kidney. Lang (11) and Kochakian (12) found that liver and kidney amino acid oxidase enzymes do not respond similarly. Lotspeich and Pitts (13) reported that the excretion of ammonia in the urine of the dog is proportional to the plasma amino acid level. They concluded that the renal amino acid oxidase is concerned with the formation of ammonia by the kidney which process plays an important role in the regulation of acid-base balance. Other investigators (14) have also found that the administration of certain amino acids produces an increased excretion of ammonia in the urine of the dog. If the ammonia is considered as a measure of the activity of AAO, since it is a product of the reaction of the enzyme, one may theorize that the amino acid oxidase activity in the kidney not only is dependent upon the various aforementioned endocrine factors, but may also vary in accordance with the amino acid level of the blood. Thus, the initial increase (Table 2)  $\frac{1}{2}$  hour after injection and subsequent decrease after  $1\frac{1}{2}$  hours in AAO activity of the normal animal's kidney may be attributed to the corresponding rise and fall in amino acid level of the blood.

This view may similarly apply for the adrenalectomized and hypophysectomized group where the findings are reversed, i.e., the greater increase in the AAO activity is attained at the  $1\frac{1}{2}$  hour period. In these instances, since the liver oxidase activity, in the absence of the activating mechanism of the pituitary-adrenal cortex system, cannot partake normally in lowering the amino acid level any longer, the effect of the continued blood amino acid elevation manifests itself in the kidney but not until a period of time has elapsed.

Russell and Wilhelmi found (10) that the kidneys of adrenalectomized rats showed a decreased AAO activity, and that administration of adrenal cortical extract to these animals increased AAO activity. Our control adrenalectomized animals which were injected with a "dry needle" did not show this decrease in 30 minutes after "injection" but did manifest a decreased AAO activity below that of normal animals in  $1\frac{1}{2}$  hours after injection with "dry needle". (see Table 4.)

A possible cause for this discrepancy will be discussed later when the effect of epinephrine on the AAO system is discussed.

### C. Blood-Amino Acid, Glucose, Urea Hematocrit (see Table 5).

AAO activity may be correlated with the level of amino acid nitrogen, urea nitrogen and glucose in the blood. In normal animals (see Table 5), the amino acid nitrogen rose from 12.1 mg. to 18.5 mg. per cent in  $\frac{1}{2}$  hour after





injection of the amino acid mixture and declined to 15.4 mg. per cent in  $1\frac{1}{2}$  hours indicating rapid deamination. The changes in the blood amino acid nitrogen level can be linked with the respective changes in urea nitrogen and glucose levels of the blood. While urea nitrogen was not greatly changed, 4.7 mg. per cent after  $\frac{1}{2}$  hour, it was elevated by 12.5 mg. per cent in  $1\frac{1}{2}$  hours after amino acid injection. Similarly, in normal animals, there was no increase in blood glucose in  $\frac{1}{2}$  hour but a 10.8 mg. per cent increase after  $1\frac{1}{2}$  hours. These results probably indicate that the increased deamination of amino acids led to an increased formation of ammonia and consequently of urea and that simultaneously increased gluconeogenesis had taken place. Acceleration of these metabolic processes in normal animals had started in  $\frac{1}{2}$  hour and were well established in  $1\frac{1}{2}$  hours after the injection of the casein hydrolysate.

In the adrenalectomized animals, the increase in these transformations seemed to be slowed down or inhibited. For example, there is no decrease in amino acid nitrogen blood level in  $1\frac{1}{2}$  hours (20.5 mg. per cent in  $\frac{1}{2}$  hour and 22.3 mg. per cent in  $1\frac{1}{2}$  hours) after the injection of the amino acid mixture. These amino acid nitrogen levels were distinctly higher than those found in normal animals (18.5 and 15.4 mg. per cent respectively). Apparently, the adrenalectomized animals are unable to accelerate properly the metabolism of the injected amino acids. These findings are in agreement with the generally accepted assumption that certain factors secreted by the adrenal cortex enhance protein catabolism.

