

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

by

Ralph N. Cagan, Capt., M.C., John L. Gray, Biochemist and H. Jensen, Ph.D., Chief Biochemist

from

Medical Department Field Research Laboratory Fort Knox, Kentucky 16 May 1949

\*Sub-project under Study of the Physiological Effects of Cold. Approved 24 Sept. 1942. M.D.F.R.L. Project No. 6-64-12-02-(9).

SHOUTHERDER ENGROCKE HE PERSON SO SOURTHER SHY

Test.

telon M. Canan, Cart., M.C., John L. Grey, Mischenist and

STORE

Medical Department Field Reserve Labourstory Fort Room, Kentucky 16 July 1989

and-project under Stady of the Physiological Effects of Cair. Approved at dept. 19A2. I.D.F.R.L. Project No. 6-64-12-02-(7)-

#### 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 adrenal ectomized and hypophysectomized animals, similarly treated, the liver amino acid oxidase activity was not augmented. Administration of an adrenal cortical extract to normal and adrenal ectomized 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.

Project No. 5-64-12-02 Sub-broject MoFfEL 02-(9)

16 May 1949

#### TOLEDREA

THE INFLUENCE OF CHILD INDOCADES SECRETIONS

IDELEO.

In the course of studios designed to investigate the effects of stream of typotherats, stout and irradistion) on the amino soid oxidese equates of the liver and stimeny, it became necessary to mose the inflicance of the along the level and of certain endorting somethers on the solutions of this engine system. Alterations in the expense of the markers assembly to the markers assembly to the markers assembly to the markers activity.

#### SHOTSE FOR COMOLUST OF

Intrapart tones! administration of an animomous cold administration of the sains sets by the sains and cold an animomous an animomous of the sains and later and indeed, the sains and indeed at the sains and the sains and indeed at the sains and the sains at the sains and the sains and the sains and the sains and the sains at the sains at the sains and the sains and the sains at the sains and the sains at the sains and the sains

the primitive may also include the company of the party of the provide and the

Thyrothese eaching blos colas becauses a cemeda almina besimpted or the chiastal or the chiast

inimportant was found to indibil amino sold outdoor activity of the

The results obtained place indicate that while the activity of the liver spine activity of the liver spine activity, at least partly, can be influenced directly by the mains activity 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 (pituitaryadrenal 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.

Submitted by:

R. N. Cagan, Capt., M.C.

J. L. Gray, Biochemist H. Jensen, Ph.D., Chief Biochemist

Director of Research

Lt. Col., M.C. Commanding

ENGINEE OF THE PARTY OF THE PAR

nt elamine rederance of the Juo beires as of point administration of the contract of the contr

It also is suggested that the possibility of an amino acid to lead to the test to investigated in cases of either liver or deriain anterine (pituitary-adrena) cortes) insufficiencies. It is postulated that amino soid polabolism in patients with liver disease or certain endperine (pituitary-adrenal cortex) deficiencies adapt to wifficiently impained to decrease significantly the rate of disapparament of the amino acids from the blood. If such proved to be the case, the rate of the amino acids from the blood. If such proved to be the case, the index of the degree of the liver or pituite fary-adrenal cortex distinction.

Squaltted by:

B. M. Cagen, Copt., M.C.

J. L. Gray, Blochamiat

Hi, Jensen, Ph.D., Chief Stochenist

Approved Carried Sales

HOLENDE A HOLENDE

++

### 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) 1-amino acid oxidase which specifically deaminates 1-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:

$$R - CH - COOH + \frac{1}{2} O_2 - R - C - COOH + NH_3$$
 $NH_2$ 
 $O$ 

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. EXPERÎMENTAL

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".

<sup>\*</sup> 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 UNTILIBIES OF CERTAIN ENDOCRIES STORESTONS

#### MOITSIMONTEL

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

"antidate", on oan be destinated to form energy producing intermediations" on oan be destinated to form energy producing intermediations "entidates" for first step in the catabolic process is the destination of the catabolic process is the destination of the catabolic step of the energy state and city of the catabolic state of the catabolic s

The activity of amino acid oxiders may be considered a measure of anino acid oxidered. The reaction is as follows:

It may be observed that ements results from this resulton. Is the liver, the same of the same and in the process of transmission. The described of saint saids is the liver is screened by an increase in the ures of the blood. Changes in the level of close ures there are no said this index of the address as the said of close ures the mad on a partial index of the address and chicken activity.

and to deliver and the state of the state of the state of the secretary of the state of the stat

"hite male rate of the Sprague-Dawley strain weighing telement 200-300 grams were employed. They were beinged for 18 hours before the experiment, but were permitted whten. Four groups of saimals were employed; normal, advensicable that the sain out in each of which two saimals were a certies of which two saimals were injected introperationessly with a 10 per cent solution of an engale casein hydrolysate" while one animal was simply plenced with a needle. "On purposes of convenience, the latter procedure will be called "dry needle".

