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The Effects of Alloxan on the Histology of the Pancreas, Thyroid and Adrenal Glands of the White Rat

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To the Graduate Council:

I am submitting herewith a thesis written by Julien L. Cagle entitled "The Effects of Alloxan on the Histology of the Pancreas, Thyroid and Adrenal Glands of the White Rat." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Samuel R. Tipton, Major Professor

We have read this thesis and recommend its acceptance:

J. Gordon Carlson, Arthur W. Jones

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 7, 1950

To the Graduate Council:

I am submitting herewith a thesis written by Julien L. Cagle entitled "The Effects of Alloxan on the Histology of the Pancreas, Thyroid and Adrenal Glands of the White Rat." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

Samuel R. Tipton
Major Professor

We have read this thesis
and recommend its acceptance:

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Accepted for the Council:

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Dean of the Graduate School

THE EFFECTS OF ALLOXAN ON THE HISTOLOGY OF THE PANCREAS, THYROID AND ADRENAL GLANDS OF THE WHITE RAT

A THESIS

Submitted to
The Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

Julien L. Cagle

August 1950

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CHAPTER I

INTRODUCTION

Prior to 1943, two methods of producing experimental diabetes mellitus in laboratory animals were known, pancreatectomy and the injection of anterior pituitary extract. In that year, the discovery of a third method, the destruction of the pancreatic islands of Langerhans by the intravenous injection of alloxan, was announced by Dunn, Sheehan and McLetchie. These workers were conducting an investigation into the pathogenesis of the renal lesion of the crush syndrome and the similar condition which occurs in mis-matched blood transfusions. Substances were being tried which influence the lower renal tubules, such as uric acid and related compounds. Among the most promising was alloxan, an oxidation product of uric acid. However, with the dosages required to obtain the renal lesions, many of the rabbits died during the first day or so with symptoms which could not be related to kidney damage. In the examination of these early mortalities, they discovered the characteristic lesions of the islets, and further investigation gave them rabbits exhibiting the typical tri-phasic blood sugar picture, with permanent diabetes resulting (ibid., 1943).

Since the publication of this paper, a tremendous amount of work has been done on the mode of action of

alloxan. Also, this convenient method of producing a permanent experimental pancreatic diabetes has stimulated research in the fields of carbohydrate metabolism and the role of insulin in metabolism. Of immediate interest was the question of whether permanent diabetes could be produced in other animals. It was soon made clear that this condition was most easily and successfully obtainable in rabbits (Brunschwig and Allen, 1944; Corkill, Fantl and Nelson, 1944; Duff, McMillan and Wilson, 1947; Duffy, 1945; Goldner and Gomori, 1944; Kendall, Meyer, Lewis and Victor, 1945; Kennedy and Lukens, 1944), dogs (Brunschwig and Allen, 1944; Carrasco-Formiguera, 1944; Goldner and Gomori, 1943; Shipley and Beyer, 1947; Thorogood and Zimmermann, 1945) and rats (Duff and Starr, 1944; Dunn and McLetchie, 1943; Gomori and Goldner, 1943; Hard and Carr, 1944; Kass and Waisbren, 1945; Lazarow, 1946). At least one thorough study of alloxan diabetes in frogs has been reported (Seiden, 1945). Cats will develop diabetes if the alloxan is given orally (Ruben and Yardumian, 1946), although oral administration is usually unsatisfactory in other animals. The failure of production of permanent diabetes in the guinea pig has been investigated in two recent studies (Collins-Williams, Renold and Marble, 1950; Johnson, 1950).

From the mass of literature available on the subject, a characteristic picture of the mode of action of alloxan emerges, in regard to the effects on the blood sugar level

and upon the pancreatic tissues. A tri-phasic response of the blood sugar is found almost invariably, although Shipley and Beyer (1947) observed a slight fall in blood glucose fifteen or thirty minutes after injection of alloxan in dogs, so that they speak of a tetra-phasic curve. In general, the tri-phasic curve consists of an initial hyperglycemia lasting for one to four hours, followed by a somewhat severe hypoglycemia at about six to twelve hours, and completed by the permanent hyperglycemia, with its onset at about eighteen to twenty-four hours after injection of the alloxan. Jacobs (1937) was apparently the first to describe the initial hyperglycemia and the subsequent hypoglycemia in rabbits. Since most of his animals died from the hypoglycemic shock, however, he did not observe the permanent hyperglycemia, nor did he discover the islet damage responsible for the effect. The first work of Dunn, Sheehan and McLetchie (1943) was then done without knowledge of Jacobs' paper, although it was acknowledged later (Dunn, 1943).

In their original paper, Dunn and his associates suggested that the first hyperglycemic phase may be the result of excessive mobilization of sugar through the adrenosympathetic system. This seems to be supported by various studies, in which the temporary hyperglycemic stage was abolished in adrenalectomized animals (Goldner and Gomori, 1944; Kirschbaum, Wells and Molander, 1945), while

Corkill and his associates (1944) found that it was abolished by ergotoxine, a substance which blocks the action of the sympathetic nervous system. This view is opposed, chiefly by Houssay and his co-workers (1945), who, finding the hyperglycemia greater when alloxan is injected directly into the portal vein, suggest that this phase is a result of a direct action of the liver. Also, they do not find ablation of it with adrenalectomy, and they have other observers to support them (e.g., Shipley and Beyer, 1947). Whatever its cause, it seems clear that this initial phase is not essential to the islet damage, since its prevention by insulin (Goldner and Gomori, 1944a and b), phlorizin (ibid.), adrenalectomy (Kirschbaum, Wells and Molander, 1945) and hypophysectomy (Bailey, LeCompte, Bailey and Franseen, 1947; Kirschbaum, Wells and Molander, 1945) makes no difference in the subsequent islet pathology. The second, or hypoglycemic phase, was thought by Dunn, Sheehan and McLetchie to be due to a stimulation of the beta cells to excess production of insulin. Houssay, Orias and Sara (1945) again argue for an effect of alloxan on the liver, probably leading to a lack of glucose production. The most generally accepted idea is that the hypoglycemia is the result of release of pre-formed insulin from the damaged beta cells. This seems to conform with the observations that, although beta cell destruction begins within five minutes after the injection of alloxan (Bailey, 1946;

