ATROPHY OF THE THYROID AND HYPERTROPHY OF THE ADRENAL IN RATS WITH ALLOXAN DIABETES[1]

LESLIE L. BENNETT AND ALEXEI A. KONEFF
Institute of Experimental Biology and Divisions of Physiology, Anatomy, and Medicine, University of California, Berkeley

TWO PLATES (FIVE FIGURES)

Since Dunn, Sheehan and McLetchie ('43) first reported the destruction of beta cells in the islets of Langerhans by alloxan, this drug has been used extensively as a method of production of experimental diabetes. It is now well recognized that alloxan is not entirely specific in its effect upon the beta cells of the islets but also produces pathological lesions in other organs such as the kidney (Dunn and McLetchie, '43; Goldner and Gomori, '43; Duff and Starr, '44), the pituitary (Thomas and Emerson, '45), and the adrenal (Thomas and Emerson, '45; Kendall, Meyer, Lewis and Victor, '45; Hard and Carr, '44). Insofar as we are aware, thyroid atrophy and adrenal hypertrophy have not previously been reported in animals with alloxan-induced diabetes.

METHODS

In our experiments all animals were male rats of the Long-Evans strain and were between 49 and 52 days of age at the start of the experiment. Diabetes was induced by the intraperitoneal injection of 200 mg of alloxan monohydrate per kilogram of body weight on each of 2 successive days. One group of animals was sacrificed 72 hours after the initial

[1] Aided by grants from the Research Board of the University of California, and the James Foundation Grant of the Medical School.

injection and a second group with persistent, severe diabetes mellitus was sacrificed 1 month later. Normal controls of the same age were sacrificed simultaneously. Since the animals with persistent diabetes failed to gain appreciably in weight, a third group of controls was placed upon a restricted diet so that their body weight changes paralleled those of the diabetic animals. With the animals under sodium pentobarbital anesthesia, blood specimens were taken just prior to autopsy and blood glucose was determined by the Somogyi micro-method ('37).

Tissues from twenty normal controls, ten acutely diabetic, seven chronically diabetic, and ten normal rats that had been on the restricted diet were examined histologically. Thyroid glands and left adrenals were fixed in Bouin's fluid, embedded in nitrocellulose, sectioned at 7 micra, and stained by the iron-hematoxylin-aniline blue method (Koneff, '36). The right adrenals were treated with osmic acid according to the Flexner and Grollman technique ('39) to demonstrate the cortical lipids and were cut in paraffin at 7 micra.

RESULTS

In table 1 are summarized the body weight, the thyroid and the left adrenal weights of the 72-hour or acute experiments. The alloxan-treated rats had blood glucose levels varying from 187 mg % to 740 mg %, and it will be seen from the table that there was a decrease in body weight and a slight decrease in thyroid weight. The change in thyroid weight was not statistically significant when the thyroid weight was expressed in terms of the body weight of the animal.

Microscopic examination revealed noticeable changes in all thyroids of this group of experimental animals. The vascularity was considerably decreased and the follicles on the whole were smaller. Not only was there a decreased amount of colloid but also the epithelial cells were flat or cuboidal. The amount of cytoplasm was less than normal and no colloid droplets, ordinarily present in the cytoplasm of follicular epithelium in the normal rat, were found. In general the nuclei

were smaller in size and were mostly oval and darkly stained rather than light and vesicular in appearance as was characteristic of the thyroid glands of the control animals. No mitoses were present. Colloidal material within the follicles was homogeneous and there was no separation of it from the epithelium by vacuole-like spaces.

TABLE 1

The body, thyroid, and adrenal weights of rats sacrificed 72 hours after the initial alloxan injection.

	CONTROL (12) [1]	ALLOXAN (12) TREATED
Onset body weight in gm	172 ± 6.0 [3]	181 ± 4.2
P [2]		.25
Autopsy body weight in gm	184 ± 6.0	161 ± 3.8
P		<.01
Thyroid weight in mg	24.3 ± 2.0	18.8 ± 1.2
P		.03
Thyroid weight in mg per 100 gm body weight	13.2 ± 1.0	11.6 ± 0.7
P		.20
Left adrenal weight in mg	16.0 ± 0.9	22.1 ± 2.2
P		.015
Left adrenal weight in mg per 100 gm body weight	8.7 ± 0.4	13.6 ± 1.3
P		<.01

[1] Number of animals in the group.

[2] From Fisher's ('36) table of t. A value of .01 or less may be considered as highly significant.

[3] Standard deviation of the mean.

The adrenals, on the other hand, were heavier than normal and in terms of body weight the difference from the control was highly significant, the mean value being 22.1 mg per 100 grams of body weight as compared with 13.6 mg for the normal controls.

