none of the previous effects on tongue, palate or mouth cavity, while the respiratory rate remained unaltered. Thus atropinization abolishes and precludes the action of ACh.

In another experiment a rectangle of ACh.Cl. 1:50 millions to the XII nuclei, followed by one of ACh.Cl. 1:10 millions augmented tremors in the tongue, initiated by intravenous eserine, and also evoked furrowing, tongue retraction and later violent generalized tongue movements; applications also evoked repeated deglutitions.

In a *non-eserinized* decerebrate preparation ACh.Cl. 1:50 millions on both XII nuclei yielded, in 35 sec. (longer than after eserinization), movements of tip of tongue, retractions and repeated deglutitions; thus, while responding to ACh., the nucleus was definitely less sensitive than after eserinization; also its responsiveness declined to further applications.

Discussion. The view is expressed here that ACh. exerts a local stimulating or facilitating action on synaptic terminations in the XII nucleus; support for this view is afforded by the following considerations: the general similarity between effects of ACh. application and faradization of nucleus; the slight depth of nucleus below floor of fourth ventricle, permitting rapid diffusion to it of ACh , the extreme dilutions (1:50 millions) of effective solutions of ACh.; the brief latency for effects of ACh. (4 to 15 sec. in some cases). The neurones of the XII nucleus are surrounded by numerous synapses derived from an extensive interneuronal plexus.[5] Thus it is inferred that ACh. diffuses and directly stimulates or facilitates these synaptic terminations and that this action is much intensified by eserinization. The specific nature of the action is further emphasized through its being annulled by atropinization.

Deglutition may be explained by the action of ACh. near the inferior fovea; faradization of this point yields reflex deglutition[6] and it was supposed that the currents impinged on afferent fibers in the *tractus solitarius,* which lies sufficiently close to the inferior fovea. Similarly the ACh. may be supposed to diffuse to, and stimulate or facilitate, synaptic afferent terminations (probably glossopharyngeal and vagal) within the nucleus of the *tractus solitarius;* fibers from this nucleus probably pass to the XII nucleus, the nucleus ambiguus and dorsal vagus nucleus, through which pathways deglutition would be evoked.

5 Cajal, S. R., *Système Nerveux,* A. Maloine, Paris, 1909, **1**, 706.

6 Miller, F. R., and Sherrington, C. S., *Quart. J. Exp. Physiol.,* 1916, **9**, 147.

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Production of Diabetes Mellitus in Rats with Alloxan.*

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Jacobs[1] observed that alloxan causes hyperglycemia followed by hypoglycemia in rabbits. Shaw Dunn and associates[2,3] have shown that the pathologic substrate of this response is necrosis of the pancreatic islets. Further study of the animals was impossible because they all died within a few days. Brunschwig, Allen, Goldner, and Gomori[4] succeeded in keeping rabbits alive for longer periods after

* This work has been supported by grants from the Douglas Smith Foundation for Medical Research of the University of Chicago and from Eli Lilly and Company, Indianapolis.

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2 Shaw Dunn, J., Sheehan, H. L., and McLetchie, N. G. B., *Lancet,* 1943, **244**, 484.

3 Shaw Dunn, J., Kirkpatrick, J., McLetchie, N. G. B., and Telfer, S. V., *J. Path. and Bact.,* 1943, **55**, 245.

4 Brunschwig, A., Allen, J. G., Goldner, M. G., and Gomori, G., *J. A. M. A.,* 1943, **122**, 966.

the injection of alloxan and found that the hypoglycemic phase is followed by temporary hyperglycemia. Bailey and Bailey[5] produced permanent diabetes in rabbits with the same chemical. Their histologic findings were complete disappearance of the islets. Goldner and Gomori[6] made dogs permanently diabetic by a single intravenous injection of alloxan. They observed degranulation and later complete disappearance of the beta cells while the alpha cells were found to be unaffected. Very recently, Shaw Dunn and McLetchie[7] reported the successful production of diabetes in rats. The histologic changes in the islets were necrosis of the central cells with survival of the peripheral cells. The latter were considered alpha cells although no special stains were applied to confirm this contention.

The experiments here described were performed independently of those of Shaw Dunn and McLetchie, after previous studies[6] had shown that rats are sensitive to alloxan.

Methods. Forty-four young white rats and 5 hooded rats, weighing 140 to 180 g, were used. It was found impossible to produce clinical symptoms by the subcutaneous injection of daily doses increasing from 50 to 175 mg/kg over a period of 8 days. Since, on the other hand, an intraperitoneal injection of 200 mg/kg was almost invariably effective, 40 rats were treated in this way.