Amigus, propered by Mead Johnson & Company, Evensville, Indiana, Marich to express our appreciation to Dr. Marren 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<sub>L</sub> (3.82 per cent), KCl (1.15 per cent) and made to pH of 7.35 with HCl and Na<sub>2</sub>HPO<sub>L</sub>. 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°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 02 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 propered by cubting out approximately three grams (weighed to the nearest 0.1 of a militarian) of theses from the entired and the these with 6 ml. animals (killed to the nearestant) and by homogenizing the these with 6 ml. of phosphate buffer (pH 7.55) for five minutes. The buffer employed was a modified trabe buffer containing MaCl (0.95 per cent), AgSO, (3.82 per cent), AGI (1.15 per cent) and made to pH of 7.35 with HGI and WapHOL. After homogenization, the minutes. The supermatant was decembed at high severe severe for the engine activity asea, Midney was proceeded in a similar names with approximately 1.2 grams of these being homogenized with 10 ml. of the came the ration of wet to dry relate of the came takes were taken to the ration of the tissue.

-loss galescome sustinct of the constance of the tissue processing of the item of the tissue sustinct of the tissue sustained t

refler neaturestab ear ydivites QAA no noldostat blas calms to desta ed.
eres aleman .noldostat to ents ens trong fi bar to to abotreg
escrittose et tross time intervals and their times processes as times.

In the Marburg manametric technique, employed for the determination of

Main Chamber - 2 al. of buffered squerostant becoments.

Side its - Equations and in Side - 0.2 al. of a O.1 aclass

- Control Clask - C.2 ml. of buffer.

Centrel Well - 0.2 al. of a 10 per cent contant lydraxide

After replacing the sir in the flasks with onywer, they were placed in the water bath and equilibrated for 10 admits at 15.8°C.

After tipping the substrate into the main chamber, remitings were taken over the chamber of the fines per starts. The oxygen uptake is expressed in staroliters of O per gram of wet through-homogenate extracted.

The in vitre and in vivo experiments have been derried out in a similar amount in order to determine the effects of insulin, adrenal cortical extract and epincephrine on the ALO system.

For the state of intracassolarly, (a) I ml. of aqueous acreas cortical extract as the intracassolarly, (a) I ml. of aqueous acreas cortical extract as the cortical entired extract the respective animal curious (Upjoba) was interthe respective animal curious at lance course, or (a) 0.3 ml. of a lance of parties of the came manner as by descending on the extention (Ferma-Davia) was injected in the came manner as by descending on the extentional cortical extract (Upjoba) or 0.05 ml. of alread cortical extract (Upjoba) or 0.1 ml. of adventions as extentions of the careaccentical cortical extract (Upjoba) or 0.1 ml. of adventions as the careaccentical extract (Upjoba) or 0.1 ml. or

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 compared adventile solubion was used in mach of the control and the experimental filests which were propered of berwise as described provided provided of the in with and in with experiments.

Plocd gincese (2), uses (3), saino acida (4) end hessinorit determinations were carried out simultaneously on blood obtained at the time the animals were described.

Animals were advennied tour edge, these animals were kept on a normal diet but for four dage, these animals were kept on a normal diet but given I per oant saline as drinking water. For the subsequent three days, they were taken oil saline and given plain water and for the last choice nours, they were starved. These animals everaged a 25 gras weight loss from operation to day of experiment.

dypophysectomized enterla were operated wis a para-brachest approach and were lengt le to Al days on a milk, chopped meat and bread diet before the expendence. This group had its own control group fed on a ministrative diet. The pypophysectomized enterla averaged 50 grams less in weight than that the time of sacrifice.

Animals were thyrothesicalised and asintained for at least 21 days before being subjected to experimental procedures. Only those enturing that everyone learness in 8.8.8, were used. The everyone learness of 2.8.8.8, were used. The everyone learness of 2.8.8.8, were used. The everyone learness of 2.8.8.8, seems the twenty-lear enturies used was 15.2 per cont (see Table 1).