Gomori and Goldner, 1945), the insulin content of the pancreas diminishes only after seven to twenty-four hours, when damage to the beta cells is well advanced, and approximately at the time when the hypoglycemia begins (Ridout, Ham and Wrenshall, 1946). Furthermore, Bailey (1946) and Lukens (1948) report the work of Hughes, Ware and Young (Lancet 6:148, 1944), who were able to reproduce the hypoglycemia by injecting an amount of insulin equivalent to that ordinarily found in the total pancreatic tissue of the animal.

There seems to be little doubt that the final permanent hyperglycemia is due to the necrosis of the insulin-producing cells.

The general histological alterations in the islands of Langerhans are essentially the same in all species of animals. Many of the variations reported may be resolved and correlated by a consideration of the time after injection and the size of the dose of alloxan. For example, hydropic changes are occasionally reported, and from the work of Duff, McMillan and Wilson (1947) and Kennedy and Lukens (1944), it appears that this type of degeneration may be seen only after a period of forty-five to ninety days duration of the diabetes. Small doses of alloxan give typical, but less severe, changes, while massive doses accelerate the necrosis (Brunschwig and Allen, 1944; Dunn,

Kirkpatrick, McLetchie and Telfer, 1943; Dunn, Sheehan and McLetchie, 1943).

A brief composite description will serve at the moment to elucidate the general histopathology involved; a more detailed description of this under the present experimental conditions will be given later. As was mentioned above, changes in the islet tissue have been noted five minutes after injection. This agrees well with the findings of Leech and Bailey (1945) that intravenously injected alloxan disappears from the blood almost completely within two minutes, and that the point of highest concentration is reached at the end of the injection period. The changes give the picture of a progressive degeneration, with disappearance of the granules from the cytoplasm of the beta cells and a general shrinkage and pyknosis of the nuclei. There is a disruption of the normal ribbon-like cellular arrangement, until after about twenty-four hours the center of the islet may consist of faintly-staining cellular debris with distorted cells and small, condensed, heavily-staining nuclei. After a few days there seem to be fewer islets, consisting chiefly of alpha cells (Brunschwig and Allen, 1944; Dunn, Kirkpatrick, McLetchie and Telfer, 1943; Goldner and Gomori, 1943; Gomori and Goldner, 1943; Hard and Carr, 1944). A paper by Hughes and Hughes (1944) presents the hypothesis that only the older and smaller beta cells are attacked by the alloxan. The new and larger cells are

thought to replace continually the destroyed ones, until finally the ability of the pancreas to produce new beta cells is exhausted.

As a general rule, the alpha cells show no evidence of injury, though the finding of fewer than normal islets indicates a complete disintegration of all insular constituents. The acinar tissue is usually unaffected.

The literature is somewhat conflicting in regard to alloxan-induced changes in the adrenal and thyroid glands. Most writers find little or no change in the adrenal (Duff and Starr, 1944; Dunn and McLetchie, 1943; Goldner and Gomori, 1943) and thyroid (Duff and Starr, 1944; Dunn, Kirkpatrick, McLetchie and Telfer, 1943; Dunn and McLetchie, 1943). Where histophatological changes are noted in the adrenal gland (Bennett and Koneff, 1946; Duffy, 1945; Hard and Carr, 1944; Kendall, Meyer, Lewis and Victor, 1945; Ruben and Yardumian, 1946) and in the thyroid gland (Applegarth, 1949; Applegarth and Koneff, 1946; Bennett and Koneff, 1946; Bennett, Koneff and Wolff, 1948), the general feeling is that such changes are a metabolic effect of the diabetes, rather than a direct action of the alloxan.

The elucidation of whether these adrenal and thyroid changes are the result of secondary metabolic effects or of direct alloxan action has been undertaken in the present experiments. The rationale of the work was, briefly, if the reported adrenal and thyroid changes could be produced

in alloxanized rats and this damage should be found to be reversible in such animals receiving injections of insulin, then it might be assumed that the original changes were due to metabolic disturbances resulting from the lack of insulin. On the other hand, if insulin injections give no indications of reversibility of the adrenal and thyroid pathology, these changes could be due to a direct action of the alloxan, thus making alloxan specifically toxic to these endocrine organs. In general, the experimental results reported here suggest that the former is true, since the changes induced tend to be relieved with the administration of insulin.

CHAPTER II

MATERIALS AND METHODS

Female rats (except for one group of males) of the Rockland strain were used. The experiments were run in groups of four, litter mates being chosen wherever possible. However, it was felt that all members of the rat colony are genetically similar, due to inbreeding, so that litter mates were not considered essential as long as weights were about the same. Each experimental group was treated in the following manner: one animal served as a control and three were injected with alloxan, one of these later receiving insulin. The animals were alloxanized according to the method of Kass and Waisbren (1945). This involves the starvation of the animals twenty-four to forty-eight hours preceding the injection. Alloxan monohydrate, obtained from the Eastman Kodak Company, was dissolved in distilled water in the amount of 19.9 mg per ml of solution. One ml of this solution for each 100 grams of body weight was then injected intraperitoneally. This gives a dosage of 199 mg/kg of body weight. The alloxan solution was freshly prepared just before injection of each group. Glucose solution was substituted for the drinking water during the first twelve to twenty-four hours after injection in order to combat the hypoglycemia. The animals were observed for signs of hypoglycemic shock, and those exhibiting such signs

received an intraperitoneal injection of 5 cc of 5 percent glucose solution. It may be noted that this was necessary in only a few cases. At all times, food (Rockland Rat Diet) and water were given ad libitum. The animals were placed in individual metabolism cages over glass funnels so that the urine could be collected.