Furthermore, the increases in blood urea ( $\frac{1}{2}$  hour, 7.9 mg. per cent;  $1\frac{1}{2}$  hours, 8.3 mg. per cent) and glucose ( $\frac{1}{2}$  hour, minus 10.5 mg. per cent;  $1\frac{1}{2}$  hours, minus 20 mg. per cent) of the adrenalectomized animals after the injection of amino acids are, with the exception of urea at  $\frac{1}{2}$  hour, not as great as compared to the increases in the same blood constituents of normal animals (urea, 4.7 mg. per cent and 12.5 mg. per cent; glucose, minus 6.3 mg. per cent, plus 10.8 mg. per cent) subjected to the same treatment (see Table 5). The observation that one gets a significant increase in urea formation at all in the adrenalectomized animals may be surprising at first thought, since the lack of adrenal cortical secretion would lead one to believe that little or no deamination takes place. However, it must be recalled that the ability of the kidney to deaminate was not found to be as impaired as that of the liver in the adrenalectomized animals (see Table 2). Thus, ammonia formed in the kidneys of these operated animals may be utilized in the liver for the formation of urea. In addition, the impaired excretory capacity of the kidneys in these animals may account for retention of some urea and this may account for the apparently greater increase in blood urea after  $\frac{1}{2}$  hour.

In hypophysectomized animals, the amino acid nitrogen level,  $1\frac{1}{2}$  hours after amino acid administration (16.0 mg. per cent) is at the same level found in normal animals similarly treated (see Table 5). The control animals in the hypophysectomized group had an amino acid nitrogen level comparable to the normal controls (12.5 mg. per cent). The ability of the hypophysectomized animals to deaminate injected amino acids at a rate comparable to that observed in the normal animal may be due to the absence of the pituitary growth factor as noted previously. Furthermore, the absence of this factor may explain the



apparently accelerated protein catabolism as shown by the findings that blood urea nitrogen is increased 12.6 mg. per cent (46.8 to 59.4) and glucose 16.6 mg. per cent (45.5 to 62.1) during this period (see Table 5). The increases are about the same as those found in normal animals after injection, and are greater than those values found in adrenalectomized animals. However, the absolute values for blood urea are increased and for glucose are decreased in the hypophysectomized animals.

By the same criteria, thyroidectomized animals manifest the ability, although somewhat retarded, to metabolize amino acids. From Table 5, it may be observed that the lowering of the amino acid nitrogen level was not quite as prompt or as effective but eventually, after injection of amino acids, the amino acid nitrogen level decreased from 21.7 mg. per cent in  $\frac{1}{2}$  hour to 16.0 mg. per cent in  $1\frac{1}{2}$  hours. Similarly, the urea nitrogen levels, although increased ( $\frac{1}{2}$  hour, 28.1 to 31.7 mg. per cent;  $1\frac{1}{2}$  hours to 39.3 mg. per cent) do not quite attain the increase that the normal animals showed in  $\frac{1}{2}$  hour and  $1\frac{1}{2}$  hours. Finally, the blood glucose levels in these animals increased in  $\frac{1}{2}$  hour (3.9 mg. per cent) but decreased greatly (12.6 per cent) in  $1\frac{1}{2}$  hours after injection.

It can be noted from Table 5, that the hematocrit is increased about 6 to 8 per cent in the injected normal animals. This finding may be explained by the relative dehydration caused by the transfer of water from the hypotonic environment of the vascular compartment into the hypertonic environment of the peritoneal cavity into which a 10 per cent solution of amino acids had been injected. It should also be noted that the adrenalectomized animals showed a greater increase in hematocrit (8 to 12 per cent) after injection, than did normals.

#### D. General Observations

From Table 4, it can be seen that those control normal and thyroidectomized animals, which only were pierced with a "dry needle"  $\frac{1}{2}$  hour previous to being sacrificed, showed a decreased amino acid oxidase activity in the liver and kidney when compared with animals sacrificed  $1\frac{1}{2}$  hours after injection. Since this decrease was not manifested in adrenalectomized animals similarly treated and since it is associated with increased blood glucose levels (Table 5), one may suspect that the secretion from the adrenal medulla may be responsible for the transient decrease in amino acid oxidase activity. The results of experiments with epinephrine both in vitro and in vivo support this possibility (see Table 3). When a non-commercial epinephrine solution containing only pure crystalline epinephrine was used, the inhibitory effect lasted only for 15 to 20 minutes. This difference in effect may be due to the presence of antioxidants in the commercial solutions.

It is also conceivable that the above mentioned inhibition may be due to the action of insulin on the AAO. The increased blood sugar levels caused by an epinephrine reaction may result in an increased insulin secretion. Although certain investigators (15,16) have been able to show insulin inhibition of this enzyme, our technique was unable to demonstrate this effect either in vitro or in vivo. (See Table 3).



