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Experimental Diabetes Produced by Alloxan.

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Experimental selective necrosis of the islands of Langerhans has recently been recorded by Dunn, Sheehan, and McLetchie.[1] In a limited number of animals the injection of alloxan produced a selective necrosis of islet tissue and the epithelium of the convoluted tubules with characteristic physiological symptoms. These observations were confirmed and extended to dogs and other species including man.[2,3,4] The obvious value of an experimentally produced diabetes and a search for the mechanism of action of alloxan led us to the following studies.

Influence on Blood-Sugar Level. Rabbit. Twenty-five animals were used for studies on the influence of alloxan on blood-sugar level. A dose ranging from 10 mg per kg to 300 mg per kg intravenously in a 5% solution was administered and blood samples taken for analysis at intervals thereafter.[5] The compound was well tolerated and even in large doses no untoward symptoms were observed for a period of 5 hours. Both fasted and unfasted animals were used. The data of these experiments are summarized in Table I.

An analysis of the data in Table I reveals that these animals group themselves into 2 classes. With small doses up to 50 mg per kg there is little or no influence upon blood-sugar level. Larger doses appear to produce in most animals an immediate rise in blood sugar to concentrations of 200 mg % or greater followed by a subsequent return to, or below the normal value within 24 hours and this, in turn, is followed by a secondary rise to diabetic level, which is maintained for weeks. An ideal curve is given in Fig. 1. Jacobs[6] previously observed the initial rise in blood sugar concentrations and the subsequent fall to low levels but did not extend his observations beyond 24 hours, hence, the secondary rise was not observed. Not all animals injected respond uniformly. Our observation is that if a diabetic response is to be obtained in an animal, the initial curve produced within the first 24 hours will demonstrate this fact. A refractory animal generally will not respond to successive doses of alloxan.

In order to determine the source of the elevated blood glucose liver glycogen deter-

TABLE I.
Influence of Alloxan on Blood-Sugar Level of Rabbit.

No. of animals	Dosage, mg/kg	Response	Blood sugar mg per 100 cc blood: Control, avg	2-4 hr after inj.	Daily avg thereafter
2	10	Negative	127	134	102
3	25	"	116	118	115
5	50	1—diabetic	106*	259*	268*
13	100	8—diabetic	109*	267* (185-400)	295* (247-396)
2	300	Hypoglycemic convulsions	107	39	death in 5 hr

* Average values for diabetic animals only.

1 Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **11**, 384.

2 Gomori, G., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 287.

3 Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1165.

4 Brunschwig, A., and Allen, J. G., *Cancer Research*, 1944, **4**, 45.

5 Folin, O., *J. Biol. Chem.*, 1928, **77**, 421.

6 Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937-38, **37**, 407.

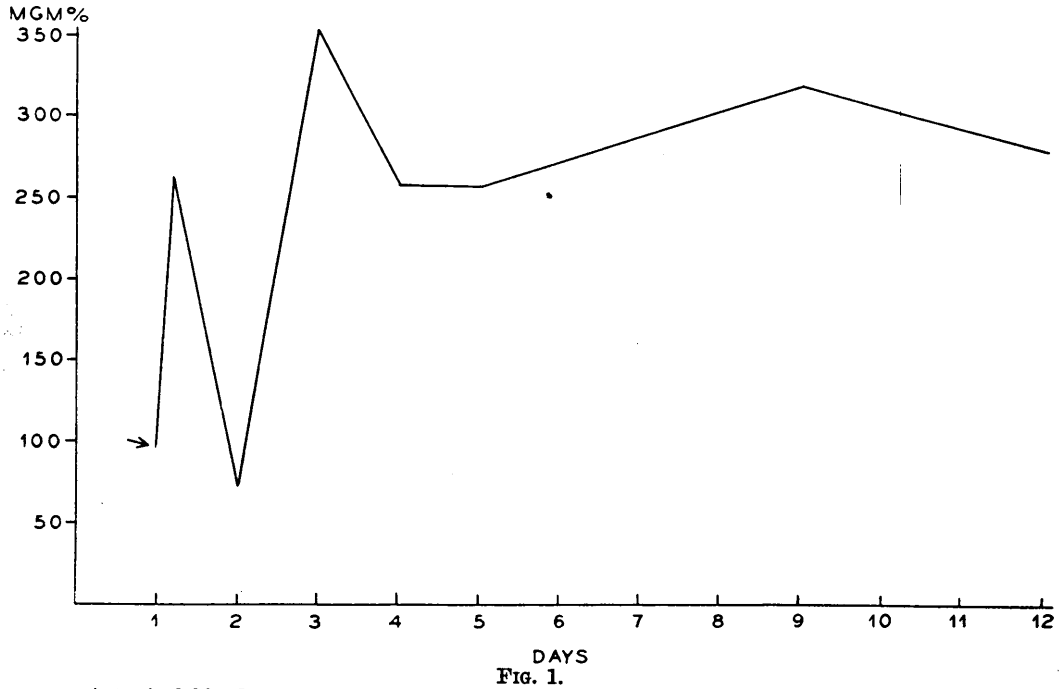


FIG. 1.
A typical blood-sugar curve obtained after intravenous injection (arrow) of 100 mg per kg of alloxan.