At approximately twenty-four hour intervals, the urine was measured and tested for glucose by Benedict's test. Relative concentrations were noted by the customary arbitrary signs, plus 1 to plus 4 (Hawk, Oser and Summerson, 1947). At about forty-eight hours, one of the animals which showed definite symptoms of being diabetic (glycosuria and polyuria) was injected with 6 - 10 units of Squibb Protamine Zinc Insulin. The one injection was deemed sufficient because of the finding of Allen (1938 - 1939) that 10 units of Protamine Zinc Insulin may continue its effect for as long as fifty-eight hours.

The animals were sacrificed approximately ninety-six hours after injection with alloxan. Each animal was first injected intraperitoneally with 1 cc of 4 percent Nembutal solution. As soon as this had quieted the animal, a matter of about five minutes, blood for glucose analysis was obtained by cardiac puncture. The visceral cavity was then opened, portions of the pancreas and the entire left adrenal removed and placed in Formalin-Zenker solution for fixation. The right adrenal was treated according to the special

technique developed by Flexner and Grollman (1939), using osmic acid reduction as an indicator of adrenal cortical activity. Both adrenals were cut in half for better fixation. Finally the thyroid gland was dissected out and also placed in Formalin-Zenker. All tissues were imbedded in paraffin, the pancreas sectioned at 3 - 7 micra and the other tissues at 7 micra. Pancreas sections were stained by the technique of Gomori (1939), using chrome alum hematoxylin and phloxine as a means of differentiating the cellular components of the islands of Langerhans. The left adrenal, thyroid and other pancreas sections were stained with Heidenhain's iron-hematoxylin. The right adrenals required no staining. The texts of Bensley and Bensley (1938), Guyer (1949) and Lillie (1948) were used as guides for the histological procedures of preparing and staining the tissues. Blood glucose was determined by the Nelson and Somogyi method (Nelson, 1944; Somogyi, 1945), although the protein-free blood filtrates were prepared by the method of Folin and Wu (Hawk, Oser and Summerson, 1947). The concentrations were determined by means of the Klett-Summerson photoelectric colorimeter.

The pancreas slides were analyzed with respect to the following items: normal or abnormal acinar tissue, normal or damaged beta cells, normal or damaged alpha cells and predominant cell type (alpha or beta) in the islets. In this way, tissues from diabetic animals could readily

be distinguished from the non-diabetic. The slides of the thyroids and the osmic acid treated adrenals were separated into their original experimental groupings and studied on a comparative basis. Points examined in the thyroids were size of follicles, condition of epithelial cells (height of cell and intensity of nuclear stain) and presence or absence of vacuoles in the colloid of the follicles. The osmic acid lipid-reduced adrenals were checked for separation of the cortex into zones and the intensity of reduction in these various zones. The adrenals stained with iron-hematoxylin were examined for evidence of cellular damage to the cortex and medulla and for the "chromaffin reaction" in the medulla.

CHAPTER III

RESULTS

The ease with which diabetes may be obtained with alloxan was illustrated in the present experiments. Of the thirty rats injected, only one was refractory to the alloxan, an incidence of 96.9 percent effectiveness. Results obtained by the early experimenters in this field were variable until standardization of the method by Kass and Waisbren (1945). Of the twenty-nine diabetic animals thus obtained, three died before the end of the experimental period, a mortality of 10.3 percent. These deaths are attributed to a direct effect of the alloxan, since they showed pale, blood-depleted livers and kidneys, which are ordinarily associated with alloxan toxicity. None of these three died from hypoglycemic shock, because they all died forty-eight hours or more after injection and all showed severe glycosuria before death. Upon injection, all experimental rats showed some signs of physical discomfort. This was localized to the abdominal region and was probably caused by irritation of the tissues by the acid alloxan solution. Since all recovered from this discomfort in a matter of four to six hours, it apparently had no connection with the deaths reported above.

Because the blood sugars were determined under non-fasting conditions, a great deal of variation might be

expected even in the normal controls. Table I gives these values. These are also shown in the bar graphs given as Figure 9. The values for the controls range from 78.24 mg to 214.85 mg per 100 cc of blood. When these extremes are omitted, the other values are found to be from 113.62 to 136, a fairly compact range. Such non-fasting values do not appear inordinately high, since the fasting normal is about 60 - 120 mg percent (Gomori and Goldner, 1943). Kass and Waisbren (1945) accept 180 mg percent as the upper limit of normality for non-fasting rats, while Lackey, Bunde, Gill and Harris (1944) found an average of 136 mg percent under non-fasting conditions for the rats in their experiments. No explanation can be given for the two extreme values noted for the controls in Table I, although they are probably due to factors which cannot be analyzed, such as the amount of food ingested just prior to sacrifice and the emotional state of the animal. Data charts reveal that rat #0 (having the low blood sugar) lost weight during the experiment, from 230 grams at the beginning to 150 grams at sacrifice ninety-six hours later. The other control with the high blood sugar, #4, showed a weight gain of 35 grams during the experimental period. In general then, normal blood sugar conditions were found to prevail in the group of controls.

