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The effect of adrenalectomy and replacement therapy upon the blood sugar level of the alloxan diabetic rat

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THE EFFECT OF ADRENALECTOMY AND REPLACEMENT THERAPY UPON
THE BLOOD SUGAR LEVEL OF THE ALLOXAN DIABETIC RAT

A THESIS
SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY
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THE DEGREE OF MASTER OF SCIENCE

BY
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CHAPTER I

INTRODUCTION

The existence of a functional relationship between the anterior pituitary, the pancreas and the adrenal cortex with respect to the control of carbohydrate metabolism is well established. The role of the adrenal cortical hormones in the regulation of carbohydrate metabolism was pointed out by Britton and Silvette ('32). Long and Lukens ('36) demonstrated the improvement of diabetes which resulted from adrenalectomy. Ingle ('41) established the diabetogenic potency of pure cortical hormones. Although not all details of the mechanisms are known, participation of the adrenal cortex in the control of carbohydrate metabolism is unquestionable.

The present investigation was prompted primarily by the wish to determine the extent to which adrenalectomy would ameliorate the hyperglycemia of the alloxan diabetic rat and the degree of exacerbation of the hyperglycemia by the administration of adrenal cortical extract to the diabetic-adrenalectomized animal. As the work progressed, another aspect of the problem was attacked by means of adrenal transplants and the administration of desoxycorticosterone acetate.
CHAPTER II

REVIEW OF LITERATURE

Bueding, Fazekas and Himwick (1943) found that there was an absence of a normal pyruvate response after the administration of glucose to the depancreatized dog. From this finding, they believed that insulin may be concerned with the earlier phases of glucose oxidation. Further evidence indicating that in certain tissues insulin may be concerned with early oxidative processes has been provided by the experiments of Price, Cori and Colowick (1945). According to these workers, one of the functions of insulin is to overcome the inhibitory action of diabetogenic hormones on the enzyme hexokinase. More recent reports of Weisberg, Caren and Levine (1949) suggested that insulin may increase cellular permeability for glucose. The work of Stetten and Boxer (1944) seemed to indicate that insulin may be concerned with the synthesis of fat from carbohydrate.

Somogyi (1951) found that intravenous injections of small doses of insulin to healthy persons produced changes in the blood sugar level which presented the mirror-image of the changes produced by consecutive administrations of glucose. The response following repeated glucose feeding has been ascribed to the stimulation of the secretion of insulin by hyperglycemia, with the consequence that the mobilized insulin held down the hyperglycemic effect of successive glucose doses. Insulin produced a counterpart of this picture. The initial insulin-hypoglycemia stimulated the pituitary-adrenal axis to increased mobilization of epinephrine, with the consequence that successive insulin did exert considerably less hypoglycemic effect than the first.

In discussing the mechanism of the action of insulin, Young (1948)
contends that the mechanism of glucose control, which maintains the blood sugar level at a remarkably constant value in health, and in which insulin plays a very important part includes the conversion of glucose to tissue glycogen and to fatty acids, as well as to the release of glucose into the circulation when the blood sugar level is low.

Anderson and Long ('47) found that when the pancreas was perfused with blood which had a low glucose content, insulin was not secreted in an amount detectable by the assay method used. A high glucose level in the perfusate stimulated the islet cells directly to secrete insulin.

Drury and Pauls ('43) found that insulin caused diabetic rats to store large quantities of sugar. There were large increases in the glycogen content of the liver and muscles but only fifteen per cent and nine per cent respectively of the blood sugar were accounted for by the extra-glycogen in these tissues. There was no evidence that any appreciable part of the sugar which was not deposited as glycogen was oxidized. According to the investigators, only a negligible amount could be accounted for by possible protein synthesis; transient storage as carbohydrate was normally stored as fat and it was considered the most likely possibility that this was the fate of the bulk of the sugar stored under the influence of insulin.

