AN ABSTRACT OF THE THESIS OF

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The specific problem studied was the effect of adrenal insufficiency upon the deamination mechanism in the white rat. Adult rats of the Evans-Wister strain, weighing from 140 to 220 grams were used. The animals were adrenalectomized according to the recent standard procedure using ether as the anesthetic. Adrenalectomized rats were maintained on Rubin-Krick solution which is an approximately isotonic solution of sodium chloride, potassium chloride, calcium chloride and magnesium chloride. They stayed in good condition on this solution only; a few animals died when not being run on an experiment. However, many of the adrenalectomized animals died during an experiment. All adrenalectomized animals were autopsied to check the completion of the removal of the adrenals. Normal rats were used as controls throughout the study.

At the beginning of the study it was assumed that if adrenal insufficiency decreased the rate of deamination, then the percentage of urea-ammonia nitrogen of the total nitrogen being excreted in the urine would be lowered. However, in the discussion two objections to this assumption were brought up.

The urea-ammonia nitrogen was determined by the Van Slyke - Cullen urease aeration method. The total nitrogen was determined by the Kjeldahl method. The urine was collected in separatory funnels attached to large glass funnels upon which the small experimental cages were placed. The feces were separated by a small wire cone placed at the apex of the large funnel. The collected urine was diluted to 100 ml by rinsing down the sides of the large funnel in order to make the collection quantitative. All calculations were based upon this 100 ml.

The different substances were fed by means of a stomach tube. Quantitative amounts based upon the surface area were measured by means of a calibrated syringe to which the stomach tube was attached. Concentrated solutions or suspensions of the different solutions were used. The volume of the stomach tubed material was kept as low as possible. Glucose, dl-alanine, fresh eggwhite, l-cysteine, d-arginine monohydrochloride and dl-glutamic acid were fed individually

in different experiments after the animals fasted for about 15 hours. In one set of experiments the rats were placed directly upon the experimental cages without any preliminary fasting. Essentially these animals were fed, ad libidum, their normal diet.

Only in the last set of experiments was there any real drop in the percentage of urea-ammonia nitrogen. In all of the other experiments the adrenalectomized animals showed almost identically the same percentage of urea-ammonia nitrogen as the normal animals.

No definite conclusions were drawn as to the effects of adrenalectomy upon the mechanism of deamination, but the results apparently agree with the recent work of Evans.

THE INFLUENCE OF ADRENALECTOMY ON DEAMINATION

by

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"But a speculatively inclined student who concerns himself with second-hand knowledge will, of course, easily succumb to the temptation to let his imagination get the better of his critical sense."

E. Nordenskiöld
The History of Biology

THE INFLUENCE OF ADRENALECTOMY ON DEAMINATION

INTRODUCTION

When a new vitamin, enzyme, gland or similar principle is discovered, many theories are proposed to explain its function. The adrenals have suffered this fate to a great extent and they are still being subjected to many theories several hundred years after their discovery.

The purpose of this study is to attempt to clear up one of these theories, namely: Does the adrenal cortex influence the rate of deamination of amino acids? The previous work on this particular problem is meager and apparently conflicting. Evans (25) using a different experimental technique than that used in this work, obtained negative results. Using kidney tissue slices in the Warburg apparatus, Russell and Wilhelmi (67) obtained results that can be interpreted as indicating that the adrenal cortex increases the rate of deamination of amino acids. The work of Samuels, Butts, Schott and Ball (69), who worked with liver glycogen formation, can be interpreted in the same way.

The experimental work in this study consisted of feeding normal and adrenalectomized rats different substances containing amino nitrogen and following the rate

of deamination by analysis of the urine nitrogen. The total nitrogen was determined by the Kjeldahl method and the urea-ammonia nitrogen by the urease and aeration method of Van Slyke and Cullem.

HISTORICAL

EARLY HISTORY. The history of the adrenals is an interesting one. It involves brilliant researches in all phases of the natural sciences, especially in biochemistry, physiology and pathology.

Before 1855, only the anatomy of the adrenals was known, their physiology was a blank space in human knowledge. It is true that there were theories as to the function of the adrenals, but these theories had no foundation and they clouded the picture rather than cleared it. Experimental methods were yet to be devised.

In 1855 an English physician by the name of Thomas Addison described (1) the disease of the adrenals which now bears his name. Addison's disease had been observed before this time but its relation to the adrenals was unknown and it was confused with several other diseases. Addison's classical description of this syndrome was so complete that only a few minor details have been added. However, the chemistry and control of Addison's disease is just now begining to be understood.

When Brown-Sequard read Addison's publication, he was prompted to adrenalectomize laboratory animals in 1855 (17) and thus he became the first worker to do experimental work with the adrenal glands. His adrenalectomized

animals died in the relatively short time of a few hours to several days after the operation. Brown-Sequard promptly postulated that the adrenals were essential for life. Other workers often got their adrenalectomized animals to live for long periods of time. So they objected to Brown-Sequard's theory and the resulting controversy as to how long an animal can survive after adrenalectomy is still raging after 86 years. The truth is slowly being brought to light and the controversy is almost over.

From 1856 until the turn of the century the comparative anatomy of the adrenals was worked out quite well. Also the histology of the adrenals was investigated and the medulla distinguished from the cortex. Many of the common laboratory animals were adrenalectomized. It was soon discovered that some species could live for several days or longer, while other species could live only for a few hours after adrenalectomy. For a long time it was believed that the rat could live without the adrenals. In some of the vertebrates outside the mammals the medulla is found to be completely separated from the cortex. By removal of the medulla only, or the cortex only, the conclusion was soon reached that the cortex was essential for life but not the medulla. This conclusion is held at present.

In 1894 a powerful pressor substance was discovered in the adrenal medulla by Oliver and Schaefer (60). Due to the relatively high concentration of this pressor substance in the medulla, it was isolated, crystallized, identified, and synthesized within a decade following its discovery. This substance goes by several names: the two most common being epinephrine and adrenaline (a trade name). Adrenaline found a permanent place in therapy almost immediately because of its remarkable physiological effects. The functional importance of epinephrine to the organism is still a matter of speculation although several functions have been assigned to it.

The adrenal cortex was almost completely neglected until the role of the medulla and adrenaline had been analyzed. The physiologists knowing that the cortex and not the medulla was essential for life realized that the physiology of the former should be investigated. So the all out assault upon the adrenal cortex started; an assault which is progressing at a terrific rate. At the present time approximately 200 original papers are appearing per year. These papers except for a few are directly related to the cortex.

It was not until the last decade, when two important discoveries were made, that the real progress was made. One was a thorough biochemistric investigation of the

blood and urine of adrenalectomized animals and patients with Addison's disease. The second was the preparation of active cortex extracts. Most of the investigations since 1930 have corresponded with either one or both of these steps. The result is that some of the more important features of Addison's disease and adrenal cortex insufficiency are now quite well understood. The intermediary metabolism is understood only to a slight degree. This thesis deals with this phase of the subject.

In this paper, the term "Addison's disease" means a syndrome existing in human beings, caused by a deficiency of the adrenal cortical hormones in contradistinction to the term "adrenal insufficiency". This is defined as the syndrome existing in adrenalectomized animals caused by a deficiency of the adrenal cortex hormones. The term "cortin" is used to designate the <u>whole</u> extract of the cortex and not just one of the fractions such as the "amorphous fraction".