#### I B.HAT

#### STATES JAMES OF OUR OUTSTONDENTS AT ETAR STATES SERVE

Par Sonare Meter Number Per Hour Antr

unber of Animals & Raduction

B.I BOLTON

16

35:25

Another group to determine whether any offect on the saint action to determine the saint offers were found when the results were from the results were control when the results were compared with those of unoperated control animals.

#### DIESUDEIG OWN BISCUSSIC

#### seshing bina onlea nevil .

"row Table 2, it is obvious that administration of amino acids to normal enimals caused a pronounced increase in the liver amino acid ordeses activity. However, neither advantagecomized 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. Paschkis 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 2 hour but only a negligible increase after 12 hours. A similar type of disagreement

amino acid level on the liver maino acid octore is probably mediated through the pituitery-adrenal system. The nature of the spinished and the pituitery-adrenal contex stimulation ofter amino acid administration the pituitery-adrenal context at incirculation whether the elevated blood amino acid lavel causes a direct or incircut elimilation of the enterior cituitery cannot as yet be answered. Passiciti and Schwener (5) posteleted that the pituitery produces a "protein metabolism hormone", which is religioned on stimulation by a protein meal and may be found in the union union. Thus, they conclude that protein itself furnishes its own trigger mechanism for its metabolism.

vilvios esablæs add do noiseroes isotroo-lineads add lo Joelle ent elect . Esaceta ent in the outre of the lect . Esaceta enter in the outre of the land to the second entered entered

It may be observed in hypophysected animals that the surface of normal middle see the live increase in activity is probably due to the capear in a tivity is probably due to the capear and the plantary growth factors and is in agreement with the capear about the province of the plantary growth factors and is in agreement with the capear and that the province and initially and the temporal produces increased fatty edd by factor and the the produces increased fatty edd of the time in the produces increased to normal animals. These investigators any coefficient the province any element in the formar postulate, for in our experiments the hypophysical and animals aboved an increased animo acts animals and that the typophysical animals aboved an increased animo acts animals and that when a partially partial and deplay of the periods of that when a partially partial and reparation of saterior the periods of observations was given to neghrotherical and animals and that any agreements for alter the administration of an animals and the confidence of t

In thyroidestonies and and each state of the liver which, nowever, were not as promounced as in normal anishing (see Table 2), According to Deans and Greep (8), thyroidestony leads to an strophy of the adrenal context.

This may explain why the increase in liver aniso acid oxidest acids, the chyroidestonies anished a secret as in normal animals. Thyroidestonies control animals is not as great as in normal animals. Thyroidestonical animals showed a degreesed AAO settetty of their liver thyroidestony degreeses liver also activity of Flein (9) who found that thyroidestony degreeses liver AAO activity.

#### E. Kidney Asino Noid Oct lees

online revit est to bond as alles to the results, as loud for the test and online revit est to bond on the state online said online and the results of the r

was also observed in the kidney amino acid oxidase activity of adrenalectomized and hypophysectomized animals. There is no increase after hour
in the adrenalectomized animals but a 59 per cent increase in the amino
acid oxidase activity after le hours. Again, peculiarly, an increase in
activity of 29 per cent occurs in the kidney of hypophysectomized animals
after le 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) hour after injection and subsequent decrease after la 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 adrenal ectomized and hypophysectomized group where the findings are reversed, i.e., the greater increase in the AAO activity is attained at the land 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 12 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, Clucose, 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 2 hour after

entering and typophysococomined animals. There is no increase after i hour second and typophysococomined animals. There is no increase in the administrative animals but a 59 per cent increase in the administrative animals in the administrative animals in the administrative animals anim

Apparently, the collection between the factors included and the filler, leng (1) and length and the titler and the filler, and the filler, and the filler, and the filler, and the construction of the construction and states and the construction of construction of construction of the construction of construction of the construction of construction of construction of the construction of

This view may similarly apply for the edrensisobesised and hypophysectionized group where the findings are reversed, i.e., the greater increase
in the AAO activity is attained at the 1g hour pariod. In these instances,
since the liver ordines activity, is the absence of the activating meanwise
of the pituitary-edrenal cortex system, cannot particks normally in lesering
the enine sold level any lenger, the affect of the continued blood asino
acti elevation menticete itself in the iddney but not uptil a period of the

bealmed occidental to evental end dans (Of) have immediable has lieuwed femous to motivate the test has publicated to the control of the cont