Jensen and Hart ('42) have shown that substances capable of producing an anti-insulin response may be differentiated by their influence on liver glycogen. They support the contention that a distinction should be made between a specific and a non-specific anti-insulin effect. According to them, an anti-insulin response can be produced by substances (epinephrine and anterior pituitary extract) which may bring about only a redistribution of the carbohydrate stores in the test animal without any formation of new
carbohydrate. A specific anti-insulin response may be elicited by substances (adrenal cortical extracts) which are capable of producing an increase in the deposition of liver glycogen by forming glucose from non-carbohydrate sources. Various urinary preparations were found to produce an elevation in blood sugar and an anti-insulin effect in normal rabbits and mice. They were found, however, not to increase the glycogen content of the liver under similar experimental conditions. In contrast, certain endocrine principles, eliciting an anti-insulin effect, have been found to promote the deposition of liver glycogen.

Evans ('41) found that when rats were injected with glucose, with or without insulin, cardiac glycogen was increased by glucose but not further increased by the addition of insulin. For such glucose injected animals, the liver glycogen was lowered by insulin and progressively so as the dose was increased; the effect occurred even during hyperglycemia.

Hechter, Levine and Soskin ('41) have studied, by the Warburg technique, the formation of glycogen in the diaphragm muscle of rats in vitro. When no insulin was added the rate of glycogen formation was the function of the concentration of sugar in this medium. In the presence of added insulin the rate of glycogen formation was high even at comparatively low sugar levels. As the sugar concentration was increased the catalytic effect of insulin became less and less evident. The effect of insulin was negligible at 400 mg./% concentration of sugar. According to these workers, insulin acted by catalyzing the conversion of glucose into an intermediary metabolite which preceded and was necessary for both glycogen formation and sugar utilization.

Selye ('40) found that doses of insulin which caused pronounced hypo-
glycemia and shock in rats fasted for shorter or longer periods had relatively little effect after forty-eight hours of starvation. In fact, in some cases he found that the blood sugar level actually increased following treatment with this hormone. The period of maximum insulin resistance was found to precede the period of "fasting hyperglycemia" in the rat; yet it was possible that the spontaneous transitory increase in blood sugar and the temporary insulin resistance might have been correlated phenomena.

In connection with the above suggestion, Chidsey and Dye ('35) have demonstrated that an increase in insulin secretion followed the injection of epinephrine. Experiments showed that epinephrine was more effective in raising the blood sugar of the depancreatized dog given insulin in adequate amounts for normal purposes than in a normal dog. Epinephrine administration was followed after several hours by high liver glycogen and low muscle glycogen in normal dogs, and by low glycogen values of both liver and muscle in depancreatized-insulinized dogs. Epinephrine appeared to stimulate in some fashion an increased secretion of insulin in a normal dog. According to them, the high liver glycogen values after the stimulation of epinephrine were due to the extra-secretion of insulin, which was also responsible for the slower rise in blood sugar following epinephrine administration to a normal dog than in one which could not augment its insulin supply.

Experiments performed by Joslin and Dublin ('36) with depancreatized dogs have demonstrated that insulin was produced in a little more than one-half the normal amount in the absence of the pancreas. There are, maybe, two sources of insulin, the tissue cells of the body and the islets of Langerhans in the pancreas, which has suggested to them that in mild cases
of diabetes the cause may be in cellular and not pancreatic deficiency.

Tuttle ('29) believed that pancreatic insulin was held inert by trypsinogen until the latter was released by the production of secretion when food was taken, which caused its discharge into the duodenum. The pancreas in his view, is an organ specialized to produce and store a surplus supply of insulin to meet the demands of the carbohydrate intake at mealtime.

DeTakats and Cuthbert ('35) obtained an average rise of 61% in sugar tolerance after cutting the splanchnic nerves to the adrenals in dogs. To them, these observations indicated that the adrenal medulla exercises some regulating power over blood sugar.