There is no strict order in which to discuss this subject. The different phases are so closely interwoven that it would be impossible to write about one phase and ignore the rest. Since Addison's disease is the real historical beginning of the study of the adrenals we will begin with it.

ADDISON'S DISEASE. In order to have a better background for the problem involved, it is necessary to know something about Addison's disease andadrenal insufficiency. Addison's disease is a relatively rare condition. In the United States, up to 1936, about 300 to 400 persons died annually from this disease (33). In some countries this disease appears to a greater extent than in others (49). From 75 to 80 per cent of the cases have been found to be caused by tuberculosis of the adrenal cortex. The prognosis is always unfavorable and death usually occurs from 6 months to 4 years after the outset of the disease. By the use of salt and cortex extract therapy, which has come into use in recent years, death due to Addison's disease can usually be postponed indefinitely. However, secondary complications often occur which cause the death of the patient.

The patient, in most cases, slowly looses his health and becomes languid and weak. He is indisposed to either mental or bodily exertion. His appetite is impaired or entirely lost. The body wastes without presenting the dry and shrivelled skin and extreme emaciation usually attendant on protracted malignant diseases. Pain and uneasiness from the region of the stomach is suffered periodically and vomiting is not uncommon. A pigmentation of the skin

is generally present. This pigmentation has never been observed in animals suffering from adrenal insufficiency, although the other symptoms of Addison's disease occur. The patient succumbs under a gradually increasing cachexia, and frequently with stormy terminal manifestations.

The metabolism of Addison's disease will be discussed under the metabolism of the adrenal cortex. Much literature has accumulated on this disease and the reader is referred to Rowntree and Snell (65), or to some of the later works (33,46,46,51,53,73) for a more complete description.

The etiology of Addison's disease has been the subject of much speculation. Thomas Addison originally reported that the cause of this disease was tuberculosis of the adrenals. The tuberculosis bacillus was not discovered until some years later. Addison's conclusions were based upon the gross examination of the diseased glands. Approximately 75% of the autopsied victims of Addison's disease show that the adrenals actually are invaded by the bacillus of tuberculosis. If Addison had a larger number of cases he, no doubt, would have discovered this fact. And what about the other 25%? This is where the speculation comes in. In some cancer plays a part; in others, infections of one type or another, and in some cases there are <u>no apparent lesions</u>.

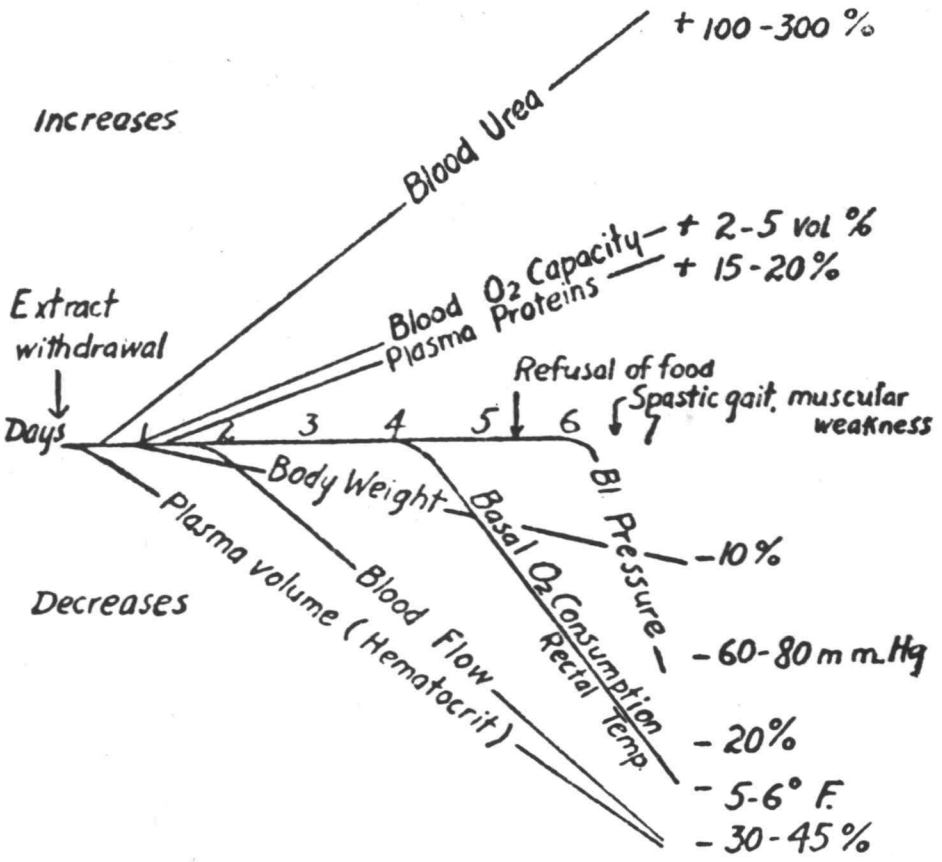
ADRENAL INSUFFICIENCY. To study the physiology of the adrenal cortex from Addison's disease would be a very difficult task because of the lack of controls and the secondary complications often found. To study uncomplicated adrenal insufficiency, animals are adrenalectomized and the resulting syndrome is carefully watched. Chemical analyses of the blood and urine are made often in the course of the experimentally induced disease. Cortin, different fractions of cortin, inorganic salts, and other substances are administered to either normal or adrenalectomized animals and their effects noted. Principally studied are the changes in the chemical composition of the blood and urine. The interrelationship of the adrenal glands to the other endocrine glands can not be easily studied, since interpretation of the results is very difficult because of the extremely complicated situation and deficiency in knowledge.

Grollman in his book on the adrenals (33) gives an excellent description of the syndrome following adrenalectomy. After recovery from the anesthesia, the animal is alert, eats and drinks, and is apparently in good condition. The chemistry of the blood is apparently unchanged and the blood pressure is normal. Gradually, however, symptoms of adrenal insufficiency begin to appear.

The animal becomes apathetic, refuses food, and may vomit. The muscular movements become slow and uncertain with increasing weakness of the hind legs. The animal's gait becomes unsteady and eventually it lies prostrate. As the insufficiency progresses, the body temperature falls in warm-blooded animals, the skin becomes cold, and the mucosae turn pale. Muscular twitches and convulsions are common. The respiration is first rapid, then slow. Anuria may be present. Death occures in comma, with respiratory paralysis while the heart is still beating.

One can see adrenal insufficiency is very similar to Addison's disease with its secondary complications. The biochemistry of the two conditions, as we shall see, is almost identical.

PHYSIOLOGY OF THE ADRENAL CORTEX. In 1933 Loeb and his co-workers (51) reported their results on the effects of adrenalectomy and the changes in the chemical composition of blood and urine. The experimental animals were dogs. Their results can best be summarized by means of the graph shown on page 11. It is advisable to study this graph carefully since it contains much information which would require many pages of reading material to convey the same idea.



From Loeb (51) and Best and Taylor (13).