A possible cause for the AAO system is discussed later when the

#### O. Elgod-latin Actd. Cluncas, Ures Hemiltonit (see Table 5)

and all the control of the control of the level and all the control of and the control of the co

injection of the amino acid mixture and declined to 15.4 mg. per cent in 12 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 ½ hour, it was elevated by 12.5 mg. per cent in 12 hours after amino acid injection. Similarly, in normal animals, there was no increase in blood glucose in ½ hour but a 10.8 mg. per cent increase after 1½ 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 ½ hour and were well established in 1½ 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  $l_2^1$  hours (20.5 mg. per cent in  $l_2^1$  hour and 22.3 mg. per cent in  $l_2^1$  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 ( hour, 7.9 mg. per cent; 12 hours, 8.3 mg. per cent) and glucose (2 hour, minus 10.5 mg. per cent; 12 hours, minus 20 mg. per cent) of the adrenalectomized animals after the injection of amino acids are, with the exception of urea at 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 & hour.

In hypophysectomized animals, the amino acid nitrogen level, la 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

in lost ten on the same and mirrors and decimed to 15.4 mg, and ten in the blood amine and be not seen and seed and the respective changes in the bloods and places and lovel can be linked with the respective changes in the unitarity and places and places and the blood. While the antitropen was not greatly can the places and the blood alternation. Statismity, in across each to 16 neuro alternation of the blood alternation of the indicates that the increased the places alternation of antine last the increased formation of among and the tense last tenses and the same and that alternation of among and that alternative processed formation of among and alternative of the case and the characters in 16 normal and make the same places in 16 normal and were well established in 16 normal and action the destrict of the case of the processes in hydrolysates the interest of the case in hydrolysates.

In the adverse entonised entenis, the increese in these transformations seemed to be closed down or inhibited. For example, there is no decrease in amino sold nitrogen blood level in 15 hours (20.5 mg. per cent in 15 hours) after the injection of the amino sold nitrogen levels were distinctly higher than alxeurs. These amino sold nitrogen levels were distinctly higher than those found in normal entenis (16.5 and 15.4 mg. per cent respectively). And adventively, the adrenal entenis enimed animals are unable to scoolarsts properly with the menerally accepted assumption that certain factors secreted by the adrenal cortex encames protein estabolism.

Furthermore, the interested in blood uses (§ hour, 7,9 mg. per comity to the pours, minus 20 mg. per cent) and glacese (§ hour, minus 20 mg. per cent) of the advanced estants of the struct of the sounded control of the struct of the sounded control of the struct of the sounded of the sound the the sound of the sound the sound the sound the sound of the sound of the sound the sound of the sound of the sounded of the sounded the s

In hypophysectomised animals, the amine seld nitrogen level, le hours siter amine sold administration (16.0 mg. per cent) is at the same level found in normal animals similarly treated (see Table 5). The comparable to in the hypophysectomised animals acted alice of the hypophysectomised the normal controls (12.5 mg. per cent). The ability of the hypophysectomised animals to desintate injected amine acted as rate comparable to that observed in the normal animal may be due to the absence of the pituitery growth factor as noted previously. Forthermores, 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 adrenal ectomized 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  $\frac{1}{2}$  hours. Similarly, the urea nitrogen levels, although increased ( $\frac{1}{2}$  hour, 28.1 to 31.7 mg. per cent;  $\frac{1}{2}$  hours to 39.3 mg. per cent) do not quite attain the increase that the normal animals showed in  $\frac{1}{2}$  hour and  $\frac{1}{2}$  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  $\frac{1}{2}$  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 adrenal ectomized 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" hour
previous to being sacrificed, showed a decreased amino acid oxidase activity
in the liver and kidney whem compared with animals sacrificed 1½ 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).

apparently accelerated protein catalogues as shown by the findings that blood area mitrogen is increased 12.5 mg. per cent (45.8 to 59.4) and gineces 15.6 mg. per cent (45.5 to 52.1) during this period (see Table 5). The increases are about the came as those found in normal animals after infection, and are greater than those values from the adventication and respectively values for blood area are increased and for glucose are decreased in the importanteed animals.

By the same orthorts, thyrotelessand animals mention to be the stilling although somewhat retended, to metabolist animo acide. From Table 5, it may be observed that the lowering of the animo acid attrough or as effective but eventually, after injection of acide acide, as prompt or as effective but eventually, after injection of acide acide, the sense animo acid in i hour to acide. Similarly, the unes aftergen levels, although the unes aftergen levels, although the unes after the law on the law of the cont. It is increased do not quite attend in the acide the normal animals animals increased and in these animals increased the hours after the cont) to the bours after the serves are determined animals animals increased to the hours after injection.