Also from Table I, it may be seen that the alloxanized animals showed a mean value for terminal blood sugar of 528.56 $\pm$ 132.22 mg percent. Of the animals receiving insulin

TABLE I

BLOOD SUGAR VALUES FOR CONTROL ANIMALS, ALLOXAN DIABETIC ANIMALS AND ALLOXAN DIABETIC ANIMALS RECEIVING INSULIN TREATMENT

	Animal Number	Initial Weight	Terminal Non-Fasting Sugar
		g.	mg %
Group I	0	230	78.24
	463	260	113.62
Controls	333	218	119.85
	153	190	125.44
	00	132	125.96
	695	170	126.25
	x	268	133.80
	643	169	136.00
	4	165	214.85
			Mean = 130.44 ± 34.97*
Group II	237	210	296.10
	380	220	385.32
Alloxan	641	190	429.00
Treated	679	160	443.68
	642	210	456.00
	646	180	484.80
	681	190	522.64
	373	220	523.38
	352	200	530.74
	361	200	575.28
	478	170	632.44
	351	180	643.40
	461	200	668.30
	391	260	817.80
			Mean = 528.56 ± 132.33*
Group III	355	210	47.83
	676	160	73.32
Alloxan	275	180	139.08
and	455	240	271.70
Insulin	696	170	294.92
	486	200	306.44
	240	220	324.30
	648	182	395.00
	3	200	453.15
			Mean = 256.19 ± 131.18*

*Standard deviation from the formula $s = \sqrt{\frac{\Sigma x^2}{n-1}}$ (Snedecor, 1948).

injections, one was near the hypoglycemic shock level, and two were in about the same range as the controls. The other animals in this group exhibited glucose levels of 271.70 to 453.15 mg percent. Including all animals in the analysis, the mean was found to be 256.19, with a standard deviation of 131.18 mg percent. The "t" test shows a highly significant difference between this and the group of untreated diabetics. The "p" value from Fisher's table (Snedecor, 1948) was less than .01.

The general picture of necrosis of the islets was found in each of the alloxanized rats. Figure 2 shows a typical islet from one of these, rat #642, untreated with insulin and having a terminal blood sugar of 456 mg percent. This may be compared with Figure 1, which is a photograph including about one-fourth of a section of a normal islet from control animal #4. At this level, a few granular alpha cells may be seen at the periphery with the main portion of the islet occupied by beta cells. A detailed description of another damaged islet will illustrate more specifically the nature of the pathology. The tissue was taken from rat #496, which had a terminal blood sugar of 294.92 mg percent. This animal received one injection of 8 units of Protamine Zinc Insulin at forty-eight hours after the alloxan, but no difference in islet pathology with such treatment was noted. During the final twenty-four hour period of the experiment, the urine glucose and urine volume were somewhat reduced

because of the influence of the insulin, but pronounced glycosuria and polyuria on the second and third days indicated the diabetic condition. The section of the islet under observation is roughly oval in shape, measuring about 0.15 mm x 0.08 mm. The section at this level shows 67 nuclei, of which 43 are alpha and delta (which are indistinguishable with the Gomori staining technique) and 24 are beta cells. The alpha cells show their normal ribbon-like arrangement and are located generally around the periphery of the islet. These cells are large, with red-staining cytoplasmic granules, and have large rounded nuclei with an open vesicular appearance. The beta cells are located in the center and to one side of the section of the islet. Only two or three normal appearing nuclei are present, and even these cells show marked fragmentation of the cytoplasm. Most of the remaining nuclei are shrunken, heavily condensed and deeply staining. Many present the appearance of being flattened and twisted. A few are nothing more than tiny dark dots. The cytoplasm is vacuolated and disrupted with almost complete loss of any cellular outline. The islet shown in Figure 2 shows an even greater ratio of alpha to beta cells (approximately 20:6) with less cytoplasmic debris in evidence.

No variations from normal were noted in the acinar tissue.

The observations on the thyroid tissues in general bear out the work of Bennett and Koneff (1946) and Bennett, Koneff and Wolff (1948). The changes in these diabetic animals were less striking than the illustrations of their work, probably because their photographs are from animals which had been diabetic for one to fifteen months. However, certain changes could be noted, particularly on a direct comparison of slides from control and experimental animals within any experimental group. In general no significant differences in the size of the follicles were noted. The most obvious variations from the normal in the untreated diabetic rats were the absence of vacuoles in the colloid of the follicles and a greater number of follicles with flattened epithelial cells and smaller, more compact, more deeply staining nuclei in these cells. In those animals receiving insulin injections, the slides generally indicated a return toward the normal condition in regard to these factors. Photomicrographs show these findings, Figure 3 being from rat #0, a control animal with a final blood sugar of 78.24; Figure 4, rat #478, an untreated diabetic with terminal blood glucose of 632.44 mg percent; and Figure 5, from rat #455, receiving 8 units of Protamine Zinc Insulin at forty-eight hours and having a terminal non-fasting blood sugar of 271.70 mg percent.

Flexner and Grollman (1939) by means of osmic acid reduction have found characteristic lipid deposition in the

adrenal cortex. They differentiate the cortex of the white rat adrenal as follows. The outermost zone is composed of the zona glomerulosa and a thin layer of cells from the underlying fasciculata. Lying in the fasciculata is the next zone, which is almost free of substances reducing osmic acid. The remaining fasciculata is divided into an outer portion, comprising about two-thirds of the zone, which is fairly rich in reducing substances, and an inner portion comparatively poor in this respect. The innermost zone is the reticular, a thin area next to the medulla, and containing little osmiophilic materials. Again on a basis of a comparative study of the slides, these zonings were noted in the controls but were found to be obliterated in the untreated diabetics. In these, there is a heavy and generalized reduction of osmic acid. In the insulin-treated animals, there is evidence that the reducing materials are somewhat less than in the untreated diabetics. In some cases, as in #240, which is reproduced in Figure 8, some evidence of zones may be seen. Figure 7 is from an untreated diabetic and shows the heavy and uniform reduction. Figure 6 is the control from this group.

The chromaffin reaction of the medulla seemed to show little variation in the groups of animals.

There was no evidence of cellular damage in either the cortex or medulla of the adrenals of the alloxanized animals.