Cannon, McIver and Bliss ('24) showed that hypoglycemia in dogs evoked epinephrine discharge which raised the blood sugar. However, they noticed that under conditions of little stress, medullo-adrenalectomized and sympathectomized dogs regulated their blood sugar very well.

Houssay, Lewis and Malinelli ('24) demonstrated that insulin stimulated the secretion of epinephrine, which in turn tended to counteract the hypoglycemia. They also found that the release of epinephrine caused by insulin depended upon intact splanchnic nerves since it failed to occur when they were cut. In connection with this point, La Barre ('37) attempted to explain the mechanism by an effect on the brain. Perfusion of the brain, whose circulation was isolated from the rest of the body, with hypoglycemic blood, produced an increase in epinephrine secretion.

According to Hartman and Brownell ('49) it is generally believed that epinephrine has certain specific effects when administered internally. It raises metabolism as shown by an increase in heat production, oxygen consumption and carbon dioxide production. It breaks down the glycogen in
skeletal muscle and liver, thus increasing sugar and lactic acid in the blood, and eventually bringing about a shift of carbohydrate from muscle to liver. There is an increased oxidation of fat, and an increased catabolism of protein which result in an increase in the utilization of carbohydrate.

Britton and Silvette ('32) found that extracts of adrenal cortex produced hyperglycemia in the normal and adrenalectomized animal; the blood sugar raising-ability was a direct function of the amount of the hormone injected and the elapsed time. According to these workers, the adrenal cortex was apparently concerned with the storage and utilization of carbohydrate, and possibly with some phase of protein metabolism.

Britton and Silvette ('32) also found that, if enough time elapsed after removal of the glands for complete development of the changes ensuing from adrenal insufficiency, profound disturbances occurred. Nearly all processes involved in carbohydrate metabolism appeared to be retarded. Even the appetite for dextrose was definitely decreased.

Bierry and Malloizel ('08) reported low blood sugar in adrenalectomized animals. This finding did not appear to be invariable but depended upon the stage of insufficiency as well as the species concerned. In some the blood sugar level was not greatly modified until terminal symptoms appeared.

Bøggild ('25) observed a fall in blood sugar after adrenalectomy in rats. Kuriyama ('18) found that the blood sugar fell to the lower normal range average value within a few weeks after operation.

Wyman and Walker ('29) found that coincident with the appearance of marked manifestation following adrenalectomy in the rat, the blood sugar fell to between 50 and 60 mg./% and reached as low as 30 mg./% in terminal
convulsion. In chronic adrenal insufficiency with marked symptoms, they noticed that the blood sugar fell to the lowest of the normal values, and occasionally below. The presence of accessory cortical tissue or successful transplants kept the value within normal range.

Cori and Cori ('27) found that adrenalectomized rats that developed no symptoms showed a subnormal ability to mobilize blood sugar under stress. After fasting the animals for twenty-four hours there was a greater fall in the blood sugar level than that which occurred in normal animals. Exercise caused the development of hypoglycemia in the rats which survived five months after adrenalectomy.

Britton and Corey ('41) have shown that the comparison of the concentrations of blood sugar and glycogen of the liver, skeletal muscle and heart in adrenalectomized, pancreatectomized, and adreno-pancreatectomized cats under certain conditions point directly to the conclusion that the adrenal cortex is highly important in regulating carbohydrate levels in the body. The effects of adrenal cortical extract and insulin in conjunction with glucose administration have been tested in several series of experiments by these investigators.

They observed that cats showed very severe losses of blood glucose and liver, muscle and cardiac glycogen when untreated. They were able to form only slight amounts of glycogen from glucose given orally over periods up to six days. No increase in liver glycogen occurred when insulin was given with glucose, but small rises in muscle and cardiac glycogen occasionally occurred. Large increases in blood glucose and glycogen contents were produced by extracts of the cortex used with glucose.