As judged by analysis of sections stained with the general method, the histological changes in the adrenals of this group

consisted of a somewhat increased vascularity and a greater than normal vacuolation of cells in the inner third of the cortex. Many of the cells in this region were increased in size. Sections of glands treated with osmic acid showed that the narrow subglomerular lipid-free zone had been filled with osmiofilic droplets while the inner third of the cortex contained quite a heavy deposit of lipid (fig. 4). No variation from normal in the size of lipid droplets was noted.

In table 2 are summarized the body weight, the thyroid and the left adrenal weights in the chronic experiments. It will be

TABLE 2

The body, thyroid, and adrenal weights of chronically diabetic rats.

	NORMAL CONTROLS (10) [1]	RESTRICTED FOOD CONTROLS (10)	DIABETIC (15)
Onset body weight in gm	166 ± 7.3	171 ± 4.4	179 ± 5.3
P [2]		.55	.20
P [3]			.20
Autopsy body weight in gm	301 ± 10.7	177 ± 5.9	189 ± 10.5
P		<.01	<.01
P			.35
Thyroid weight in mg	40.3 ± 2.6	23.0 ± 1.3	16.0 ± 0.8
P		<.01	<.01
P			<.01
Thyroid weight in mg per 100 gm body weight	13.3 ± 0.8	13.0 ± 0.5	8.8 ± 0.5
P		.75	<.01
P			<.01
Left adrenal weight in mg	20.2 ± 0.7	13.9 ± 0.7	21.0 ± 0.7
P		<.01	.45
P			<.01
Left adrenal weight in mg per 100 gm body weight	6.7 ± 0.1	8.0 ± 0.5	11.7 ± 0.6
P		.02	<.01
P			<.01

[1] Number of animals in the group.

[2] Compared with the normal controls.

[3] Compared with the restricted food controls.

seen that the diabetic animals had a mean gain in body weight of only 10 gm as compared with 135 gm for the normal controls for the same 31–34 day period. The animals on a restricted food intake gained an average of only 6 gm. The absolute weight of the thyroid was significantly reduced in the restricted food intake group. This reduction was proportionate to body weight, the thyroid weight to body weight ratio being 13.3 mg per 100 gm and 13.0 mg per 100 gm for the normal control and restricted food controls respectively.

Only a slight difference in morphological appearance of the thyroids of rats maintained on restricted food intake was seen when these glands were compared with those of normal control animals. There was only a slightly lower follicular epithelium and a slightly greater amount of colloid in proportion to the mass of epithelial tissue.

In the diabetic animals the blood glucose varied from 381 mg % to 510 mg % and the thyroids were quite small. Their absolute weights averaged 16.0 mg as compared with 40.3 mg for the normal controls, or as compared with 23.0 mg for the restricted food controls. This reduction in weight was highly significant whether considered on an absolute weight basis or relative to body weight.

Morphological changes in the thyroids of this group were not as uniform as those in the glands of animals sacrificed at 72 hours. In the majority of cases the thyroids showed alterations similar in nature to those previously described for the 72-hour group but they were more marked, attaining in most instances the contrast with the normal depicted in figures 1 and 2. The glands of three animals in the 30-day group did not show any pronounced abnormality although the blood glucose of these rats was elevated. The glands which showed the greatest change were those from the animals with the most severe diabetes as judged by the degree of hyperglycemia and the failure of normal growth.

The adrenal weights of the chronically diabetic group were essentially the same as those of their controls but it should be noted that the body weight of the controls was 112 gm greater

than that of the experimental animals. Therefore, relative to body weight, the adrenals of the diabetic animals were definitely enlarged, being 11.7 mg per 100 gm body weight as compared with 6.7 mg per 100 gm body weight for their controls.

In this group of rats, as in the 72-hour group, histological evidence of stimulation of the adrenal cortex was present as judged by the increased vascularity and the heavier than normal deposit of lipid. However, there was a difference in the distribution of osmiophilic material throughout the cortex. The heaviest deposit was found to occupy quite evenly the outer half of the cortex, including the lipid-free zone. In contrast to this, the inner half contained much less lipid and, in some glands did not show any perceptible change from normal (figs. 3 and 5). The adrenals of rats maintained on the restricted diet showed no deviation from normal as to lipid distribution or content, although they were heavier than normal relative to the body weight of the animals.

