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Alloxan Diabetes in Hypophysectomized Rats.*

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Since diabetes can be produced in certain animals by the injection of alloxan[1,2,3] or by the injection of crude anterior pituitary extract,[4-7] this investigation was undertaken to determine whether alloxan produces its diabetogenic effect through the pituitary gland.

For many years an association between the pituitary and diabetes mellitus has been suggested by (1) the high incidence of diabetes and glycosuria among acromegalics,[8] (2) the production of experimental diabetic animals by the injection of crude anterior pituitary extract,[4-7] (3) the marked hypersensitivity to insulin exhibited by hypophysectomized animals,[9] (4) the amelioration of the diabetes in depancreatized dogs or toads following hypophysectomy,[10,11] (5) the suggestion of pituitary overactivity in children[12a] in the few months preceding the onset of diabetes as evidenced by rapid dental and osseous development beyond that expected for their age.

Although both alloxan and crude anterior pituitary extract (A P E) produce islet cell destruction and diabetes when injected into dogs, there are some striking differences in their action, the most outstanding of which are as follows:

(1) The injection of APE produces diabetes only in the dog and in the partially depancreatized rat or cat,[13] whereas alloxan has already been shown to produce diabetes in the dog, rat, cat, rabbit, pigeon, monkey, sheep and turtle.[12b]

(2) Hyperglycemia is imperative for the production of diabetes with APE.[13] The use of insulin, phlorhizin or a low carbohydrate diet which prevents hyperglycemia also prevents the development of diabetes. Alloxan however produces islet cell necrosis and diabetes irrespective of hyperglycemia.[14]

(3) Alloxan produces islet cell destruction and diabetes within 24 hours[15] after injection of a single dose, whereas APE must be injected daily for several days or weeks.[4]

(4) Pancreatic islet lesions produced with a single diabetogenic dose of alloxan show degenerative changes from the start with destruction of all or nearly all of the beta cells within 24 hours.[15] No similar rapid islet destruction follows APE but rather a gradual degranulation with the development of extensive hydropic degeneration of the beta cells.[16]

The production of diabetes by the injection

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16 Duff, G. L., *Am. J. Med. Sci.*, 1945, **210**, 381.

of repeated small doses of alloxan produces some degranulation and hydropic degeneration in the beta cells but not to a degree comparable to that following the administration of APE.[15]

In view of these findings, it seemed appropriate to investigate whether the presence of the pituitary is essential for the production of alloxan diabetes. This report deals with hypophysectomized animals injected with alloxan.

Materials and Methods. White rats of the Hisaw strain, obtained from the Biological Laboratory of Harvard University, were used. They were maintained on Purina Fox-Chow and water. After hypophysectomy glucose water was also made available at all times. (See Discussion.)

Hypophysectomy was done by one of us (C.C.F.) using a parapharyngeal approach through the base of the skull, the technique of which has been described in detail elsewhere.[17]

Approximately 2 weeks after operation, a control micro blood sugar determination (Folin-Malmros method) was done on blood obtained by cutting off the tip of the rat's tail. Blood samples were obtained near the middle of the day throughout the experiments and no attempt was made to withhold food. (See Discussion.) A freshly prepared 5% solution of alloxan (Eastman Kodak Company) was then administered subcutaneously in an initial dose of 150 mg per kg in 15 rats and 100 mg per kg in 4 rats. When no hyperglycemia occurred, the initial dose was repeated or the dose was increased by 25 or 50 mg per kg. Animals were considered diabetic when the blood sugar exceeded 200 mg % 24 or more hours after the injection of alloxan.

In evaluating the completeness of the hypophysectomy the chief clinical criterion used was failure to gain in weight. At autopsy a careful search was made for remnants of the pituitary and, if any were found, the animal was omitted from the series.

In most instances the animals were killed with ether, and autopsied immediately, the fresh tissues being fixed usually in Bouin's and Helly's fluid. In most cases sections were made of all the important organs. The pancreas was examined in all the rats included in the final series. Gomori's chrome-alum hematoxylin stain[18] proved to be most satisfactory for differentiating the beta and alpha cells in the islets of Langerhans.

Results. The final series consisted of nineteen rats. These were divided into groups on the basis of their clinical behavior, as follows:

Group A—Diabetes; animals sacrificed soon after onset	5 rats
Group B—Diabetes; animals allowed to survive for months	3 "
Group C—Transitory diabetes	7 "
Group D—Apparently resistant to alloxan in doses given	4 "

Group A—*Diabetes; Animals Sacrificed Soon After Onset.* This group comprises 5 rats sacrificed after being diabetic for periods varying from 2 to 28 days. Four of these animals had blood sugar levels over 400 mg %, after a single dose of 150 mg alloxan per kg. The fifth animal received doses of 100, 125, and 150 mg per kg at approximately weekly intervals without obvious effect but developed hyperglycemia after 175 mg per kg was given. The incidence of diabetes in this group does not appear to differ from that in normal rats injected with alloxan. (See Discussion.)

Group B—*Diabetes; Animals Allowed to Survive for Months.* The 3 rats in this group remained diabetic for relatively long periods, one for 2½ months, and 2 for 8½ months. One of these required a dose of 300 mg per kg before diabetes was definitely established. All 3 animals showed some fluctuation in blood sugar levels, all having occasional blood sugar values below 200 mg %. This point is commented upon in the discussion below. One animal developed cataracts in both eyes.

Group C—*Transitory Diabetes.* Six of the 7 animals in this group showed a single high blood sugar a few days following the first injection of alloxan and then one or more

[17] Franseen, C. C., Brues, A. M., and Richards, R. L., *Endocrinology*, 1938, **23**, 292.