Pancreatectomized animals when untreated showed practically normal
amounts of liver, skeletal and cardiac muscle glycogen almost up to the
time of death. They were able to form liver glycogen from glucose
solution alone. More muscle glycogen appeared to be deposited under the in-
fluence of insulin, and slightly more liver glycogen under adrenal cortical
extract.

Adreno-pancreatectomized cats which were untreated often showed low
blood sugar and liver glycogen levels when symptoms appeared somewhat
similar to the conditions observed in adrenal insufficiency. Adrenal
cortical extract given with glucose brought about large deposits of glycogen
in liver and cardiac tissues.

Britton and Corey also observed that adrenal cortical extract was able
to form liver glycogen in the absence of the pancreas; insulin did not do so
in animals following loss of the adrenal cortex. It appeared to these
workers that the carbohydrate hormone of the cortex stimulated markedly
hepatic glycogenesis, while insulin may have affected favorably the forma-
tion of glycogen in muscles.

Zuelzer (101) found in cats and dogs that extirpation of the pancreas
at the same time that the adrenals were tied off, provoked little or no
glycosuria. Seven years later Frouin (108) showed that pancreatectomy pro-
duced less glycosuria than usual in dogs from which all but a small portion
of the adrenal tissue had been removed.

Hedon and Giraud (120) observed in the pancreatectomized dog that the
reduction in blood sugar by adrenalectomy did not occur until after a
twenty-four hour delay. Moscata (122) found that after pancreatectomy the
adrenals increased in size and in epinephrine content. To him, this obser-
vation indicated that there exists an interrelationship between the pancreas
and the adrenals.

Hartman and Brownell ('34) found that adrenal cortical extract maintained the hyperglycemia and glycosuria of adrenalectomized-pancreatized animals at diabetic levels as long as they ingested food and provided they had not lost too much weight. However, the ketones of the blood and urine were diminished, but they could not replace the ketogenic function by the administration of cortical extract.

Embden and Kalberlah ('06) noticed that adrenalectomy decreased the fat accumulation in the liver which was a characteristic of the diabetic state. They found that blood ketones were derived principally, if not entirely, from the processing of fat in the liver. To these workers, it seemed useless to look for diabetic ketonemia unless one could first show the characteristic fat content of this organ.

Lukens and Kohan ('38) confirmed the fact that hyperglycemia and glycosuria could be maintained in adrenalectomized-depancreatized animals. They showed that the extra sugar excreted by these animals could be fully accounted for by an increased nitrogen excretion. In all of their experiments it was noticed that the dosage required was exceedingly large in comparison with that needed to maintain an adrenalectomized animal in good health.

Houssay and Biasotti ('36) found that injections of an extract of the anterior lobe of the hypophysis into pancreatectomized-adrenalectomized toads restored the blood sugar to diabetic levels. It appeared to these workers that the factor in the pituitary did not act through the adrenals.

Houssay and Rietti ('37) noticed that the ketogenic substance of the anterior pituitary was effective in rats during the first six days after
adrenalectomy and on the fourteenth day, but had little effect during the intermediate period unless adrenal cortical extract was injected. Neither demedullation of the adrenals nor removal of the thyroid suppressed the ketogenic function of the anterior pituitary.

Janes and Friedgood ('45) found that following adrenalectomy there was either a marked reduction of, or a complete disappearance of the symptoms of alloxan diabetes. Their results on alloxan diabetes are in agreement with the observations of Long and Lukens ('36).
CHAPTER III

MATERIALS AND METHODS

In the present investigation twelve male hooded rats (250-325 gm. in weight) of the Long-Evans strain were used in four series of experiments. In all series the animal was placed in a closely fitted tin tube sufficiently ventilated at one end for the head and with a hole at the opposite end large enough for the animal's tail to extend through it to the outside. The animal was conditioned to the tube and the luke-warm water into which his tail was placed for several minutes prior to the collection of blood from it. The excess water was removed from the tail by absorbent cotton.