DISCUSSION

The deviations from normal in the thyroids of our alloxan-treated rats were those associated with hypofunction of the gland, and the question arises as to whether these abnormalities were the direct result of the action of alloxan on the thyroid or whether they were secondary to the deranged metabolism of the diabetic animal. We feel that the latter is the correct interpretation. We have not found acute necrotic lesions in the thyroid of the type that alloxan produces elsewhere. Since there was no cell destruction and alloxan is rapidly inactivated by the body (Goldner and Gomori, '44) it is difficult to see why restoration to normal did not occur in the subsequent 30 days if the changes were the direct effect of alloxan. In support of our belief that the atrophy is the result of the diabetes per se is the observation that thyroid weights of partially depancreatized rats are decreased if there is a marked hyperglycemia (Foglia, '45) and that flattening of the follicular epithelium is known to occur in the

diabetic dog (Licini, '09). Even more convincing is the fact that in preliminary experiments we have found that insulin largely prevented the thyroid changes seen at 30 days, although our data are too few to permit statistical analysis and insulin administration was stopped 2 days before termination of the experiment.

On the other hand the enlargement of the adrenal was associated with histological evidence of cortical hyperfunction. Foglia ('45) has also reported an adrenal enlargement in partially depancreatized rats with severe hyperglycemia but he presented no histological studies. A continued hypertrophy or hyperfunction of a month's duration could hardly be accounted for by an acute direct toxic effect of the alloxan, but is more likely the result of the altered metabolism of the diabetic animal.

SUMMARY

In rats with alloxan induced diabetes there was an hypertrophy of the adrenal and an atrophy of the thyroid. It was concluded that neither of these changes was the result of a direct effect of alloxan upon these organs but rather that they were secondary to the resulting diabetes mellitus.

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PLATE 1

EXPLANATION OF FIGURES

Photographs of figures 1 and 2 were taken with 8 mm objective; magnification × 230. Iron-hematoxylin-aniline blue stain.

1 Thyroid gland of a normal, 82-day-old male control rat.

2 Field selected from a section of the thyroid gland of a rat injected with alloxan 1 month previous to sacrifice. Note the decreased vascularity, small follicles, low epithelium, small, compact nuclei, small amount of colloidal material, and absence of colorless droplets in the follicular colloid.

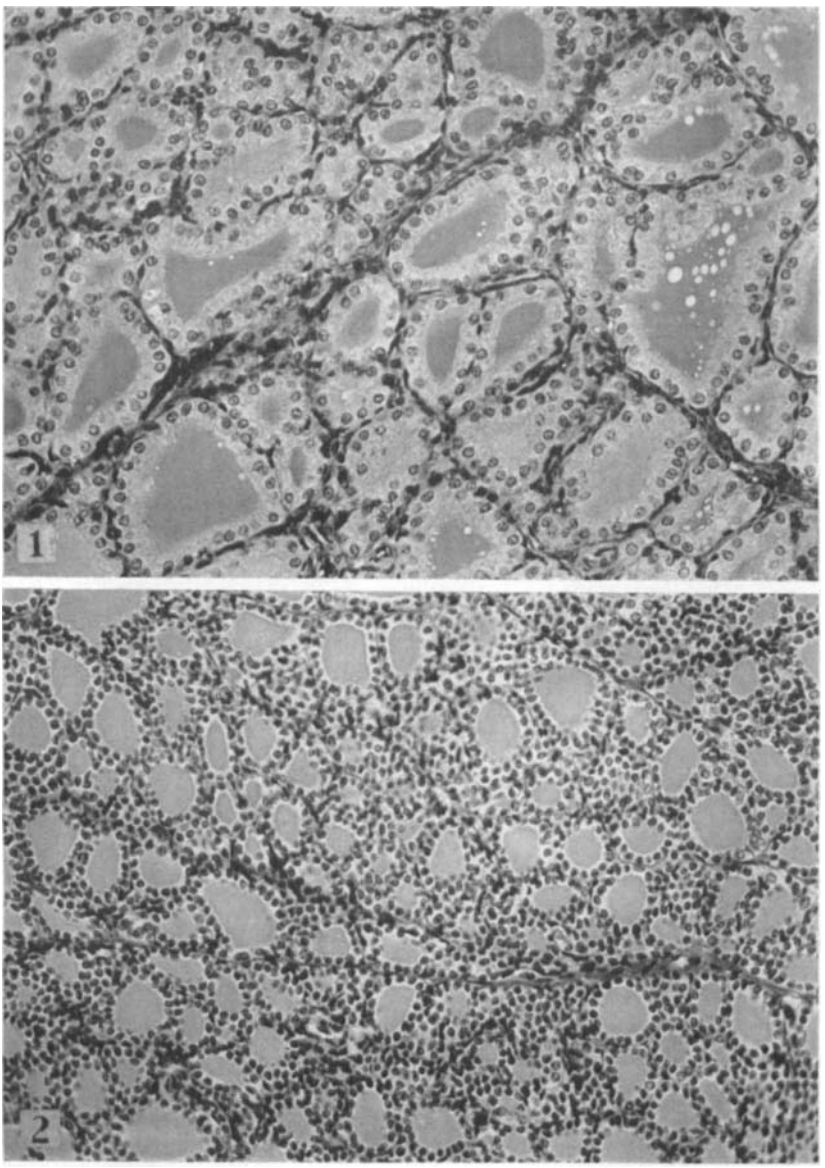
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PLATE 2