#### IV. SUMMARY AND CONCLUSIONS

1. Data have been presented relating blood amino acid level and certain endocrine secretions to the activity of amino acid oxidase in the liver and the kidney of rats.
2. Administration of casein hydrolysate to normal animals produces an increase in the amino acid oxidase activity of the liver and kidney in these animals.
3. Administration of casein hydrolysate to adrenalectomized or hypophysectomized animals does not produce this increase in the amino acid oxidase activity of liver.
4. Adrenal cortical extract accelerates the activity of this enzyme in the liver in vitro and in vivo and in the kidney in vitro.
5. The pituitary-adrenal cortex complex mediates the stimulus for the acceleration of amino acid oxidase activity of the liver observed after amino acid administration.
6. The nature of the mechanism of the pituitary-adrenal cortex stimulation after amino acid administration remains to be investigated.
7. Livers of hypophysectomized animals show increased amino acid oxidase activity which may be due to the absence of the growth factor of the pituitary.
8. Thyroidectomized animals show a decreased amino acid oxidase activity of the liver but an increased activity of the kidney. Administration of amino acids stimulates the liver oxidase.
9. Epinephrine inhibits amino acid oxidase activity of the liver. The effect of insulin is doubtful.
10. The amino acid oxidase activity of the liver and the kidney may respond similarly to certain endocrine stimuli. However, it appears that the blood amino acid level may influence the kidney oxidase activity directly but not that of the liver.

#### V. RECOMMENDATIONS

In order to complete the concept of the effect of endocrines on the enzyme, amino acid oxidase, it is suggested that further work be done with hypophysectomized and castrated animals. These experiments should be carried out similarly to those described in this report.

It is further recommended that an investigation on the tolerance of humans to amino acid administration be initiated. The experiments to be carried out on normal humans and on those with either adrenal or liver insufficiency. It has been related in this report that the metabolism of amino acids is inhibited in animals with adrenal deficiency. It is postulated that amino acid metabolism in patients with liver or certain endocrine.



(hypophysis-adrenal cortex) disfunctions might be sufficiently impaired to decrease significantly the rate of disappearance of amino acids from the blood. If such proved to be the case, the rate of removal of intravenously administered amino acids may serve as an index of either liver or hypophysis-adrenal cortex functions.

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(pyruvate-thiamine complex) disintegration might be catalytically catalyzed  
 to decrease significantly the rate of disappearance of amino acids from  
 the blood. It was proved to be the case, the rate of removal of lactate  
 venously administered amino acids may occur as an index of either liver  
 or pyruvate-thiamine complex function.

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TABLE 2

## MICROLITERS AND % INCREASE IN AMINO ACID OXIDASE ACTIVITY AFTER AMINO ACID INJECTION

Values Equal Microliters and % Increase in Microliters of Oxygen Uptake Per Gram of Tissue Homogenate Extract

Tissue	Minutes After Injection	Normals	Adrenalectomized	Hypophysectomized	Thyroidectomized
LIVER	30	14 = 50%	0 = 0%	—	9 = 33%
	90	15 = 38%	2 = 4%	-31 = -38%	3 = 11%
KIDNEY	30	119 = 89%	-24 = -9%	—	-43 = -14%
	90	18 = 7%	107 = 59%	66 = 29%	-93 = -27%



TABLE 3

EFFECT OF CERTAIN HORMONES ON AMINO ACID OXIDASE OF LIVER  
IN VITRO AND IN VIVO

	MICROLITERS OF OXYGEN UPTAKE			
	Figures in Parentheses Equal Number of Animals			
	Buffer	Alanine	Difference (AAO)	Control (AAO)
ADRENAL CORTICAL EXTRACT				
<u>In Vitro</u>	300	425	125 ± 18 (6)	59 ± 8 (14)
<u>In Vivo</u> (Adrenalectomized)	301	343	42 ± 3 (2)	21 ± 3 (2)
<u>In Vivo</u> (Normals)	219	279	60 ± 4 (4)	27.5 ± 1.5 (2)
ADRENALIN				
<u>In Vitro</u>	232	257	25 ± 2.6 (5)	59 ± 8 (14)
<u>In Vivo</u>	230	253	23 ± 6 (4)	41 ± 4.5 (20)
INSULIN				
<u>In Vitro</u>	242	274	32 ± 9 (13)	59 ± 8 (14)
<u>In Vivo</u>	203	246	43 ± 4 (8)	57 ± 9 (4)



TABLE 4

TOTAL RESPIRATION AND AMINO ACID OXIDASE ACTIVITY OF CONTROL  
AND EXPERIMENTAL ANIMALS  
Microliters of Oxygen Uptake Per Gram of Homogenized Tissue Extract