It can be noted from Table 5, that the bemateerit is increased about 5 to 8 per cent in the injected normal animals. This finding may be explained by the relative debydestion coursed by the transfer of water from the hypotenic anvironment of the vascular compertment into the hypotenic environment of the peritoneal cavity into which a 10 per cent solution of mains solds had been injected. It should also be noted that the siredalsolucionistic showed a greater increase in newatteerit (3 to 12 per cent) after injection, then didnormals.

#### D. General Cheervallone

From Table 1, to the party were pleased with a "dry needle" a now of providing a coloring actification only were pleased with a "dry needle" a now of providing to being accrification should be a decreased and no said oxidate activity in the liver and indney when domeane with animals accrificated 1, home after animals accrificated the decrease was not manifested in advension to activity levels (Table 5), one may accommend that the secretarian from the advension and provide the translation for the translated decrease in amine and oxidate activity has responding to the translated decrease in anima and in the secretarian according to the indicatory of according only pure crystalline apparatus and according to a solution and according to the indicatory of secretarian and only for 15 to 20 almites. This difference is elections the to the presence of antioxidants in the commendate activities.

It is also describe that the above mentioned inhibition any be due to the setion of insulin on the AAC. The increased blood auger levels eased to the setion of insulin continuity is an increased insulin secretary. Although dertain investigators (15,16) have been able to show insulin inhibition of this ename, our technique was unable to demonstrate this effect 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 piutitary-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.

#### SUMMER AND CONCLUSIONS

- the favel blos cains book relating blood amine acid lavel and content of anima and content to the content of anima and the ideas of rate.
- 2. Administration of casein hydrolysate to normal animals produces an impresse in the amino acid ominase setivity of the liver and kidney in these animals.
- -cute to berimistration of casein hydrolysake to adrended think to be acted as the color of the color of the color of the colors of the colors
  - A. Adrenal cortical extract accelerates the activity of this engine in the liver in vitro and in vivo and in the kidney is vitro.
- 7. The plutitary-advenal cortex complex mediates the character the conserved after acceleration of amino acid oxidese activity of the liver observed after amino acid administration.
- 6. The nature of the nechanism of the pibultary-adrenal cortex stimi-
  - After our to the prophyse consists animals show increased animals of the prowin factor of the picutary.
  - 1. Thyroidestonded animals show a desiresed amino acid oxidese setivity of the kidney. Administration of smino acids stimulates the liver oxidese.
    - 9. Epinephrine inhibite amine sold exidens spinitr of the liver.
  - 10. The saint sold oridess sorivity of the liver and the kiney may respect the three similarity to cortain endocrine stimuli. However, it appears that the blood andno sold level may influence the kidney exidess activity directly but not that of the liver.

#### EROTTA GREATMONTE TONS

on order to complete the concept of the effect of endocrines on the entries, and to confident, it is suggested that forther sork be done with hypophyseotomized and castrated anihals. These experiments should be corried out similarly to those described in this report.

to experience and no nollegitewint as the description of the during to be experimented to be experimented to be experimented to the experiment of the experiment of the control out on normal breather that the control of the control

(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.

#### VI. BIBLIOGRAPHY

- 1. Blanchard, M., et al. 1-Amino Acid oxidase of animal tissue.

  J. Biol. Chem. 155: 421, 1944.
- 2. Somogyi, M. D. Determination of blood sugar. J. Biol. Chem. 106: 69, 1945.
- 3. Van Slyke, D. D. and G. E. Cullen. Determination of urea by the urease method. J. Biol. Chem. 24: 117, 1916.
- 4. Frame, E. G., J. A. Russell and A. E. Wilhelmi. The colorimetric estimation of amino nitrogen in blood. J. Biol. Chem. 149: 255, 1943.
- 5. Paschkis, K. E. and A. Schwoner. Output of protein metabolism hormone of pituitary anterior lobe. .Endocrinology. <u>26</u>: 117, 1940.
- 6. Szego, C. M. and A. White. The influence of growth hormone on fasting metabolism. Endocrinology. 44: 150, 1949.
- 7. Russell, J. A. and M. Cappiello. The effects of pituitary growth hormone on the metabolism of administered amino acids in nephrectomized rats. Endocrinology. 44: 333, 1949.
- 8. Deane, H. W. and R. O. Greep. Cytochemical study of adrenal cortex in hypo- and hyperthyroidism. Endocrinology. 41: 243, 1947.
- 9. Klein, J. R. Effect of thyroid feeding and thyroidectomy on the oxidation of amino acids by rat kidney and liver. J. Biol. Chem. 128: 659, 1939.
- 10. Russell, J. A. and A. E. Wilhelmi. Metabolism of kidney tissue in adrenalectomized rat. J. Biol. Chem. 137: 713, 1941.
- 11. Lang, K. The effect of the level of protein intake on the activity of oxidation enzymes in the organs. Klin. Wchnschr. 24-25/55-56: 868, 1947.
- 12. Kochakian, C. D. and M. N. Bartlett. The effect of crystalline adrenal cortical steroids, dl-thyroxine, and epinephrine on the alkaline and acid phosphatases and arginase of the liver and kidney of the normal adult rat. J. Biol. Chem. 176: 243, 1948.
- 13. Lotspeich, W. D. and R. F. Pitts. The role of amino acids in the renal tubular secretion of ammonia. J. Biol. Chem. 168: 611, 1947.