CHAPTER IV

DISCUSSION

The terminal non-fasting blood sugar levels of the diabetic rats in the present experiments averaged 528.56 ± 132.33 mg percent, with a range of about 300 to over 800. The blood sugar values tend to be higher in alloxanized animals than in those which are pancreatectomized, with extremes up to 1000 mg percent and over occasionally reported (Houssay, Orias and Sara, 1945; Goldner and Gomori, 1943). This leads one to think that alloxan must be exerting some effect other than destruction of the insulin-producing tissue. Thorogood and Zimmermann (1945) observed that, although the insulin requirement of alloxanized dogs is higher than depancreatized ones, the former are able to survive longer without insulin treatment and are less likely to develop ketosis. On this basis, they propose that the pancreas secretes a second endocrine factor (possibly the alpha cells being the site of this) which acts to increase the blood sugar but to prevent ketosis in the animal deficient in insulin. Much more likely, it seems, is the idea that alloxan has a direct, or possibly indirect, effect on the liver, and through this mediation the blood sugar level is kept high. Houssay and his co-workers (1945) have long maintained the importance of the liver effect in the blood sugar picture with alloxan. Damage is frequently

found in the liver, and Goldner and Gomori (1943), noting fatty degeneration in this organ, suggest the possibility of a direct action of alloxan on the liver parenchyma. In the present experiments a gross pathological condition of the liver was sometimes noted, consisting of a bleached, bloodless appearance of the organ. No histological study was made of these tissues. This condition was usually found in animals which showed high blood sugars and were sick and moribund toward the time of sacrifice. It was also found in those which died a day or two before the time for sacrifice. Johnson (1950) found that, though guinea pigs failed to develop permanent diabetes, those which showed a temporary diabetes (of five or six days duration in her experiments) had an impairment of glucose tolerance for several weeks, the curves suggesting those typical of liver dysfunction, as worked out by Soskin (Soskin and Levine, 1946).

Though the insulin-treated rats showed relatively high blood sugar values, except for three animals, the mean for this group (256.19 ± 131.18 mg percent) is considerably lower than that for the untreated animals (528.56 ± 132.33). The indications are that not enough insulin was injected to control the diabetes in these cases. There was no evidence that resistance to the insulin was present. In general, it has been shown that alloxanized animals respond well to insulin treatment, the diabetes being easily controlled by proper dosage. Although little work has been reported with

insulin treatment of alloxan diabetic rats, much work has been done with rabbits and dogs. Duffy (1945) with rabbits and Goldner and Gomori (1943) and Thorogood and Zimmermann (1945) with dogs note that alloxanized animals require more insulin for control of the blood sugar than do depancreatized ones. Reasons for this were discussed above. No difference was found in the islet pathology of the insulin-treated and untreated rats. An example of this may be seen from the description of an islet from a treated rat (#496), as given in "Results," and the photograph of an untreated animal (#642), Figure 2.

The adrenal and thyroid changes after alloxan treatment seem to be the result of metabolic disturbances from the ensuing diabetes rather than to a specific toxicity of the alloxan itself. The general findings supporting such a view are (1) the lack of cellular damage, similar to that produced in the pancreas, and (2) the regression of the pathological changes under the influence of insulin treatment.

The histological study of the thyroids of the alloxan diabetic rats suggest a condition of hypofunction. More follicles than in the normal controls show flattening of the epithelium and intense staining of the nuclei, which indicate a decrease in functional activity. This may be seen in Figure 4. An interesting feature is the lack of vacuolization in the follicular colloid material of these

animals. These results are in agreement with the work of Bennett and Koneff (1946) and Bennett, Koneff and Wolff (1948), but are less extensive and less pronounced than their papers indicate, perhaps because of the greater length of time of their experiments. Although they offer no explanation, these workers attach some significance to the homogeniety of the colloid material. Smith and Copenhaver (1948) note that the presence of vacuoles is apparently a fixation artifact, since the colloid of living tissues does not show the vacuoles, nor are they seen when the thyroid is prepared by the freezing-drying technique. However, this artifact may be a means of indicating the viscosity of the colloid. A quotation from Maximov and Bloom (1948) seems pertinent: "Hyaluronidase (the spreading factor) is . . . present; it affects the viscosity of the colloid and varies in activity with the state of the gland." There seems, then, to be a possibility of a relationship between hyaluronidase activity and the diabetic condition, which might bear future investigating.

Figure 5 shows the indications that with insulin administration the function of the thyroid returns toward the normal condition. Most of the follicles show epithelial cells cuboidal in shape with lightly staining vesicular nuclei. There is vacuolization of the colloid. This, with the lack of cellular destruction, bears out the metabolic disturbance idea. Direct damage by the alloxan, by analogy

to the known picture in the pancreatic islets, would presumably give cellular changes which would be irreversible with insulin treatment, particularly with the short time duration of the present experiments.

The separation of the adrenal cortex into zones according to the ability of its secretions to reduce osmic acid was outlined earlier. The substances responsible for this reduction are the unsaturated lipoid materials and other substances, such as ascorbic acid and glutathione. The osmic acid is reduced by these to the oxides of osmium deposited in the gland at the site of origin. The originators of the technique have demonstrated that there is a decrease in reduction of osmic acid associated with a decrease in adrenal function, while adrenal stimulation results in an increased deposition of osmic acid reduction products. In the present study, the adrenals of the diabetic animals fixed in osmic acid showed evidence of hypersecretion. The zones were obliterated and the accumulation of osmiophilic droplets was heavier than normal, as can be seen in Figure 7. Since some hormones of the corticosterone type unquestionably have some carbohydrate-regulating function, this may be another factor in the high blood sugar levels of alloxan diabetic animals. Again, evidence of the alleviation of the hyperfunction is found with insulin treatment. Absence here, also, of cellular damage leads to the conclusion that there is no specific toxic effect

of alloxan on the adrenal gland, but that the metabolic upset from the depletion of insulin from the organism causes the functional disturbances noted.