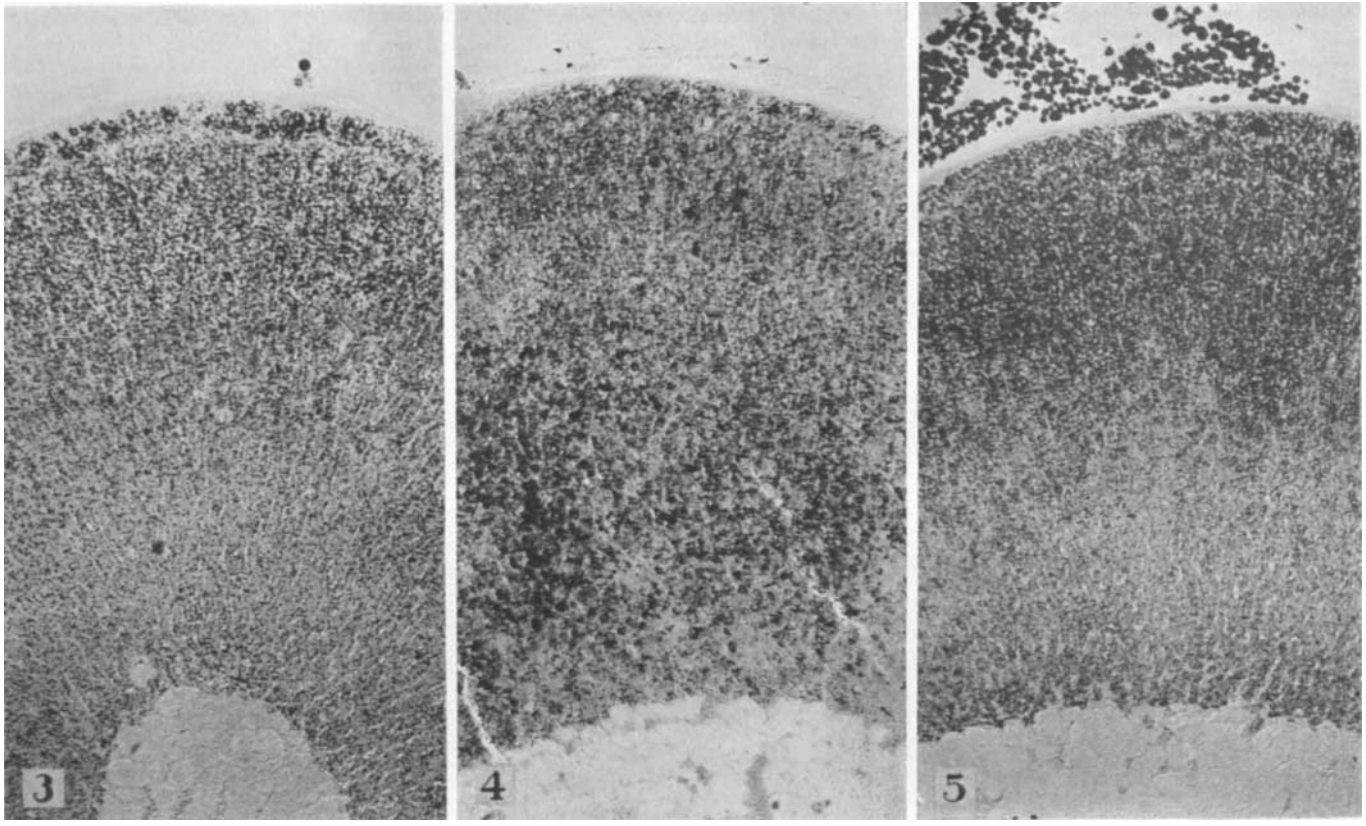
EXPLANATION OF FIGURES

Photographs of figures 3, 4 and 5 were taken with 16 mm objective; magnification 82 ×. Osmic acid impregnation according to Flexner and Grollman technique.

3 Adrenal cortex of a normal, control rat, aged 82 days. Field selected to demonstrate normal distribution of lipids and lipid-free subglomerular zone.

4 Adrenal cortex of a rat initially treated with alloxan 72 hours prior to sacrifice. Note the disappearance of the lipid-free zone and the general increase of lipid content in inner third of the cortex.

5 Adrenal cortex of a rat which had been injected with alloxan 1 month previous to sacrifice. Note the heavier than normal deposit of osmiophilic material, especially in the outer half of the cortex, and the absence of the lipid-free zone.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