About one-half centimeter of the tail was, then, clipped off and several drops of blood were collected in a small glass container. One-tenth milliliter of blood was quickly measured out, pipetted, and transferred to ten milliliters of a dilute tungstic acid solution which were in a fifteen-milliliter centrifuge tube. The pipette used for transferring the blood was rinsed out several times with portions of the solution in the tube. After stirring the mixture well, the contents of the tube were centrifuged for several minutes.

Four milliliters of the water-clear supernatant fluid were placed in a fifty-milliliter sugar tube. Then, two milliliters of potassium ferri-cyanide solution were added, followed by one milliliter of cyanidecarbonate solution, mixed by lateral shaking, and placed in a boiling water bath for eight minutes. The solution was cooled by placing it in a large beaker of cold water for from one to two minutes, after which five milliliters of dupanol coloring reagent were added and mixed by shaking. After standing
for several minutes, the solution was diluted to the twenty-five milliliter mark with distilled water, mixed well by inversion, and allowed to stand for ten minutes. Ten milliliters of the colored solution were transferred to a colorimeter tube and read in the Klett-Summerson photoelectric colorimeter against a blank set at zero. This was a modified method of Polin and Malmros by Klønshøj and Habbard ('39) for micro-determination of blood sugar.

In Series I alloxan was administered to the animal to determine whether or not it would cause a persistence of the hyperglycemia for at least four weeks.

In Series II and III, after there was a demonstrable diminution in the blood sugar level, five cubic centimeters of Upjohn's adrenal cortical extract and one cubic centimeter of Vitamin's desoxycorticosterone acetate were administered subcutaneously at different intervals, respectively. Each animal was allowed to fast from the time the compound was given until the blood sugar level was again checked. This time varied from five to six hours.

In Series IV adrenal grafts were used as replacement therapy. A longitudinal incision, approximately one-half inch long, was made in the ventral neck region. With the aid of the blunt end of a pair of forceps the skin was gently separated from the musculature to form a pouch. The adrenal autotransplant was cut into six pieces and three were imbedded on each side of the incision. The wound was sutured with cotton thread. In cases in which there was no immediate demonstrable drop in the blood sugar the animals were discarded.

In all series the normal blood sugar level of each animal was
established by taking the average of several determinations. In Series II, III and IV the animal was then unilaterally adrenalectomized by the dorso-lateral lumbar approach. In all operative procedures ether was used as an anesthetic. A few days later, the animal was rendered diabetic by an intraperitoneal injection of approximately one-hundred and seventy milligrams of alloxan per kilogram of body weight. Fifty milligrams of the drug were homogenized in one milliliter of normal saline. After a persistent state of hyperglycemia had developed, the contralateral adrenal was extirpated.

In all cases the blood sugar level was checked until the animal died.
CHAPTER IV

EXPERIMENTAL RESULTS

Twelve male hooded rats were used in four series of experiments. In Series I four animals were subjected to diabetogenic doses of alloxan to determine whether or not the drug would cause a persistence of the hyperglycemia for at least four weeks. Prior to the establishment of a persistent diabetic state, the blood sugar showed a characteristic triphasic reaction: 1. there was an immediate hyperglycemia which reached a maximum height within three hours; 2. the blood sugar level dropped tremendously by the fourth hour. This hypoglycemic state persisted for several hours and was often fatal, and 3. there was a hyperglycemic state by the second day after the administration of alloxan. Although the blood sugar level varied somewhat, the hyperglycemia supervened for thirty days. This is evident when graph 1 is examined.

In Series II three animals were used. After the second adrenal was removed from each rat there was a marked decrease in each blood sugar level on the following day. To each of these diabetic-adrenalectomized animals, adrenal cortical extract was administered at different intervals as replacement therapy. The animal was allowed to fast from the time the extract was injected until the blood sugar level was again checked. Adrenal cortical extract caused a rise in the blood sugar level of these fasting animals. The blood sugar level was higher the day after the administration of the extract than it was five hours after fasting (graph 2). The blood sugar raising-ability of adrenal cortical extract in the fasting diabetic-adrenalectomized rat was about the same as in the fasting adrenal-
ectomized animal. This may be seen when graph 2 is compared with graph 3.