As one looks at the curves, several questions come to mind; How soon after adrenalectomy do the effects of adrenal insufficiency become evident? Is there any storage of the hormones of the adrenal cortex? Which abnormalities are primary and which are secondary? We have been speaking of the hormones of the adrenal cortex but is there more than one hormone actually produced? Does the kidney function normally during this syndrome? To answer all of these questions is impossible at present.

Raab (62), working principally with heart muscle, found some evidence of storage of the cortical hormones. He has also done a considerable amount of research on the varying concentrations of the adrenal hormones in the blood stream (63). All of his work is based upon the m method of analysis which is subject to unfavorable critisism. Following adrenalectomy or the removal of cortin therapy, the effects of adrenal insufficiency, according to the graph, become evident almost immediately. Some of these effects, no doubt, are caused by the operation itself. At any rate, if there is any storage of the cortex hormones, the amount stored is small. A considerable amount of work has been done in order to connect, directly or indirectly, traumic shock with a temporary deficiency of the cortical hormone. Some success has been attained but a great deal of more research is required. See Wiggers (80)

for a recent review on this subject.

As the adrenal deficiency develops, both mineral and carbohydrate metabolism become abnormal. There is some experimental evidence (24,52) that the mineral metabolism is the primary one to be effected. Carbohydrate metabolism being affected, only after the mineral metabolism has been affected. Adrenalectomized animals can be maintained for long periods of time by the use of salt therapy, (to be discussed later). The liver glycogen and blood sugar in this treatment remain at normal levels. If the salt therapy is stopped and large quantities of glucose are fed, the symptoms of adrenal insufficiency develop rapidly, including a drop in the blood sugar and liver glycogen. Some authors claim that 'something' which is believed to control both mineral and carbohydrate metabolism is damaged or destroyed (52).

Insulin also appears to have something to do with the rapid decrease in blood sugar (22,37,40,54). The cortex hormones, especially the "sodium factor", apparently posess anti-insulin properties. Therefore, in their absence, the insulin can act with greater vigor and thus cause a rapid decrease in blood sugar. This is a good argument, but for the fact that salt therapy will keep the concentrations of blood sugar normal. Mineral metabolism must be brought into the picture. Why salt therapy main-

tains the blood sugar level is, of course, unknown. Possibly it is connected with the permeability of the cell membrane (8,19,23,56,59,71). In some experiments it has been found that the rate of diffusion is increased for sodium ions as the insufficiency develops. The hemoconcentration seen in adrenalectomized animals is believed to be due, in part at least, to this change in the rate of diffusion through membranes (38).

Harrison and Darrow (36) did some interesting work on renal function and adrenal insufficiency. They concluded that the renals are upset to some extent, such as a decrease in selective reabsorption of sodium ions in the tubules and an increased reabsorption of the potassium. The decrease in urea excretion is due mainly to the decrease in the blood pressure because of the hemoconcentration. Salt therapy is believed to have some of its beneficial effects because it increases the blood volume, which in turn results in an increase in the blood pressure. In this way the glomerulus filtration is increased and more potassium as well as urea, creatinine and uric acid are excreted. The increased potassium concentrations in the blood produce a toxic condition (27,74,78). When adrenalectomized animals are maintained upon salt therapy, the kidneys apparently function normally to a limited extent. The increased salt intake seems to keep the blood

picture normal by a "flushing out" action through the kidneys (5,7).

A large number of steroids have been isolated from the adrenal cortex. Kendall (42,43) and Reichstein (64) working independently, were the first to do this. Verzar (77) lists some 24 steroids that are supposed to have been isolated from the adrenals, or believed present in the cortex. Pfiffner, in his recent review on the adrenal cortical hormones, (61) lists five active components which have a known structural formula. They are corticosterone, dehydrocorticosterone, 17-hydroxycorticosterone, 17-hydroxydehydrocorticosterone and desoxycorticosterone. A sixth fraction, 17-hydroxydesoxycorticosterone, has not been sufficiently investigated to be classed as active, although all indications show that it is active. The main activity still lies in the amorphous fraction. The best evidence of the polyhormone theory of the adrenal cortex is that each isolated active fraction generally has a slightly different physiological activity. This is why animals maintained on cortin do much better than those kept on corticosterone or some other fraction. A terrific amount of work has been done on the physiological activity of these fractions. For reviews see (45,61). It must be remembered that in the process of isolation of these,

different fractions may have been broken off or rearranged from larger molecules and that the cortex actually produce one hormone.

EXPERIMENTAL METHODS

STOCK ANIMAL AND DIET. The animals used in this study were adult male and female rats of the Evans-Wister strain from 3 to 6 months of age from the stock colony. The animals weighed from 140 to 220 g. and litter mates were used wherever possible. At present the stock animals are kept on the diet used by Dr. J. R. Haag of the Oregon State Agricultural Experiment Station. It has the following composition:

	Percent	100 lbs.
Whole yellow corn meal	71.0	100 lbs.
Linseed oil meal	15.0	20.9
Powdered skimmed milk	10.0	13.9
Ground alfalfa leaves	2.0	2.8
Sodium chloride	0.5	0.7
Calcium carbonate	0.5	0.7
Irradiated yeast	1.0	1.4

This diet must be frequently supplemented with fresh greens. Fresh liver is strongly recommended for the breeding stock and nursing mothers, but is not added to the diet of the stock animals. Adrenalectomized animals were also kept on this diet. Rubin-Krick solution was used in the place of drinking water.

ADRENALECTOMY. Ether was used as the anesthetic, and administered at such a rate so that the animal was just kept "anesthetized". The rat was placed in a large bell jar with a layer of cotton saturated with ether in the bottom. The bell jar, of course, was kept covered. As soon as the animal stopped moving, it was removed. The ether then being administered by the use of a vial, 30mm by 90mm, with a layer of cotton also saturated with ether in the bottom. The rate of administration being controlled by placing the vial at different distances from the rats nostrils. The total length of time the rats were 'under' varied from 20 to 45 minutes. Grollman in his earlier work (29) strongly recommends the use of intraperitoneally injucted barbiturates, but in his recent work (35) he used ether. Ether was used because of its ease of administration, and it appeared to be equal to any anesthetic for adrenalectomy providing the animal is placed on a high salt diet immediately after the operation.

A combination of the most recent techniques described by Grollman (35) and Richter (82) was used. Bilateral incisions were made. The hair was cut short and a vertical incision, about 15mm in length, was made by cutting a short slit in the skin. Then inserting the tips of the

closed sissors and forcing the tips apart, the skin was spread apart. The abdominal wall was opened in the same manner. The adrenal gland was next brought to the incision by the use of a pair of small curved forceps. The adrenal with a small amount of the surrounding fatty tissue was torn from the rest of the fatty tissue with the use of two forceps. Great care was taken to prevent rupturing the adrenal. (If the gland was broken inthe process of removal, the animal was discarded because of the great possibility of the formation of accessory adrenal bodies.) The animal was then sewed up with silk thread. Wound clips were used on some of the animals, but the incisions so treated, did not heal as fast nor as well. They were, therefore, discontinued. "Semi" aseptic conditions were maintained during the operation and only one animal was known to become infected.