Ingophysic-strend during the functions sight be well-dently important describes a sight from the decrease of amino acids from the blood. If such proved to be the case, the rate of removal of functions we man acids may serve as an index of ethner liver or bypochysis-schemal courter functions.

#### DYLENGER JY

- 1. Blol. Ches. 155: 421, 1944.
- 2. Somogyi, M. D. Determination of blood sugar. J. Simi. Chem. 106: 59, 1945.
- 3. Van Slyke, D. D. and O. E. Odlien. Determination of ures by the messes method, J. Elel. Chem. 24: 117, 1916.
- i, Frame, B. C. J. A. Russoll and A. E. Wilhelmi. The colorimetric catinetics of amino siprogen in blood. J. Biol. Chem. 149: 255, 1963.
  - 5. Paschida, K. E. and A. Schwoner. Output of protein metabolism hormone of pibulcary anterior lobe. Andoorinology. Zb. 117, 1940.
  - 5. Seego, C. M. and A. White. The infidence of growth hormone on ... fasting metabolism. Madocrinology, id: 150, 1949.
- 7. Museall, J. A. end M. Cappiello. The effects of picultary growth hormone on the methodiles of administrated amino solds in nephreo-tenius rate. Esteminology, Mar 233, 1929.
- S. Desne, H. W. and R. O. Green: Cytochemical study of advenal cortex in ayro- and hyperthymoddiam. Endogrinology. 41: 263, 1947.
- 9. Klein, J. R. Effect of thyrold feeding and thyroldestony on the oxidation of amino solds by ret hidney and liver. J. Siel. Chem. 122: 659, 1939.
- 10. Bussell, J. A. and A. E. Wilhelmi. Metabolism of bidney bissue in cdrenelactomised ret. J. Riol. Chem. 177: 713, 1941.
- 11. Leng, K. The effect of the Lovel of protein intere on the activity of inidation engage in the organs. Klin. Mohnschr. 21-25/55-551 868, 1917.
  - 12. Mornstian, C. P. and M. M. Bertlett. The effect of orystalline advenet cortical aterator, cl-thyroxine, and epissphrine on the alkaline and acta phosphoteses and arginese of the liver and kidney of the nermal adult ret. J. Biol. Chem. 176: 243, 1966
  - 13. Lotspeich, W. D. and H. F. Pitts. The role of anino solds in the renal tubular secretion of amonia. V. Mol. Crem. 158: 511, 1947.

- 14. Bliss, S. Increased excretion of urinary ammonia in dog following intravenous injection of both natural and unnatural forms of certain amino acids. J. Biol. Chem. 137: 217, 1941.
- 15. Bach, S. J. and E. G. Holmes. The effect of insulin on carbohydrate formation in the liver. Biochem. J. 31: 89, 1937.
- 16. Stadie, W. C., F. D. W. Lukens and J. A. Zapp, Jr. Effect of insulin upon urea formation, carbohydrate synthesis, and respiration of liver of normal and diabetic animals. J. Biol. Chem. 132: 393, 1940.

- - Engly 5. J. and F. G. Holgne. The effect of insulfa an carcoignizate formulation in the liver. Blochen. J. 11: 39, 1987J
- Statio, C. D. F. D. T. Indens and J. A. Repp. Jr. Effect of insulin upon more d'orestion, combande synthesses, and respiretion of liver of doront and disbetic entrain. J. Biol. Chem. 1321 793, 1910.