Tissue	Time After Injection Dry Needle <sup>a</sup>	Normal			Adrenalectomized			Hypophysectomized			Thyroidectomized		
		Buffer	Alanine	Oxidase Activity	Buffer	Alanine	Oxidase Activity	Buffer	Alanine	Oxidase Activity	Buffer	Alanine	Oxidase Activity
LIVER	0	211	270	59 ± 8 (10)	--	--	--	--	--	--	--	--	--
	30	208	235	27 ± 3.3 (8)	197	226	29 ± 6.1 (9)	--	--	--	205	223	18 ± 2.0 (4)
	90	235	275	40 ± 4.4 (9)	171	198	27 ± 5.1 (5)	204	285	81 ± 9 (5)	201	228	27 ± 3.1 (4)
KIDNEY	30	206	341	135 ± 13.5 (6)	197	462	265 ± 17 (6)	--	--	--	164	475	311 ± 18 (4)
	90	220	472	252 ± 21 (13)	198	380	182 ± 14 (5)	181	412	231 ± 15 (5)	201	551	350 ± 21 (4)
As Above After Injection of 5 cc. Amigen Intraperitoneally													
LIVER	30	226	267	41 ± 4.5 (20)	225	249	24 ± 4.3 (19)	--	--	--	219	246	27 ± 3.0 (8)
	90	224	279	55 ± 3.1 (21)	179	207	28 ± 4.0 (7)	200	250	50 ± 5.7 (5)	239	269	30 ± 4.3 (8)
KIDNEY	30	205	459	254 ± 36 (19)	169	400	231 ± 25 (14)	--	--	--	195	463	268 ± 22 (8)
	90	207	477	270 ± 31 (28)	187	476	289 ± 20 (7)	173	470	297 ± 30 (9)	194	451	257 ± 5 (8)





TABLE 5

## CHANGES IN BLOOD CONSTITUENTS DURING EXPERIMENTAL PROCEDURES

Blood Constituent	Minutes After Injection	Figures in Parentheses Equal Number of Animals											
		Normals		Adrenalectomized		Hypophysectomized		Thyroidectomized					
		Control	Injected	Control	Injected	Control	Injected	Control	Injected				
HEMATOCRIT %	30	52.5 ± 0.6(4)	55.4 ± 1.0(8)	55.5 ± 1.3(3)	60.0 ± 1.4(5)	--	--	49.4 ± 1.5(4)	51.2 ± 0.8(8)				
	90	52.4 ± 0.4(4)	56.5 ± 1.5(6)	55.5 ± 0.8(3)	63.0 ± 2.0(5)	--	--	50.5 ± 0.9(3)	55.0 ± 2.0(6)				
AMINO ACIDS Mg. % Amino Acid N <sub>2</sub>	30	12.1 ± 1.3(5)	18.5 ± 1.6(8)	12.6 ± 1.3 (7)	20.5 ± 1.3(13)	--	--	11.9 ± .9(3)	21.7 ± 3.1(6)				
	90	12.9 ± 1.4(9)	15.4 ± 0.2(12)	14.5 ± 0.6 (3)	22.3 ± 1.8(5)	12.5 ± 0.5(4)	16.0 ± 1.2(7)	12.2 ± .4(4)	16.0 ± 1.6(8)				
UREA Mg. % Urea N <sub>2</sub>	30	18.9 ± 0.8(4)	23.6 ± 1.8(9)	40.8 ± 2.0(3)	48.7 ± 3.1(4)	--	--	28.1 ± 0.9(3)	31.7 ± 1.8(7)				
	90	17.7 ± 0.3(5)	30.2 ± .8(8)	40.2 ± 2.6(3)	48.6 ± 2.8(2)	46.8 ± 2.2(3)	59.4 ± 1.6(6)	29.1 ± 1.5(3)	39.3 ± 2.1(5)				
GLUCOSE Mg. %	0	68.2 ± 0.2(3)	--	--	--	--	--	58.5 ± 3.0(3)	--				
	30	72.9 ± 2.1(3)	66.6 ± 2.4(8)	46.5 ± 2.3(3)	36.0 ± 6.1(5)	--	--	73.7 ± 1.0(4)	77.6 ± 0.8(8)				
	90	66.0 ± 3.0(5)	76.8 ± 2.7(10)	47.8 ± 2.0(3)	27.8 ± 1.1(5)	45.5 ± 1.5(2)	62.1 ± 3.6(4)	59.2 ± 2.5(4)	46.6 ± 3.4(8)				

Date	Particulars	Particulars												
		1	2	3	4	5	6	7	8	9	10			
1911	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1912	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1913	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1914	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1915	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1916	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1917	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1918	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1919	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1920	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1921	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1922	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1923	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1924	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1925	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1926	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1927	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1928	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1929	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1930	...	...	...	...	...	...	...	...	...	...	...	...	...	...

STATE OF NEW YORK