A single dose of alloxan was injected intraperitoneally. A freshly prepared 5% solution in distilled water was used. Six animals were put in individual metabolism cages on the first day and killed by decapitation at intervals from the third hour on. Three or 4 more animals were placed in metabolism cages on each of the following 5 days. Their urine was collected for 24 hours and analyzed for sugar and ketone bodies. At the end of this period the animals were weighed and killed. The blood was analyzed for sugar only, since its amount was not sufficient for more detailed studies. The pancreas was fixed in a modified Bouin's solution (4 g of potassium chromium sulfate dissolved in a mixture of acetic acid, 5 cc; 40% formaldehyde, 25 cc; saturated aqueous solution of picric acid, 75 cc) which was found to permit a better differentiation of the cell types in the islets than other fixatives. The liver and the kidneys were fixed in 80% alcohol. All tissues were embedded in paraffin. Sections of the pancreas were stained with chrome hematoxylin and phloxin,[8] with Mallory-Heidenhain's azan stain and with Mallory's phosphotungstic hematoxylin as modified by Gomori.[8] Sections of the liver and kidneys were stained for glycogen with the Bauer-Feulgen[9] stain.

Results. Three white rats were found dead and cold in their cages and were discarded. In 5 animals no blood sugar determination was done. The 5 hooded rats were found to be resistant to alloxan under the conditions of these experiments. Fig. 1 shows the chemical findings in the remaining 27 animals and in 9 normal controls. Only 4 animals of this group were found to be resistant to alloxan. All the others showed hyperglycemia and glycosuria. Ketonuria was observed from the third day on and was present on subsequent days in all animals which had a blood sugar level above 300. Considerable polyuria was quite common; the daily amount of the urine often exceeded 10% of the body weight and reached 40 cc in one instance. Some of the animals had a pink urine on the first day after the injection. Many of the animals looked quite sick, especially after the first 2 days, but ate well until ketonuria set in with complete loss of appetite. They lost an average of 30% of their original weight in 6 days.

Histology. The following description applies to sections stained with chrome hematoxylin and phloxin.

Some nuclear pyknosis of the beta cells was observed as early as 3 hours after the injection. The 8 hours' specimen showed marked pyknosis of most of the beta nuclei with shrinkage of the cytoplasm. At 20 hours the overwhelming majority of the beta cells were greatly shrunken, and in some cases the cell cords were disintegrated into individual cells; in

[5] Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1165.

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

[7] Shaw Dunn, J., and McLetchie, N. G. B., *Lancet*, 1943, **245**, 384.

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

[9] Bauer, H., *Z. mikr.-anat. Forsch.*, 1933, **33**, 143.