[18] Gomori, G., *Am. J. Path.*, 1941, **17**, 395.

blood sugars below 200 mg %. Three of the rats later responded to a larger dose of alloxan with a blood sugar over 200 mg %, but all 3 again had a subsequent blood sugar less than 150 mg %. In other words, the blood sugar levels showed wide fluctuations and a striking tendency to revert to normal.

Group D—*Animals Apparently Resistant to Alloxan.* The 4 animals in this group were given from 2 to 5 injections of alloxan, 2 of them receiving a maximum dose of 200 mg per kg and the other 2 a maximum dose of 300 mg per kg without developing hyperglycemia. It is noteworthy that one animal showed apparently complete loss of beta cells from the islets of Langerhans and another showed an acute degenerative change in the beta cells probably due to the last injection of alloxan.

Pathological Findings. In 2 animals (one from Group B, one from Group D), the histological preparations were unsatisfactory owing to postmortem change. The islets of Langerhans in the remaining 17 rats showed loss of beta cells in varying degree, with apparent complete loss of beta cells in the majority, the islets being composed of alpha cells staining pink with the Gomori chrome-alum hematoxylin method. A few surviving beta cells were present in some islets of 2 rats in Group A, one in Group C, and one in Group D. No definite necrosis or other lesion of organs such as liver, kidney, and adrenal was noted. The expected findings in the hypophysectomized animals—*e.g.*, atrophy of gonads, adrenals, and thyroid—were also present.

Discussion. Hypophysectomized rats fail to grow and their fur remains fine and soft. Their weight tends to remain constant and they show an abnormal response to both insulin and to adrenalin. These animals show marked hypersensitivity to insulin and respond to about one-tenth the dose of insulin required to act in normal animals.[19] Adrenalin produces less hyperglycemia than it does in the intact animal. Since hypophysectomized rats show a marked tendency to hypoglycemia and since the administration of alloxan is followed by a transitory hypoglycemia, especial care must be taken to prevent fatal hypoglycemia in hypophysectomized rats given alloxan. For this reason, glucose water was made available.

Perhaps the most striking feature of the experimental results is the large number of animals showing diabetes which was either transitory or fluctuating. These included not only the rats in Group C (transitory group), but also the 3 rats in Group B (long duration), all of which showed one or more normal blood sugars during their course. In other words, even in the permanently diabetic hypophysectomized animal, the blood sugar showed a definite tendency to revert to normal.

In unoperated animals made diabetic with alloxan, a certain number may be expected to exhibit a transitory course, but the fluctuations were not so striking as in the present series. In a previous series of 50 control rats given a dose of 150 mg in this laboratory, the results were classified as follows:

	%
Permanent diabetes	58
Transitory diabetes	12
Resistant to 150 mg	22
Died before first blood sugar	8

In this control group the rats exhibiting permanent diabetes showed sustained high blood sugar levels, without reversion to normal, while those classed as transitory showed high blood sugar levels for a week or so, the subsequent ones being within the normal range. In other words, the striking fluctuations seen in the hypophysectomized animals were not present in the controls.

It is also noteworthy, as remarked above, that all the animals in which good histological preparations were obtained showed destruction, to a greater or less degree, of the beta cells in the islets of Langerhans; *i.e.* the anatomical lesion was present regardless of the apparently mild course of the diabetes.

The phenomenon of amelioration of diabetes following hypophysectomy is, of course, not new, being well known in the "Houssay animal", where hypophysectomy is followed

[19] Russell, J. A., *Physiol. Rev.*, 1938, **18**, 2.

by improvement in the diabetes produced by pancreatectomy. The results of the present experiments are, therefore, not surprising and it would seem that there is no more evidence that alloxan acts through the pituitary than there is that pancreatectomy exerts its effect in this way.

The general question of the relation of the pituitary to carbohydrate metabolism is well discussed by Russell,[19] Houssay,[20] and Lukens.[21]

A preliminary report on the production of alloxan diabetes in hypophysectomized rats was published from this laboratory in 1945.[22] As far as we are aware, the only other reports on alloxan diabetes in hypophysectomized animals are those of Kirschbaum *et al.*,[23] of Duff and Starr,[24] and of Gaarenstroom.[25]

Kirschbaum, Wells and Molander[23] found that hypophysectomized rats injected with alloxan developed severe hypoglycemic convulsions but no experiments were carried beyond 6 hours and no histological sections were reported.

Duff[16] describes unpublished observations by Duff and Starr in which hypophysectomized rats were given alloxan and some survived the initial hypoglycemic stage. Duff states that "although histologic studies showed destruction of the islets of Langerhans quite as extensive as in intact animals, there was no evidence of diabetes during the periods of survival. The blood sugar remained at more or less normal levels, but there were irregular fluctuations somewhat above and below the normal limits."

Gaarenstroom[25-27] reported that extirpation of the hypophysis in animals already made diabetic with alloxan led to a marked decrease or even total disappearance of glycosuria and a fall in blood sugar, if the animals were fasted. After administration of sugar the blood sugar rose again to high levels.

Conclusions. 1. Diabetes was produced by the injection of alloxan in 15 of 19 hypophysectomized rats. 2. The diabetes so produced tended to be either transitory or fluctuating in severity and it is suggested that this phenomenon represents the "Houssay effect" seen in depancreatized hypophysectomized dogs. 3. Extensive loss of beta cells in the islets of Langerhans was noted in all animals in which satisfactory histological preparations were obtained. The presence of hypophysis is therefore not necessary for the production of what appears to be the essential lesion in alloxan diabetes.

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