MAINTENANCE OF ADRENALECTOMIZED ANIMALS. Adrenalectomized rats maintained on an ordinary diet, die in about 6 to 10 days depending upon the condition of the animal, the temperature of the room, the anesthetic used, and many other factors. Loeb (51) noted that the blood sodium rapidly decreased and the urine sodium increased as the insufficiency developed. He, therefore, came to the conclusiom, that perhaps the survival time could be increased by increasing the amount of sodium in the diet. His

conclusions were correct; the survival period was increased almost indefinitely.

In 1934 Rubin and Krick (66) made a through investigation of the increase in the survival period and found that a mixture of sodium chloride, potassium chloride, magnesium chloride, and calcium chloride had the most beneficial effects. This solution is now known as Rubin-Krick solution; it has the following composition:

Tap water	20 liters
Sodium chloride	140 g
Potassium chloride	7 g
Magnesium chloride	3 g
Calcium chloride	6 g

The solution given in the place of drinking water was the one used in this study. Many investigations into the relationship of the survival period and salt therapy have been made since the classical paper of Loeb (51). Some of the more recent studies are (4,6,21,30,57,58,79). All of these investigators come to the same general conclusions, but they differ someshat in minor points. For instance, how much potassium should be given, or should part of the sodium chloride be replaced by sodium bicarbonate?

Why does this salt therapy have such a beneficial effect? Just what is the mechanism of the reaction? The answers to these questions are unknown at present. There

have been answers given but they are poor theories and fail to stand up under experimentation. The author thinks that these questions will have to be answered before much progress can be made in the more complicated intermediary metabolism of protein, fat and carbohydrate metabolism in adrenal insufficiency.

If the adrenalectomized rats are to live for any great length of time, the temperature of the room must be kept at approximately 80 degrees F. The heat center seems to be disturbed in some manner(75). The temperature of the room was held close to 80 degrees by the use of thremostatically controlled auxillary electrical heaters with fans. An automatic temperature recorder was used for a week during the coldest part of the winter and the temperature was found to remain fairly constant.

The rats were maintained for at least 10 days after adrenalectomy before they were put on an experimental feeding.

FASTING AND STOMACH TUBING. Before the animals were fed on an experimental diet, e.g. dl-alanine, they were fasted from 12 to 24 hours (depending upon the experiment). When the animals were fasted 24 hours the death rate during the experiment was much higher than when a 12 to 15 hour fast was used. Numerous investigators have proved that the liver glycogen drops very rapidly in

fasting adrenalectomized animals and that at the end of 24 hours only a trace of it can be found. The blood sugar also drops considerably (3,15,18,24,26,28,34,50.52). This may be the reason for the high mortality rate.

In some of the experiments the animals were stomach tubed at 12 hour intervals and in others at 8 hour periods. The 8 hour feedings seemed to agree much better with the animals than the longer intervals. Larger doses were given in the 12 hour feedings. This may have caused water intoxication to which adrenalectomized animals easily succumb (72).

The quantity of food fed was based upon the body surface of the animal which was calculated by Lee's method (48). The formula is; Surface area=$K \cdot W^{0.60}$ K, the constant for rats, is 12.54, and W is the weight in grams. Surface area is given in square centimeters when these units are used. The concentrations of the experimental food were such that either one ml. or o.5ml. was equal to 100 sq. cm. of body surface. The total volume of any one stomach tubing was kept below 3 ml. These volumes were measured in standard medical syringes. The rubber stomach tube being placed over the needle.

It was noticed that adrenalectomized animals, especially the larger ones, were very easily "lunged", that is, the rubber tube goes down the trachea instead of the esoph-

agus resulting in the death of the animal within 2 minutes. It is easy to tell in a normal rat if the tube is going down the right channel, but in the adrenalectomized animals it is much more difficult. The tube, on several occasion, descended the trachea as easily as it normally goes down the esophagus. An autopsy convinced the author that the rats were actually lunged. The author does not know the reason why these animals were lunged so easily and nothing was found in the literature. It is possible that the trachea is dilated to a slight extent.

COLLECTION OF THE URINE. Wire cages with 3 flanges which fit over 200 mm. diameter glass funnels were used. Attached to the bottom of the funnel for collection of the urine sample was a separatory funnel. A layer of light mineral oil in the separatory funnel lessens evaporation. A wire cone in the large funnel served to separate the fecal material from the urine. The urine was collected in a 100 ml. volumeteric flask. By washing down the funnel with distilled water the sample was made up to the graduation mark. The urine was filtered through cotton to remove extraneous matter. Urine that was contaminated with blood or fecal material was, of course, discarded. All of the calculations were based on this 100 ml. volume. In a few instances, volumes other than 100 ml. were used because of the size of the rat or the size of the dose of the

experimental food.

ANALYSIS OF THE URINE. Because of the lack of time, only the total nitrogen and the urea-ammonia nitrogen were determined. The total nitrogen was determined by the Kjeldahl method. 5ml. samples of urine were used; the digestion catalyst was one part cupric sulfate to 4 parts potassium sulfate. Controls and blank runs showed only a slight trace of ammonia in the reagents. Using a standard solution of urea, about 99.9% recovery of the nitrogen was obtained. In the later part of the work, the 0.1 N sodium hydroxide was standardized by the use of a standard ammonium chloride solution and by running a regular Kjeldahl distillation. The value obtained was 0.1001 N. (Another student, using an orthodox method of standardization, obtained a value of 0.1000 N.)

The method of Marshall (55) and Van Slyke and Cullan (76) was used for the urea-ammonia nitrogen determinations. These are based upon the use of urease. The blank runs on the reagents showed no nitrogen present and the percentage recovery on known urea samples was almost as good as that obtained of the Kjeldahl apparatus. The technique used was as follows; Four ml. samples of urine were pipetted into the aeriation tubes; about one ml. of water was added to increase the volume; 5 drops of caprylic alcohol to prevent foaming and one crushed Arlco-Urease (obtained from

the Arlington Chemical Co. of Yonkers, New York) tablet were added. The tube was covered by an inverted 50 ml. beaker and allowed to stand the required length of time with an occasional shaking. The urease tablets contain enough phosphate buffer to prevent the escape of the ammonia. The collecting tubes were prepared by adding 25 ml. of 0.02 N sulfuric acid, 2 drops of caprylic alcohol and mehtyl re indicator. The connecting tubes must be kept dry. At first considerable difficulty was had, but this vanished when the connecting tubes were kept dry. When the hydrolysis of the urea is complete, the beaker is removed, 4 to 5 g. of powdered anhydrous potassium carbonate is added, and the connecting tube put in place. This operation should take not more than 3 seconds, otherwise the chance of ammonia escaping becomes too great. The aeration is then started and allowed to continue for an hour. Occasional shaking of the apparatus is desirable to hurry the process of solution of the carbonate. The contents of the collecting tube are transferred to a flask and titrated in the usual manner.

In some of the experiments individual analysis of the urine samples were not run. The samples from several rats were pooled and the analysis was then preformed. This saved much time when preliminary experiments were

being run or when the urine samples were being collected in 4 or 8 hour periods instead of the usual 24 hours. Evans used this method on the analysis of rat blood (25). The proper correction for the surface area, number of rats, volume of sample, and etc, were made. The results obtained by this method, of course, were easily reproduced.