The "chromaffin reaction" is found in the adrenal medulla when the organ is fixed in solutions containing dichromate (such as Formalin-Zenker) or chromic acid (Maximov and Bloom, 1948). This is the formation of a brown stain, which is believed to be due to the oxidation and polymerization of epinephrine. The reaction was found to be present in all animals, no differences being detected in the various groups.

The discomfort noted on injection of the alloxan into the peritoneal cavity was mentioned earlier. This may have been due to local acid irritation, since the alloxan solution was found to have a pH of about 3. An attempt to raise the pH by addition of NaOH resulted in inactivation of the alloxan. Animals injected with such a solution showed no irritation reaction, but the continued excretion of normal amounts of sugar-free urine for several days indicated that the alloxan was ineffective. Three days later, these same animals were given doses of unmodified alloxan solution, and the characteristic symptoms of diabetes ensued.

Since only one group of males was used in this experiment, no comment on sex differences in response to alloxan can be made. In general, no such differences have been observed in the literature, but a recent work appearing

in abstract (Beach, Bradshaw and Blatherwick, 1950) indicates that female rats are more susceptible than males, both as to incidence and severity of the diabetes.

CHAPTER V

SUMMARY

In the present study, diabetes was produced in white rats by the intraperitoneal injection of alloxan in the dosage of 199 milligrams per kilogram of body weight. This resulted in elevated blood sugar levels, which could be controlled by the injection of insulin. Damage to the islands of Langerhans of the pancreas was found to consist of a general necrosis of the beta cells, leaving islets composed almost entirely of alpha cells at ninety-six hours after alloxan treatment. No alpha cell or acinar pathology was noted.

The thyroid glands of the diabetic animals were found to have homogeneous colloid in the follicles. A large number of these follicles showed flattened epithelial cells with small, compact, densely staining nuclei. This is indicative of hypofunction of the gland.

The adrenal glands showed evidence of increased secretion of the cortical substances capable of reducing osmic acid.

The pathological conditions of both these organs tended to be alleviated by the injection of Protamine Zinc Insulin. No cellular damage of the adrenal or thyroid was noted.

The observations made suggest that the adrenal and thyroid pathology associated with alloxan diabetes is due to a secondary metabolic effect arising from the depletion of insulin from the organism, rather than to a direct toxic action of the alloxan itself.

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APPENDIX

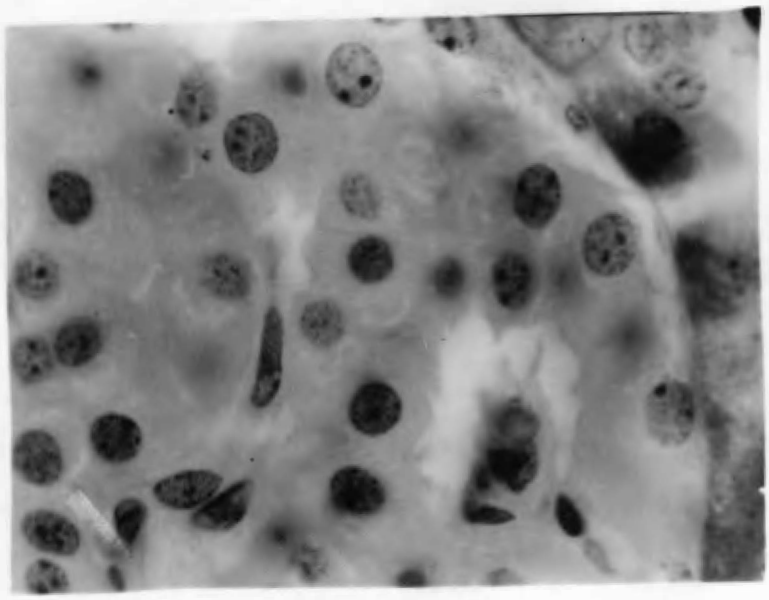

Figure 1

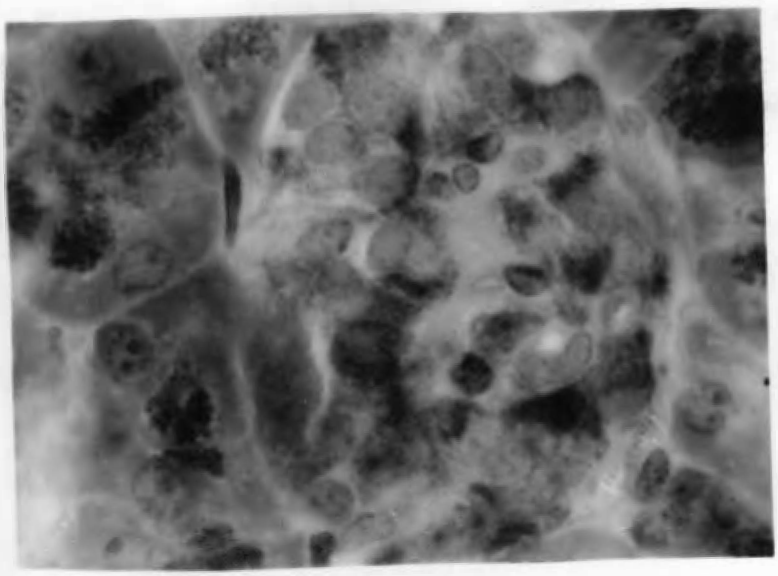

Figure 2

Figure 1. Section of pancreatic islet from normal control animal #4. Gomori's chrome alum hematoxylin stain.

Figure 2. Section of pancreatic islet from alloxan-treated animal #642. Chrome alum hematoxylin stain.