In Series III three animals were used. The same procedures were employed in this series as in Series II except desoxycorticosterone acetate was used as replacement therapy. After a five-hour fast there was a slight drop in the blood sugar level followed by a rise on the first day after the administration of the compound. When the animal was fasted for five hours without the administration of desoxycorticosterone acetate, there was a drop in the blood sugar level comparable to the one seen when the compound was given. This can be observed in graph 4.

In Series IV autografts of the adrenal gland were used as replacement therapy. Efforts to keep diabetic-adrenalectomized rats alive with such tissues yielded favorable results in only one case. In this particular case, and others, one cubic milliliter of 1.0% saline was injected intraperitoneally when the blood sugar level approached the hypoglycemic range. The saline restored the animal from prostration. Such restoration was accompanied by an increase in the blood sugar level slightly above normal.
CHAPTER V

DISCUSSION

There are wide differences of opinion regarding the extent to which tissues can oxidize carbohydrate in the absence of insulin. It is generally agreed at this time that the depancreatized animal can oxidize some carbohydrate in the midst of plenty. There are no satisfactory methods for measuring the oxidation of glucose in the intact animal and determinations based upon eviscerated animals and minced tissues in vitro may be subjected to criticism.

In the normal resting animal the adrenal cortex functions quietly and gives no recognizable signs of its action. During adrenocortical insufficiency or excess, the changes from normal are ubiquitous. The functions and structures of most, if not all, tissues in the body are affected. According to Ingle ('52) there was no demonstrable metabolic process known to be abolished by adrenocortical insufficiency, and no new process was known to be created when these hormones were administered in excess. He proposed that these and other hormones may enter into body economy by affecting the rates of metabolic processes.

According to Selye ('47) the adrenal cortex can undergo wide excursions in its activity. Its size and secretory activity are greatly increased above normal when the animal is subjected to noxious stimuli (stress). When injury is severe, increased amounts of adrenal cortical hormones are required or the animal will die.

A direct participation of the adrenal cortex in the hormonal control of carbohydrate metabolism has been the subject of vigorous debates for a
number of years. The interest in this problem has been centered not only on the interpretation of the alterations in carbohydrate metabolism that occur in adrenalectomized animals but also on their relation to the functions of the adrenal cortex. This problem would appear to be capable of a simple solution were it not for the fact that other changes follow adrenalectomy that at first appear to be of greater significance than the disturbances in carbohydrate metabolism that have been reported.

The present investigation was undertaken to study the effect of adrenalectomy and replacement therapy upon the blood sugar level of the alloxan diabetic rat. Male animals were chosen because some investigators (Beach et al., '52) have found that the females of some strains are hyper-sensitive to alloxan and the life-span of the adrenalectomized female is longer than that of the male (Steward and Rogoff, '29). The mechanism that may account for the response in the former case is not known. In the latter case, the difference might be due to the fact that during pregnancy and pseudocyesis the organism can chemically modify sex hormones into compounds comparable to those of the adrenal cortex during insufficiency of the latter. The fact that these hormones have the same basic molecular nucleus may account for the mechanism of the conversion.

Small autografts of the adrenal gland were imbedded in the ventral neck region for several reasons: 1. vascularization and polymorphonuclear leucocytic infiltration occur more quickly in small grafts than in large ones; 2. the ventral neck region is highly vascularized; 3. going into the body cavity for a third time would precipitate deleterious repercussions upon the diabetic-adrenalectomized animal, and 4. such a location of the incision would facilitate the inconvenience of the animal in tearing away
the sutures.