AUTOPSIES. In order to be certain that no natural occuring accessory adrenal bodies are present, it is necessary to autopsy the animals after an experiment. About 4% of rats are supposed to have such accessory bodies. Many percent of them can have such bodies if the adrenals are ruptured during the extripation (33). In most cases a gross examination was made, but in the more suspicious looking cases, histological slides were prepared. By the more suspicious looking cases is meant, an animal that comes through an experiment feeling quite lively and at autopsy, shows some deposits of fat in the region above the kidneys. In the adrenalectomized animals, the absence of fatty tissue was very noticeable, especially in the peritoneal cavity. There has been some work done with the adrenals and fat. Most of the work was done on fat absorption (8,9,10,11, 12,20,81), but so far the author has found no references in literature to this defficiency of fatty tissue. However, in Addison's disease there is a general defficiency of fatty tissue.

In a number of the experiments some the adrenalectomized rats died. Some dying during the preliminary starvation period, some during the feeding period, and others shortly after the feeding period. Several animals died within a few days or a few weeks after the operation. It can be said that the mortality rate was very high. This condition made experiments quite difficult.

RESULTS

The results obtained are summarized in the table on the following page. It contains only the more important parts of each experiment. For those experiments which lasted more than one day, only the results of the first day are shown. Complete data for each experiment is given in the appendix.

Experiments 10,11,12, and 13 were the only ones that showed a marked contrast between the percentage of the urea-ammonia nitrogen in the normal animals and in the adrenalectomized animals. The experiment in which cortin was used, No. 17, shows nothing exceptional.

Egg white was used to give the animals a better balanced diet, to decrease the rate on intestinal absorption (free amino acids are absorbed relatively rapidly) and to try to detect a decreased rate of deamination when protein was used. The only beneficial effect noted was the relatively low loss of body weight of the rats during the course of the experiment.

The toxic effects of d-arginine monohydrochloride and of high concentrations of dl-alanine on adrenalectomized animals is of interest but not surprising.

SUMMARY OF THE EXPERIMENTAL RESULTS

(See the appendix for the individual experiments)

Exp. num.	Num. & sex	Condition	Substance fed and amount[a]	mg of N[c] per cm^2	% U-A N of total[b]
1.	6 F.	normal	glucose 1.87	0.345	83.6
2.	5 M.	normal	glucose 1.87	0.276	82.0
3.	6 F.	normal	alanine 2.00	0.626	81.9
4.	6 M.	normal	alanine 2.00	0.556	85.5
5.	4 F.	adrenal.	glucose 1.87	0.454	84.4
6.	3 M.	adrenal.	alanine 2.00	0.647	83.0
7.	3 M.	normal	alanine 4.00	0.970	80.4
	3 M.	adrenal.	alanine 4.00	0.704	82.7
8.	3 M.	normal	alanine 6.00	1.241	82.6
	3 M.	adrenal.	alanine 6.00	1.098	78.0
9.	no analysis---see appendix.				
10.	6 F.	normal	nofood	0.0944	87.1
	6 M.	normal	no food	0.0896	85.9
11.	4 M.	adrenal.	no food	0.0593	73.8
	4 M.	adrenal.	no food	0.0793	79.8
12.	4 F.	adrenal.	no food	0.0745	68.3
	4 M.	adrenal.	no food	0.0735	72.9
13.	6 F.	normal	no food	0.0313	73.2
	6 M.	normal	no food	0.0912	82.6
14.	7 F.	normal	eggwhite 2ml	0.529	81.3
15.	2 F.	adrenal.	eggwhite 2ml	0.610	85.5
	3 F.	normal	eggwhite 2ml	0.498	86.8
16.	2 F.	adrenal.	eggwhite 2ml	0.538	82.8
	5 M.	adrenal.	eggwhite 2ml	0.540	86.8
17.	3 F.	normal[d]	eggwhite 2ml	0.461	87.6
	2 M.	normal[d]	eggwhite 2ml	0.491	85.2
18.	3 F.	adrenal.	cysteine 2.0	0.430	80.2
	3 F.	normal	cysteine 2.0	0.419	78.1
19.	no analysis---see appendix.				
20.	3 F.	adrenal.	arginine 2.0	0.767	82.6
	3 F.	normal	arginine 2.0	0.801	85.7
21.	3 F.	adrenal.	glutamic	0.625	81.1
	3 F.	normal	acid 2.0	0.443	76.4

a--amount fed in mg per 100sq. cm per 24 hours.
b--U-A N is urea ammonia nitrogen.
c--first day or period of the experiment.
d--subcutaneous injections of cortin.

DISCUSSION

If the cortical hormones step up the rate of deamination of amino acids, then, of course, adrenal insufficiency will result in a slowing down of this mechanism provided no other compensatory mechanism comes into play.

Numerous experiments (9,12,19,23,38,57, and 71) have shown that the rate of diffusion through membranes in animals suffering from adrenal insufficiency is markedly changed for different substances. For example, Dennis and Wood (23) found that in adrenal insufficiency the rate of absorption of sodium in the intestine was greatly reduced while that of potassium was reduced only a small extent.

The rate of deamination and the total amount of deanimation taking place must be distinguished. If, for example, in a normal rat, 20 mg of ammonia nitrogen are formed each hour by deamination but deamination takes place at this rate only so long as active absorption from the small intestine is taking place and the rate drops to, say, 5 mg of ammonia nitrogen per hour when intestinal absorption is not taking place, (e.g. the rate of deamination in the normal rat is limited by the concentration of deaminizable substances present) then the mechanism for deamination is not working at full capacity; let us say it is doing only 25% of the work that it is capable of doing

over a period of 24 hours. Now take an adrenalectomized animal. The rate of absorption of the food through the intestinal wall is slowed down according to some experiments (23). If the normal deamination mechanism were present, the rate of deamination would nevertheless be slowed down because of the decreased rate at which deaminizable substances are made available but the total amount of deamination taking place in a 24 hour period would be the same.

To go a little further. If the mechanism for demination is disturbed by adrenal insufficiency to such an extent that it is capable of working only 50% of its normal capacity over a 24 hour period, the total amount of ammonia nitrogen formed by deamination during this period is the same as for a normal animal, although the deamination capacity has been reduced 50% and the rate of deamination has also been reduced. We assumed that the normal rate of deamination, over a 24 hour period, was only 25% of its capacity, then the adrenal insufficient animal according to the above example is deaminizing at 50% of its capacity.

The crude hypothesis is based on the assumption that the rate of deamination in a normal animal is based upon the rate of deaminizable substances available and the amount available varies considerable over a 24 hour period.

Furthermore, there is a maximum capacity and a maximum rate at which deamination can take place, and this is constant over a 24 hour period.

If the above hypothesis is correct, then you cannot detect the effect of deamination by urine analysis from urine collected over long periods of time, (unless the rate of deamination was reduced to a very low level). The percentage of urea-ammonia nitrogen would be the same in both cases, in normal and in insufficiency animals. Some variation could be expected if the urine were collected for short periods of time as shown in experiments 10,11, 12, and 13. But urine collected in such short periods does not give good quantitative results for the total amount of nitrogen excreted over that period, even when the bladder is "squeezed" to force urination.