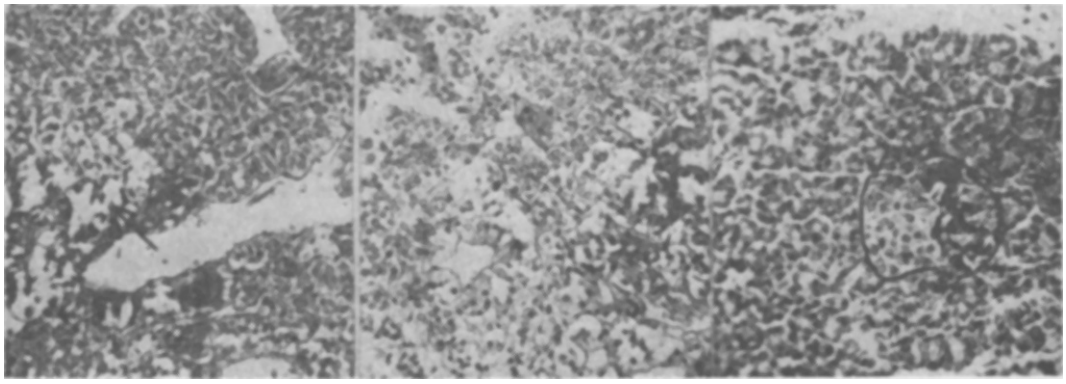
minations were made on 7 rabbits in periods varying from 3 hours to 25 days following injection. All values were well within a normal range, averaging 3.46% A similar result was observed in 10 rats sacrificed 48 hours following injection of 200 mg per kilo dosages of alloxan. This type of evidence suggests that the capacity of the liver to store carbohydrate as glycogen is undiminished, but does not satisfy the question of elevated blood sugar. However, the initial rise in blood glucose (Fig. 1) may be due to adrenal stimulation as is indicated by typical epinephrine type blood pressure curves obtained from dogs after 100 mg doses of alloxan, and from the histological findings.

It may be noted that the extreme fatty infiltration reported for the diabetic dog[7] has not been evidenced in our rabbits. Values of 12.4, 11.2, and 13.1 g % dry weight have been obtained in animals diabetic for 7, 11, and 25 days respectively.

Histological Findings. Autopsies were performed on 8 animals under nembutal or dial anesthesia. Tissues from the pancreas, liver, adrenals, and kidneys were appropriately fixed for cytological study. In gross appearance all organs were normal in these animals. Routine hematoxylin-eosin and Heidenhain-azan stains were used but, in addition, sections of the pancreas were treated with Gomori's modification of the azan technic and Bensley's neutral gentian for demonstration of the alpha and beta cells.

Our histological observations on the effect of acutely toxic doses of alloxan on the pancreas are essentially similar to those previously described for the rabbit,[1] dog,[4,7] and rat.[2] Changes noted are first, a pyknosis of the beta cells with the alpha cells unaffected, and, secondly, a shrinkage of the cells accompanied by degranulation. No histological changes were noted in the kidney or liver of these animals. A marked effect, however, was seen in the adrenal gland. In nonlocalized areas of the medulla there occurred a marked fragmentation and shrinkage of the cells with some cellular debris resulting (Fig. 2). Remaining areas of the gland appear to be unaffected but no definite statement can

[7] Goldner, M. G., and Gomori, G., *Endocrinology*, 1943, **33**, 297.



A B C

FIG. 2.

A. Adrenal medulla, rabbit, 300 mg per kilo. Fragmentation and shrinkage of cell cords in certain areas (arrow) with nuclear remnants. Adjacent areas show normal cellular arrangement. Heidenhain-azan × 100.

B. Adrenal medulla, rabbit, diabetic 7 days. Small areas show some fragmentation and shrinkage. In less affected glands vacuolation is the only noticeable effect. Hematoxylin-eosin × 100.

C. Pancreatic islet, rabbit, diabetic 25 days. The outlined islet is composed of the deeply stained alpha cells and nongranular cells. × 100.

be made with respect to a variation in the chromaffin reaction.

Microscopical findings on diabetic animals sacrificed on the 7th, 11th, and 25th days after alloxan administration show significant effects in only the pancreas and adrenal glands. The pancreas of the animals diabetic for 7 and 11 days are essentially alike. When specially stained they show apparently a normal quota of islets but many of them are small and made up entirely of granular alpha cells. In a few islets undifferentiated cells appear which are practically devoid of cytoplasm. Their nuclei, conspicuous by the reduction in cytoplasm, are normal in reaction. In the 25-day diabetic animal no pathology could be detected in hematoxylin-eosin preparations; the islets are numerous, many of normal size distribution and cellular content. Specially stained preparations show the majority of cells either alpha or nongranular (Fig. 2) but with some islets showing granular beta cells. The granular content of these cells varies considerably ranging from the normal distribution to cells with very few granules. In many islets the ratio between the alpha and nongranular cells is equal to that normally occurring between the alpha and beta cells. Small islets are composed entirely of either alpha or nongranular cells. There is a suggestion, therefore, that with the dosages employed complete beta cell necrosis does not occur but that regeneration of a functional cell is possible with an expected amelioration of the diabetes.

The adrenal medulla of the permanently diabetic animals are normal excepting for small areas in which the cells show some degree of cytoplasmic vacuolation (Fig. 2). This is neither as extensive nor exhibits the degree of shrinkage and fragmentation seen in the animals given acutely toxic doses. Absence of any medullary damage in a 25-day diabetic animal suggests that a complete recovery has ensued by this period.

Summary. The administration of alloxan in one suitable dose produces a pancreatic experimental diabetes. Satisfactory animals exhibit a prolonged high blood-sugar level that is maintained for weeks. No renal lesions are observed in such animals and the fat and glycogen content of the liver is within normal limits. Adrenal and pancreatic islet cellular changes may explain the character of the acute phase.

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