In the first series each animal that responded to alloxan exhibited a typical triphasic pattern of the blood sugar for which Goldner and Gomori ('44) have proposed the following. The initial hyperglycemia is due to the presence of an intact adrenal gland. The second phase of the reaction of the blood sugar to alloxan, the hypoglycemia, is caused either by the stimulation of the islet cells or by the release of stored insulin from the degeneration of the Beta cells. The third phase, the persistent hyperglycemia, is due to the absence of the synthesis of the insulin by the necrotic Beta cells.

The concentration of the blood sugar level of the diabetic rat was markedly reduced by adrenalectomy. The increased disappearance of glucose in the diabetic-adrenalectomized animal may be explained on three grounds: 1. a reduction in gluconeogenesis; 2. an increased oxidation of carbohydrate, and 3. an accumulation of intermediates of carbohydrates. Hartman and Brownell ('34) found that there was a retarded development of the specific dynamic action of carbohydrate following adrenalectomy. This response was due in part to slower absorption of glucose as well as to a reduction in the rate at which glucose was converted into glycogen.

In Series II it was demonstrated that adrenal cortical extract caused a rise in the blood sugar level of the fasting diabetic-adrenalectomized rat. The blood sugar level was higher the first day after the administration of the extract than it was after the five-hour fast. The rise in blood sugar during fasting might have been due to the formation of carbohydrates from non-carbohydrate precursors such as proteins or fats. Upon cessation of the hormone the animal died within a few days. These findings seem to
point directly to the fact that the drop in the blood sugar level and the
death of the animal were due to adrenal insufficiency.

In Series III, upon the administration of desoxycorticosterone acetate, the blood sugar level of the fasting diabetic-adrenalectomized rat fell slightly. On the following day the blood sugar level was higher. When the animal was allowed to fast without the administration of desoxycorticosterone acetate, the blood sugar level fell to a level comparable to the one seen when the compound was injected. This finding seems to indicate that the slight drop in the blood sugar level was not due to an oxidative power of desoxycorticosterone acetate but to fasting.

The animals used in the last two series of experiments were restored from extreme prostration time after time, and the life-span was greatly prolonged by the injection of adrenal cortical extract and desoxycorticosterone acetate. Such resuscitation was accompanied by increases in blood sugar, muscular strength and body weight.

In Series IV it was noticed that the life-span of the diabetic-adrenalectomized animal with adrenal grafts was shorter than that of the adrenalectomized rat. Upon autopsy, most of these grafts were found to be necrotic or suppurated. The mild hyperglycemia that persisted in the animal that survived may have been due to the effects of saline, of the presence of accessory cortical tissue or of regenerated adrenal grafts.

The increased mortality in these animals may be contributed to two main factors. 1. Alloxan is a toxic drug. Following its administration investigators have found that there is (a) a diminution in the activity of the complement of the blood; (b) necrosis of the proximal tubules in the kidney, and (c) hypertrophy of the zona fasciculata of the adrenal gland.
All of these are extra-pancreatic effects. 2. In order for an adrenal
graft to become functional it must (a) become penetrated by blood vessels;
(b) the capsule must be present within the adrenal graft; (c) the animal
must be bilaterally adrenalectomized (capsule included), and (d) the
adrenocorticotrophic principle of the anterior hypophysis must be active.
CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Each animal that responded to alloxan exhibited a characteristic triphasic reaction of the blood sugar: (a) an immediate hyperglycemia which probably depended upon an intact adrenal gland; (b) a hypoglycemia with a duration of several hours which might have been evidence of the liberation of stored insulin in the pancreas, and (c) a hyperglycemia that was usually persistent which might have been due to the absence of the synthesis of insulin by the Beta cells of the pancreas.

2. The concentration of the blood sugar level of the diabetic rat was markedly reduced by adrenalectomy. During terminal stages of adrenal insufficiency the blood sugar level fell far below normal. The increased disappearance of sugar in the blood after adrenalectomy may have been due to a decrease in gluconeogenesis, to an accumulation of intermediates of carbohydrates and to an increased oxidation of carbohydrates.