The results obtained in experiments 10,11,12, and 13 might be explained on the assumption that adrenalectomized animals had less food in their intestines at the beginning of the experiment than the normal rats. The amount of endogenous nitrogen, such as in uric acid and creatinine would be almost the same from day to day but the amount of exogenous nitrogen, in ammonia and urea would drop somewhat. The result would be a lowered percentage of urea-ammonia nitrogen of the total nitrogen. It would appear that the rate of deamination would be lowered

by the adrenal insufficiency. However, Groat (32) reports that the food intake of adrenalectomized animals maintained on salt therapy is the same as that for normal animals.

The results obtained in this study apparently agree with Evans (25) but disagree with Russell and Wilhelmi (67,68) and Samuels (69). The techniques used in each of these reports are different. In none of them, including this one, has the technique been simplified to a point where a true control can be run, that is only one experimental variable and the rest of the factors being held constant. In Evans' (25) work the kidneys were litigated, Russell and Wilhelmi (67,68) used tissue slices. Samuels worked on the rate of liver glycogen formation from dl-alanine and not with nitrogen excretion. In this study, as Harrison has shown (36) the uncontrolled variable was the kidneys.

Before any definite conclusions can be drawn from this experiment, the rate of deamination over a period should be investigated. Also the capacity of the deaminating mechanism for a 24 hourperiod, the exact role of the kidneys in adrenal insufficiency and the different "degrees" of adrenal insufficiency should receive further study. But the biggest question that needs to be answered is, if a decrease in the rate of deamination does actually take place in adrenal insufficiency; do the

cortical hormones affect the deamination directly or do they affect it indirectly through the general lowering of the rate of metabolism which takes place in adrenal insufficiency?

SUMMARY

1. The effects of adrenal insufficiency on the deamination mechanism in rats were studied.

2. A decrease in the rate of deamination is supposed to be indicated by a decrease in the percentage of urea-ammonia nitrogen of the total nitrogen excreted in the urine.

3. The rate of deamination was differentiated from the deamination capacity.

4. Dl-alanine, normal diet, raw egg white, l-cysteine, d-arginine monohydrochloride and dl-glutamic acid were fed to normal and adrenalectomized rats.

5. No definite decrease in the percentage of urea-ammonia nitrogen was obtained except in one series of experiments which need further investigation.

6. No definite conclusions were drawn from the results obtained in this study as the the effects of adreanl insufficiency upon the deamination mechanism.

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APPENDIX

EXPERIMENT 1

Normal females fed glucose, 1.87 mg per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 24 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml.

3 day feeding period.

URINE ANALYSIS:

Rat number	weight in g	S.A. cm^2	mg of N	mg of N per cm^2	mg of U-A* N	% U-A N of total
First day:						
1	174	277	88.3	0.319	80.3	90.8
2	146	249	89.4	0.359	72.0	80.6
3	176	279	88.3	0.317	71.4	80.8
4	160	264	93.6	0.354	74.3	79.3
5	152	256	93.6	0.366	78.6	83.8
6	182	285	100.7	0.353	86.8	86.2
Second day:						
1	-	-	86.2	0.311	64.3	74.6
2	-	-	78.6	0.316	63.2	80.3
3	-	-	42.0	0.151	35.1	83.4
4	-	-	77.0	0.292	62.2	80.9
5	-	-	80.5	0.314	66.3	82.4
6	-	-	97.6	0.342	81.2	83.2
Third day:						
1	-	-	56.8	0.205	46.9	82.6
2	-	-	61.3	0.246	47.6	77.6
3	-	-	69.5	0.249	54.8	78.8
4	-	-	56.0	0.212	46.5	83.0
5	-	-	68.0	0.266	55.2	81.2
6	-	-	61.6	0.216	50.0	81.2

* U-A N is urea-ammonia nitrogen.

EXPERIMENT 2

Normal males fed glucose, 1.87 mg per 100 sq.cm per 24 hours, in 2 feeding each 24 hours.

Fasted 19 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml.

3 day feeding period.

URINE ANALYSIS:

First day:

rat number	weight in g	cm^2 S.A.	mg of N	mg of N per cm^2	mg of U-A N	% U-A N of total
1	177	280	84.2	0.301	67.2	79.8
2	166	269	76.4	0.284	64.7	84.8
3	182	285	87.6	0.307	68.8	78.5
4	214	314	65.2	0.208	54.8	84.0
5	190	292	blood in the urine.			
6	174	277	77.8	0.281	64.7	83.1
Second day:						
1	-	-	64.7	0.231	53.0	82.0
2	-	-	73.0	0.272	57.8	79.1
3	-	-	61.3	0.215	49.1	80.0
4	-	-	75.6	0.241	61.4	81.1
5	-	-	88.5	0.303	71.0	80.2
6	-	-	77.8	0.281	63.6	81.7
Third day:						
1	-	-	83.7	0.299	66.2	79.1
2	-	-	63.8	0.237	51.3	80.5
3	-	-	34.2	0.120	27.3	79.8
4	-	-	65.0	0.207	50.0	76.9
5	-	-	69.4	0.238	55.4	79.8
6	-	-	60.5	0.219	48.0	81.0

EXPERIMENT 3

Normal females fed dl-alanine, 2.00 mg per 100 sq. cm per 24 hours, in 2 feedings each 24 hours.

Fasted 24 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml.

3 day feeding period.

URINE ANALYSIS:

First day:

rat number	weight in g	cm^2 S.A.	mg of N	mg of N per cm^2	mg of U-A N	%U-A N of total
1	170	273	172.6	0.632	143.5	83.2
2	142	245	153.0	0.624	126.1	82.4
3	174	277	168.4	0.608	138.7	82.3
4	162	266	168.0	0.631	135.0	80.4
5	152	256	165.2	0.645	137.6	83.3
6	180	281	172.6	0.614	137.4	79.8
Second day:						
1	-	-	168.1	0.616	131.1	78.0
2	-	-	156.8	0.639	123.6	78.8
3	-	-	137.4	0.496	109.7	79.9
4	-	-	147.9	0.556	119.7	80.8
5	-	-	142.5	0.556	116.2	81.6
6	-	-	153.1	0.545	125.6	82.0
Third day:						
1	-	-	149.5	0.547	122.5	82.0
2	-	-	140.4	0.573	116.6	83.1
3	-	-	150.0	0.541	125.1	83.3
4	-	-	150.8	0.566	122.9	81.4
5	-	-	174.0	0.679	143.4	82.4
6	-	-	147.4	0.524	124.8	84.6

EXPERIMENT 4

Normal males fed dl-alanine, 2.00 mg per 100 sq. cm. per 24 hours, in 2 feeding each 24 hours.

Fasted 19 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml.

3 day feeding period.