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 Cram of Tissue Homogenate Extract

§	gen in commence and commence and commence and commence and	CONTRACTOR OF CO
Thyroidectomized	9 = 33% 3 = 11%	-43 = -14%. -93 = -27%
Hypophysectomized Thyroidectomized	-31 = -38%	66 = 29%
Adrenalectomized	0 = 0%	-24 = -9% 107 = 59%
Normals	14 = 50%	119 = 89%
Minutes After Injection	30	30
Tissue	LIVER	KIDNET

STEELS OF

	2 m		
			1
			1
			1
			1
			1
			1
			1
			1
			1
			1
			a Europe de la constante de la
			a.E
			a Europe de la constante de la
			a.E
			a.E
			a Europe de la constante de la
			a Europe de la constante de la
			a Europe de la constante de la
			a.Lunalli
			a Europe de la constante de la
			a Europe de la constante de la
			Tolketran Boreste National
			Market Standin
			Tolpoptum Sometin

TABLE 3

EFFECT OF CERTAIN HORMONES ON AMINO ACID OXIDASE OF LIVER

IN VITRO AND IN VIVO

				No. of Concession, Name of Street, or other Designation, or other					
	MICROLITERS OF OXYGEN UPTAKE								
	Figures in Parentheses Equal Number of Animals								
	Buffer	Alanine	Difference (AAO)	Control (AAO)					
ADRENAL CORTICAL EXTRACT									
In Vitro	300	425	125 ± 18 (6)	59 ± 8 (14)					
In Vivo (Adrenalectomized)	301	343	42 ± 3 (2)	21 ± 3 (2)					
In Vivo (Normals)	219	279	60 ± 4 (4)	27.5 ± 1.5 (2)					
ADRENALIN									
In Vitro	232	257	25 ± 2.6 (5)	59 ± 8 (14)					
In Vivo	230	253	23 ± 6 (4)	41 ± 4.5(20)					
INSULIN									
In Vitro	242	274	32 ± 9 (13)	59 ± 8 (14)					
In Vivo	203	246	43 ± 4 (8)	57 ± 9 (4)					
NO. OF THE PARTY O	the state of the s	water to suppose the months of the same	the state of the s	No. of the Control of					

SERVICE OF OTHERMAN HORSINGS ON SERVICE OF THE SERV

TOTAL RESPIRATION AND AMINO ACID OXIDASE ACTIVITY OF CONTROL AMIC AND EXPERIMENTAL ANDMALS Microliters of Oxygen Uptake Fer Gram of Homogenized Tissue Extract

	tomized	Oxidase	1	18 # 2,0 (4)	27 \$ 3.1 (4)	(7) 81 # 116	350 ± 21 (4)		27 \$ 3.0 (8)	30 \$ 4.3 (8)		1 44	257 4 5 (8)
	Thyroldectomized	Alanine	1	223	228	527	551		246	269	5	(94	157
		Buffer	1	205	201	164	202		219	239	100	FA.	194
Animals	ctomized	Oxidase	1	-	81 4 9 (5)		231 \$ 15 (5)		1	50 ± 5.7 (5)			297 ± 30 (9)
Mumber of A	Hypophysectomized	Buffer Alanine	1	,	285	1	717	Amigen Intraperitoneally	1	250			7.10
Equal Num	H	Buffer	1	1	20%	1.	181	Intraper	1	200			173
in Parentheses E	contred	Oxidase Activity	1	29 4 6.1 (9)	27 \$ 5.1 (5)	265 ± 17 (6)	182 ± 14 (5)	of 5 cc. Amigen	24 \$ 4.3 (19)	28 \$ 4.0 (7)		04	289 # 20 (7)
Figures i	Adrenalectomized	Alanine	1	226	198	797	380	ection o	249	207		37	917
1		Buffer	1	197	17.1	197	198	ifter Inj	225	179		107	187
	Normale	Oxidase	(01) 8 7 65	27 ± 3,3 (8)	(6) 7.7 \$ 07	135 ± 13.5 (6)	252 # 21 (13)	As Above After Injection	(1) \$ 4.5 (20)	55 ‡ 3.1 (21)	(OU) 70 T (30	H	270 1 31 (28)
	Nors	Alanine	270	235	275	377	7.15		267	279	8/		11.7
		Buffer	211	208	235	506	220		226	777	S S	-	204
	Time	Dry Needle"	0	30	06	30	06		30	96	Ç		3
	Tissue			LIVER		KIDNEY			aght.	waarn		KEDIEY	