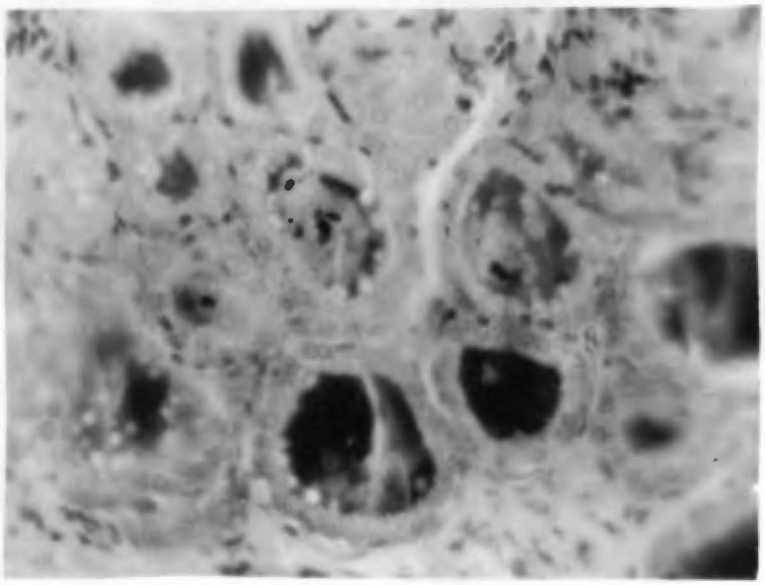

Figure 3

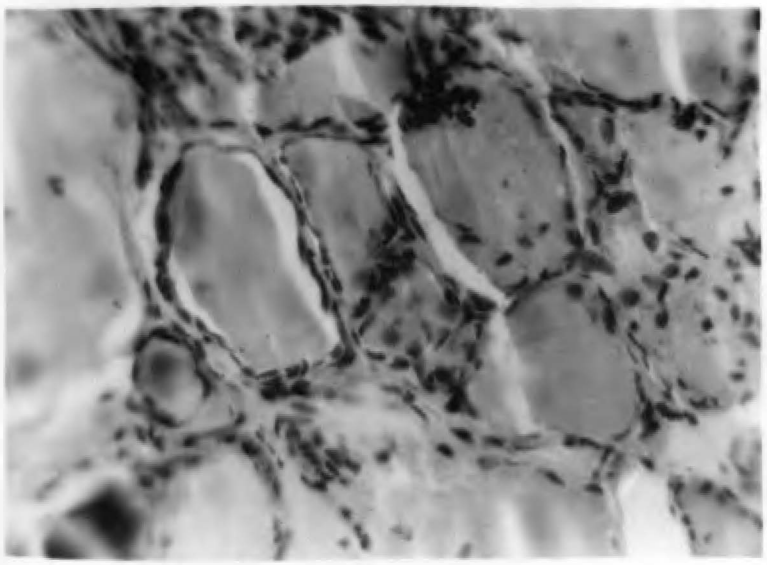

Figure 4

Figure 3. Section of thyroid from control animal #0. Heidenhain's iron-hematoxylin stain.

Figure 4. Section of thyroid from untreated diabetic rat #478. Heidenhain's iron-hematoxylin stain.

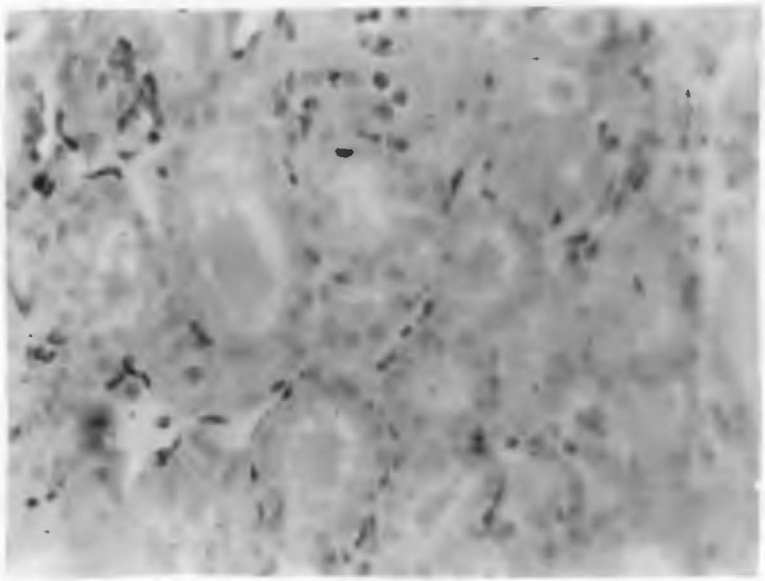

Figure 5

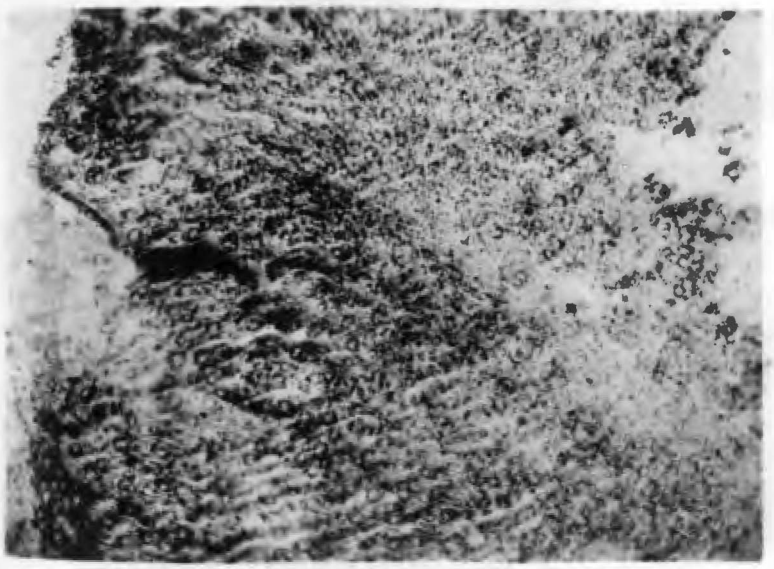

Figure 6

Figure 5. Section of thyroid from diabetic animal #455 receiving insulin injection at forty-eight hours. This section did not take the stain as heavily as the other thyroid sections shown. Heidenhain's iron-hematoxylin.