URINE ANALYSIS:

rat number	weight in g	S.A. cm^2	mg of N	mg of N per cm^2	mg of U-A N	%U-A N of total
First day						
1	169	272	145.4	0.535	124.8	85.8
2	162	265	144.0	0.543	119.9	83.3
3	181	283	166.0	0.586	144.0	86.7
4	218	317	174.8	0.551	153.7	87.8
5	188	290	170.5	0.588	145.3	85.2
6	174	277	148.2	0.535	124.8	84.2
Second day:						
1	-	-	167.0	0.614	141.8	84.8
2	-	-	144.5	0.545	111.7	77.3
3	-	-	159.7	0.564	119.4	74.9
4	-	-	176.1	0.556	139.1	79.0
5	-	-	168.1	0.579	138.8	82.4
6	-	-	166.4	0.610	133.6	80.2
Third day:						
1	-	-	161.0	0.592	134.1	83.3
2	-	-	161.6	0.610	136.7	84.6
3	-	-	139.8	0.493	122.1	87.4
4	-	-	177.2	0.558	154.2	87.2
5	-	-	168.1	0.579	145.2	86.3
6	-	-	145.7	0.529	120.6	82.7

EXPERIMENT 5

Adrenalectomized females feed glucose, 1.87 mg per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml.

3 day feeding period.

URINE ANALYSIS:

First day:

rat number	Weight in g	S.A. cm^2	mg of N	mg of N per cm^2	mg of U-A N.	%U-A N of total
1	175	278	124.8	0.448	103.4	82.8
2	148	252	108.5	0.431	94.2	86.7
3	164	267	130.7	0.488	113.0	86.4
4	172	275	123.4	0.448	100.9	81.8
Second day:						
1	-	-	66.8	0.240	54.8	82.0
2	-	-	53.4	0.212	43.8	82.2
3	-	-	96.7	0.362	81.5	84.3
4	-	-	100.3	0.365	80.9	80.7
Third day:						
1	-	-	92.2	0.332	75.6	82.0
2	-	-	Died - adrenal insufficiency.			
3	-	-	82.9	0.311	69.6	83.8
4	-	-	91.1	0.331	78.3	86.0

EXPERIMENT 6

Adrenalectomized males feed dl-alanine, 2.00 mg per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml.

2 day feeding period. Animals too weak to make it a 3 day experiment.

URINE ANALYSIS:

First day:

rat number	Weight in g	S.A. cm^2	mg of N	mg of N per cm^2	mg of U-A N.	% U-A N of total
1	182	285	218.4	0.767	183.1	83.8
2	202	303	197.5	0.652	169.6	85.8
3	172	275	143.4	0.521	114.0	79.5
Second day:						
1	-	-	179.3	0.629	151.3	84.4
2	-	-	180.9	0.596	152.3	84.3
3	-	-	139.1	0.506	113.4	81.6

EXPERIMENT 7

3 normal and 3 adrenalectomized males fed dl-alanine, 4.00 mg. per 100 sq. cm. per 24 hours in 3 feedings.

Fasted 8 hours before the first feeding.

Urine collected at the end of 24 hours, 8 hours after the last feeding.

Urine volume; 200 ml.

1 day experiment.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	mg of N	mg of N per cm^2	mg of U-A N.	% U-A N of total
1	210	310	226	0.729	188.5	83.4
2	197	299	242	0.809	202	83.3
3	189	291	167.4	0.574	136.0	81.3
Normal:						
4	220	319	329	1.032	264	80.2
5	282	370	371	1.001	298	80.3
6	218	317	278	0.877	255	80.8

EXPERIMENT 8

3 normal, 2 adrenalectomized males and 1 adrenalectomized female, dl-alanine, 6.00 mg per 100 sq. cm. per 24 hours in 3 feedings.

Fasted 8 hours before first feeding.

Urine collected at the end of 24 hours, 8 hours after the last feeding.

Urine volume, 300 ml.

1 day experiment.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	mg of N	mg of N per cm^2	mg of U-A N.	% U-A N of total
1 M	174	277	263.5	0.952	212.5	81.1
2 M	183	286	292	1.021	230	78.7
3 F	171	274	362	1.321	268	74.2
Normal						
4 M	230	328	410	1.250	339	82.7
5 M	243	339	411	1.211	337	82.0
6 M	254	348	439	1.263	365	83.2

EXPERIMENT 9

3 normal and 3 adrenalectomized males, fed dl-alanine, 8.00 mg per 100 sq. cm. per 24 hours in 3 feedings.

Fasted 8 hours before first feeding.

Urine collected at the end of 24 hours, 8 hours after last feeding.

Urine volume, 400 ml.

1 day experiment.

RESULTS:

Adrenalectomized:

Rat number	
1	died about 4 hours after first feeding.
2	comatose condition after the second feeding, removed.
3	died about 3 hours after second feeding.

Normal:

4	weak but otherwise all right.
5	" " " " "
6	" " " " "

Urine analysis was not made because of the above results. The animals probably died of adrenal insufficiency aggravated by either or both, water intoxication and the toxic action of the large amount of alanine.

EXPERIMENT 10

6 normal males and 6 normal females, urine collected during fasting period. Given only tap water and libitum.

Placed on funnels at the beginning of the fasting period.

Urine collected at the end of 4,12,24, and 36 hours of the fast.

Accumulative analysis of urine made for each sex.

URINE ANALYSIS: All results are, of course, the average for 1 rat.

End of 4 hour fast (4 hour urine collection).

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1 (6F)	198	299	28.20	0.0944	24.60	87.1
2 (6M)	261	353	31.6	0.0896	27.15	85.9
End of 12 hour fast (8 hour urine collection).						
1	-	-	70.0	0.234	63.2	87.4
2	-	-	63.9	0.182	52.2	81.4
End of 24 hour fast (12 hour urine collection).						
1	-	-	58.4	0.195	48.5	82.8
2	-	-	89.3	0.254	72.9	81.7
End of 36 hour fast (12 hour urine collection).						
1	-	-	53.4	0.179	44.5	83.3
2	-	-	68.0	0.193	56.7	83.3

EXPERIMENT 11

4 adrenalectomized males and 4 adrenalectomized females, urine collected during fasting period. Given only Rubin-Drick solution ad libitum.

Placed on funnels at the beginning of the fasting period.

Urine collected at the end of 4,12,24, and 36 hours of the fast.

Accumulative analysis of urine made for each sex.

URINE ANALYSIS: All results are, of course, the average for 1 rat.

End of 4 hour fast (4 hour urine collection).

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1 (4F)	178	281	16.65	0.0593	12.26	73.8
2 (4M)	235	333	26.38	0.0793	21.03	79.8
End of 12 hour fast (8 hour urine collection).						
1	-	-	28.53	0.1016	16.79	59.7
2	-	-	50.00	0.1505	35.85	70.7
End of 24 hour fast (12 hour urine collection).						
1	-	-	50.38	0.1794	36.05	71.6
2	-	-	108.5	0.326	85.7	79.0
End of 36 hour fast (12 hour urine collection).						
1	-	-	58.50	0.209	44.4	76.1
2	-	-	80.25	0.241	61.0	75.8

EXPERIMENT 12

(Repetition of experiment 11)

4 adrenalectomized males and 4 adrenalectomized females, urine collected during fasting period. Given only Rubin-Krick solution ad libitum.

Placed on fummels at the beginning of the fasting period.

Urine collected at the end of 4,12,24, and 36 hours of the fast.