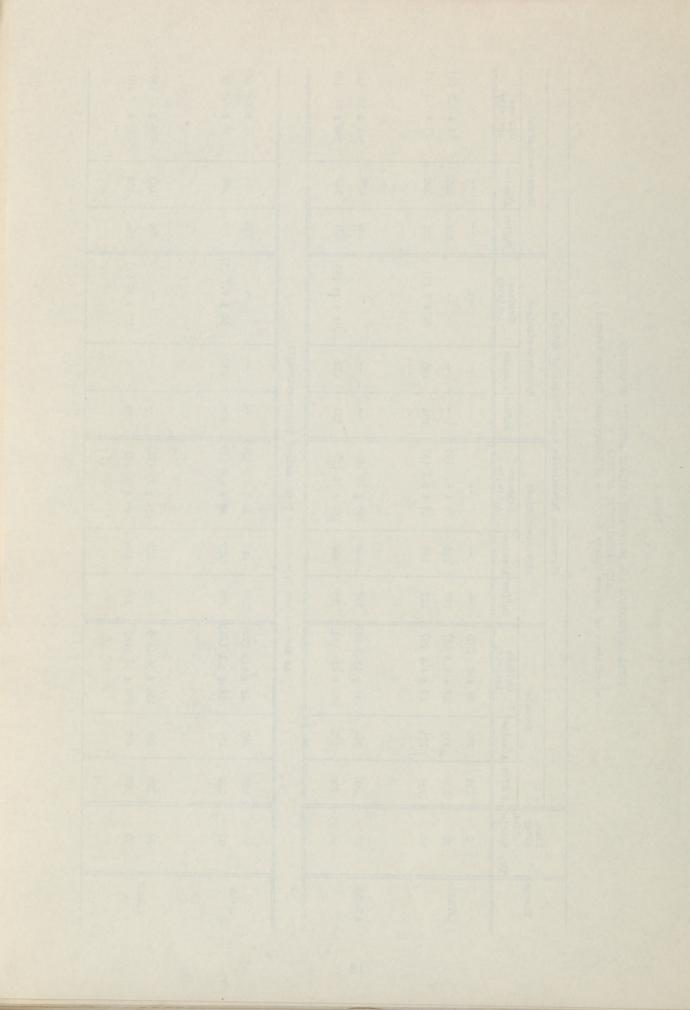


TABLE 5

CHANGES IN BLOOD CONSTITUENTS DURING EXPERIMENTAL PROCEDURES

	Thyroidectomized	Injected	55.0 \$ 2.0(6)	21.7 ± 3.1(6)	39.7 ± 1.8(7)	77.6 ± 0.8(8)
	Thyroide	Control	49.4 \$ 1.5(4)	11.9 \$ .9(3)	28.1 ± 0.9(3)	58.5 ± 3.0(3) 73.7 ± 1.0(4) 59.2 ± 2.5(4)
Animals	tomized	Injected	1 1	16.0 \$ 1.2(7)	29.4 \$ 1.6(6)	62,1 \$ 3.6(4)
Number of	Hypophysectomized	Control	1 1	12.5 ± 0.5(4)	46.8 ± 2.2(3)	45.5 ± 1.5(2)
Figures in Farentheses Equal	drenalectomized  ol Injected	60.0 \$ 1.4(5)	20.5 \$ 1.3(13)	48.7 \$ 3,1(4)	26.0 ± 6.1(5) 27.8 ± 1.1(5)	
Figures in	Adrenal	Control	55.5 ± 1.3(3)	12.6 ± 1.3 (7)	40.8 \$ 2.0(3)	46.5 # 2.3(3)
	- 100	Injected	55.4 \$ 1.0(8)	18.5 # 1.6(8)	23.6 ± 1.8(9)	 66.6 # 2.4(8) 76.8 # 2.7(10)
	Normals	Control	52.5 \$ 0.6(4)	12.1 \$ 1.3(5)	18.9 \$ 0.8(4)	68.2 ± 0.2(3) 72.9 ± 2.1(3) 66.0 ± 3.0(5)
	After	Injection	30	30	30	30 0
	Blood	Constituent	HEMATOGRIT	AMINO ACIDS  ME. %  Amino Acid  N2	UREA Mg. A Urea Ng	GLUCOSE

<b>一个一个一个一个一个一个一个一个一个一个一个一个一个一个一个一个一个一个一个</b>			
<b>一种意思</b>			
	the the		
		Carting States	