Figure 6. Section of right adrenal gland from control animal #333. Flexner and Grollman's osmic acid fixation. Medulla to right of figure.

Figure 7

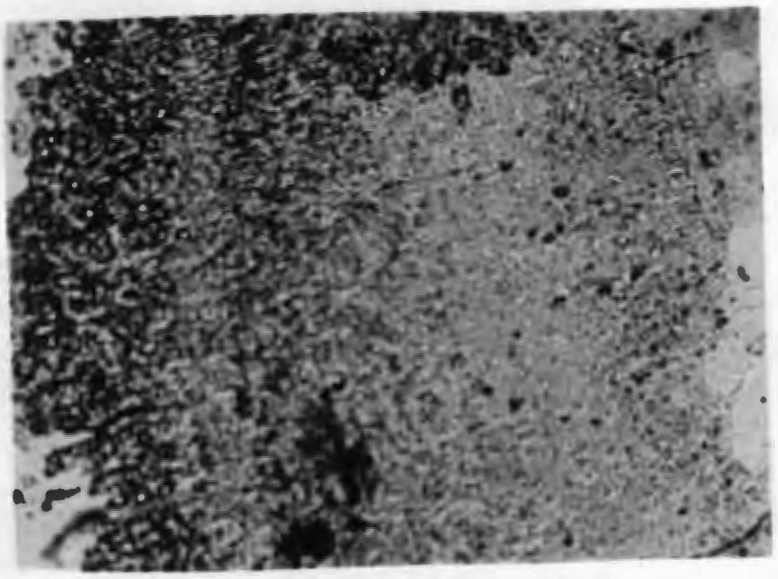

Figure 8

Figure 7. Section of right adrenal, osmic acid treatment. Diabetic animal #237, without insulin. Medulla to right.

Figure 8. Section of right adrenal, osmic acid reduction. Diabetic animal #240 receiving insulin administration. Medulla to right.

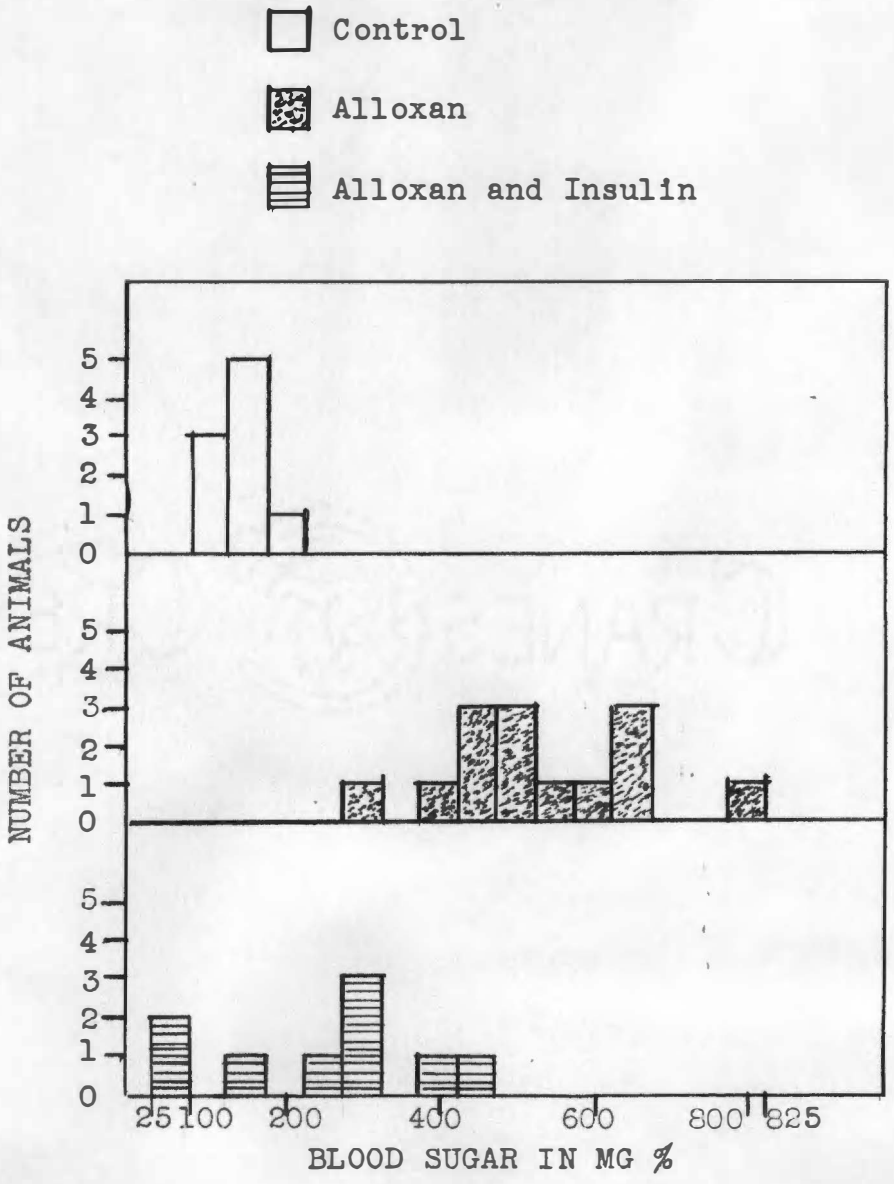

Figure 9. Blood sugar values for normal controls, alloxan diabetic rats, and diabetic rats receiving insulin treatment