3. Adrenal cortical extract caused an increase in the blood sugar level of the fasting-diabetic-adrenalectomized rat. This increase was probably due to the endowed power of gluco-corticoids to decrease the oxidation of glucose and to stimulate gluconeogenesis.

4. When desoxycorticosterone acetate was administered to the fasting-diabetic-adrenalectomized rat there was a slight drop in the blood sugar level which could have been due to fasting. On the following day the blood sugar level was higher. Fasting alone caused a drop in the blood sugar level comparable to the one seen when the compound was
5. Adrenal cortical extract, desoxycorticosterone acetate and normal saline restored the diabetic-adrenalectomized animals from extreme prostration time after time, and the life-span was greatly prolonged. Such resuscitation synchronized with increases in body weight, muscular strength and blood sugar.

6. Efforts to keep diabetic-adrenalectomized animals alive with autografts of adrenal glands yielded fruitful results in only one case. The mild hyperglycemia that persisted may have been due to the effects of saline, of the presence of accessory cortical tissue or of re-generated adrenal grafts.
LITERATURE CITED


PLATE I

(Explanation of graph)
Graph 1  This is a graph showing the mean triphasic reaction of the blood sugar of four rats subjected to diabetogenic doses of alloxan. There was an immediate rise in the blood sugar level which reached its maximum height within three hours. There was a tremendous drop in the blood sugar level by the fourth hour and finally a persistent hyperglycemia on the second day. Although there were slight fluctuations in the blood sugar level, the hyperglycemia supervened for thirty days.

Note: Ordinate axis = blood sugar level in milligrams per cent
Abscissa axis = time after the administration of alloxan in hours and days
Broken line = normal blood sugar level
Arrow = point at which alloxan was administered
PLATE 2
(Explanation of graphs)
Graph 2  This is a graph showing the effects of adrenalectomy and the administration of adrenal cortical extract upon the blood sugar level of the diabetic rat. On the first day following adrenalectomy the blood sugar level fell tremendously. Adrenal cortical extract caused a rise in the blood sugar level of the fasting-diabetic-adrenalectomized animal. The blood sugar rising-ability of adrenal cortical extract in the fasting-diabetic-adrenalectomized rat was about the same as in the fasting-adrenalectomized animal. This is seen when graph 2 is compared with graph 3.

Graph 3  This graph shows the influence of adrenal cortical extract upon the blood sugar level of the fasting-adrenalectomized rat.

Note:  Ordinate axis = blood sugar level in milligrams per cent
Abscissa axis = numbers below the axis represent the days after the administration of alloxan; numbers above the axis represent the days after adrenalectomy (graph 2). The numbers below the axis in graph 3 represent the days after adrenalectomy
Broken line = normal blood sugar level
Arrows = the points at which alloxan, adrenalectomy and the administration of adrenal cortical extract took place
ADX = adrenalectomy
ACE = adrenal cortical extract
XXX = the day on which the animal died
PLATE 3
(Explanation of graph)
Graph 4  This is a graph showing the influence of adrenalectomy and
the administration of desoxycorticosterone acetate upon the
blood sugar level of the diabetic rat. On the first day following
adrenalectomy there was a tremendous drop in the blood sugar
level. When desoxycorticosterone acetate was administered to the
fasting-diabetic-adrenalectomized animal there was a drop in the
blood sugar level. On the following day the blood sugar level
was higher. Fasting alone caused a drop in the blood sugar level
comparable to the one seen when the compound was injected.

Note:  Ordinate axis = blood sugar level in milligrams per cent
Abscissa axis = numbers below the axis represent the days after
the administration of alloxan; numbers above the
axis represent the days after adrenalectomy
Broken line = normal blood sugar level
Arrows = the points at which alloxan, adrenalectomy and
the administration of desoxycorticosterone
acetate took place
ADX = adrenalectomy
DCA = desoxycorticosterone acetate
XXX = the day on which the animal died