Accumulative Analysis of urine made for each sex.

URINE ANALYSIS: All results, are of course, the average for 1 rat.

End of 4 hour fast (4 hour urine collection).

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1 (4F)	167	279	20.8	0.0745	14.22	68.3
2 (4M)	228	326	24.0	0.0735	17.53	72.9
End of 12 hour fast (8 hour urine collection).						
1	-	-	37.8	0.135	25.63	67.8
2	-	-	48.7	0.149	32.19	66.4
End of 24 hour fast (12 hour urine collection).						
1	-	-	52.3	0.187	36.55	69.8
2	-	-	81.7	0.253	60.2	73.7
End of 36 hour fast (12 hour urine collection).						
1	-	-	58.7	0.251	44.8	76.2
2	-	-	86.9	0.266	66.4	76.3

EXPERIMENT 13

(Repetition of experiment 10)

6 normal males and 6 normal females, urine collected during fasting period. Given only tap water ad libitum.

Placed on funnels at the beginning ofthe fasting period.

Urine collected at the end of 4,12,24, and 36 hourss of the fast.

Accumulative analysis of urine made for each sex.

URINE ANALYSIS: All results are, of course, the average for 1 rat.

End of 4 hour fast (4 hour urine collection).

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1 (6F)	199	300	9.40	0.0313	6.90	73.3
2 (6M)	244	339	30.90	0.0912	25.56	82.6
End of 12 hour fast (8 hour urine collection).						
1	-	-	54.4	0.182	42.2	77.4
2	-	-	46.5	0.137	34.20	73.6
End of 24 hour fast (12 hour urine collection).						
1	-	-	65.0	0.217	47.1	72.6
2	-	-	60.3	0.178	46.7	77.2
End of 36 hour fast (12 hour urine collection).						
1	-	-	66.8	0.223	52.7	78.8
2	-	-	73.8	0.218	58.3	78.8

EXPERIMENT 14

7 normal females fed fresh egg white*, 2 ml per 100 sq. cm. per 24 hours, in 2 feeding each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml for each rat.

2 day feeding period.

URINE ANALYSIS: (Accumulative analysis and divided into 2 parts, all results are, of course, the average for 1 rat.)

First day:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1 (3F)	177	279	157.2	0.563	127.6	81.2
2 (4F)	183	285	141.2	0.495	115.2	81.4
Second day:						
1	-	-	155.5	0.557	142.6	91.7**
2	-	-	160.5	0.562	132.2	82.3

* It is very difficult to obtain a homogeneous egg white solution.

** Probably an error in analysis.

EXPERIMENT 15

2 adrenalectomized females and 3 normal females fed fresh egg white, 2 ml. per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml. for each rat.

1 day feeding period.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1	182	285	174.9	0.613	151.0	86.4
2*	180	283	85.8	0.303	72.5	84.5
Normal:						
**3 (3rat)	185	288	143.3	0.498	124.5	86.8

* Died at the time of the second feeding; stomach tubed.

** Accumulative analysis. Results are, of course, the average of one rat.

EXPERIMENT 16

1 adrenalectomized male, 2 adrenalectomized females and 5 normal males fed fresh egg white, 2 ml per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml for each rat.

1 day feeding period.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1 (M)	188	290	76.8	0.265	65.3	85.1
2 (F)	148	252	110.8	0.439	91.6	82.7
3 (F)	188	290	185.0	0.637	153.5	82.8
Normal						
4 (2M)*	188	290	155.4	0.537	134.6	86.6
5 (3M)*	238	334	181.2	0.542	157.5	87.0

* Accumulative analysis. Results are, of course, the average for one rat.

EXPERIMENT 17

3 normal females and 3 normal males, all with cortin injections, fed fresh egg white, 2 ml per 100 sq. cm. per 24 hours, in 2 feeding each 24 hours.

Cortin ("Eschatin"--prepared by the Parke, Davis and Co.) injection was given at the time feeding. Subcutaneous injections, 0.1 ml per 100 sq. cm. being given.

Fasted 24 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml for each rat.

1 day feeding period.

URINE ANALYSIS:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cum^2	Mg of U-A N.	% U-A N of total
1 (3F)*	185	287	132.2	0.461	115.8	87.6
2 (2M)*	195	297	145.7	0.491	124.0	85.2
3 (1M)**	212	312	85.7	0.274	75.2	87.7

* Accumulative analysis. Results are, of course, the average for one rat.

** Last the first 12 hours of urine collection.

EXPERIMENT 18

3 adrenalectomized females and 3 normal females fed l-cysteine*, about 2 mg per 100 sq. cm per 24 hours, in 2 feeding each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml for each rat.

1 day feeding period.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1	167	270	120.5	0.446	94.2	79.1
2	146	249	99.5	0.403	80.8	81.2
Normal:						
3	158	262	108.2	0.412	84.8	78.5
5	163	267	110.8	0.414	89.4	80.7
4	204	305	131.5	0.431	97.8	75.0

* Approximately a 10% suspension of l-cysteine(the natural occuring isowere) with about 2% gum tragacanth.

EXPERIMENT 19

Normal and adrenalectomized males fed d-arginine monohydrochloride (the natural occuring isomere), 4.00 mg per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 15 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

1 day feeding period.

RESULTS:

12 hours after the first feeding,,the adrenalectomized animals had a bad diarrhea and the normal animals were beginning to show the symptoms of diarrhea. Paper towels were fed to the rats in hopes of stopping this condition. 12 hours after the second feeding all of the animals had a bad diarrhea; the urine sample was contaminated. One adrenalectomized animal died shortly after the experiment.

EXPERIMENT 20

Normal and adrenalectomized females fed d-argenine monohydrochloride (the natural occuring isomere), 2.00 mg per 100 sq. cm. per 24 hours, in 2 feeding, each 24 hours.

Fasted 17 hours before first feeding but the animals were given paper towels at the beginning of the fast to help prevent diarrhea.

Urine collected every 24 hours, 12 hours after last feeding.

Urine volume; 100 ml for each rat.

1 day feeding period.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1	168	271	219.7	0.812	186.5	84.9
2	160	264	213.8	0.818	174.5	81.5
3	158	262	176.0	0.672	143.0	81.3
Normal:						
4 (3F)*	177	280	224.2	0.801	193.2	85.7

* Accumulative analysis. Results are the average for one rat.

EXPERIMENTS 21

Adrenalectomized and normal females fed dl-glutamic acid, 2.00 mg per 100 sq. cm. per 24 hours, in 2 feedings each 24 hours.

Fasted 16 hours before first feeding.

Urine collected every 24 hours, 12 hours after the last feeding.

Urine volume; 100 ml for each rat.

1 day feeding period.

URINE ANALYSIS:

Adrenalectomized:

Rat number	Weight in g	S.A. cm^2	Mg of N	Mg of N per cm^2	Mg of U-A N.	% U-A N of total
1	201	302	216.5	0.717	173.7	80.3
2	198	299	167.8	0.560	136.8	81.5
3	154	258	153.5	0.597	125.7	81.8
Normal:						
4 (3F)*	172	275	122.0	0.443	93.2	76.4

* Accumulative analysis. Results are the average for one rat.