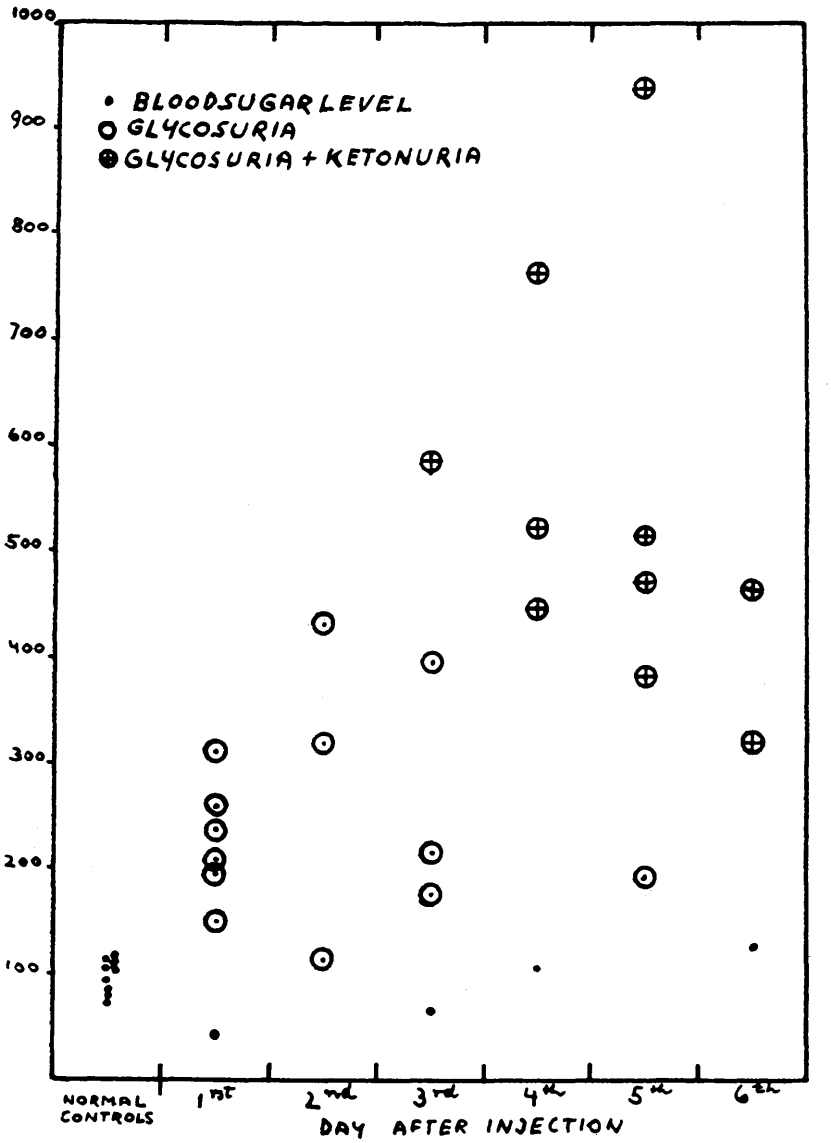
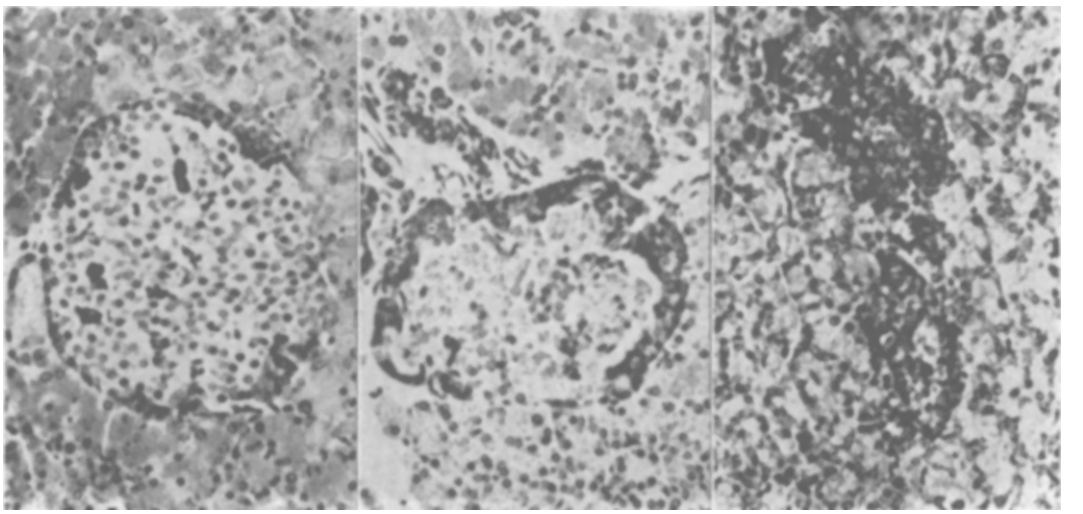


FIG. 1.

Hyperglycemia, glycosuria, and ketonuria in 27 rats after intraperitoneal injection of alloxan (200 mg/kg).

other cases the cords coalesced into an almost homogeneous debris in which individual cells could not be recognized. On the second day these changes became more accentuated, and, at the same time, the beta cells started to lose the typical staining of their granules and to be transformed into pale bluish-gray shadows. From the third day on these shadows disappeared very rapidly. On the fourth and fifth days only an occasional beta cell could be seen. At the same time agranular cells of an unidentifiable type appeared in large numbers and filled the areas previously occupied by the necrotic beta cells. At the periphery the alpha cells, which seemed to have escaped all injury, also proliferated considerably. By the fifth and sixth days the islets were entirely free from debris, and with the routine hematoxylin-eosin stain looked practically normal. However, special stains showed that they consisted of agranular and alpha cells only; beta cells were completely absent. No vacuolation



A B C

FIG. 2.

Microphotographs of pancreatic islets of rats (phosphotungstic acid hematoxylin stain).
A. (Left) Normal islet. Dark (alpha) cells at the periphery.
B. (Center) Necrosis of the central light (beta) cells on the second day. Dark peripheral (alpha) cells normal.
C. (Right) Sixth day. The islets consist almost exclusively of dark (alpha) cells.

of the islet cells was seen at any stage of the process; mitoses were extremely rare. The ducts and the acinar parenchyma showed no change.

The liver contained very little glycogen after the second day but was normal otherwise in all cases except 2 in which focal necroses were observed.

The kidneys did not show any change of importance. From the fourth day on deposition of glycogen in Henle's loops was quite common.

The organs of the hooded rats were completely normal. Out of the 4 white rats that failed to respond 2 showed no changes at all, while the 2 others showed slight degenerative changes of the beta cells.

Comment. Shaw Dunn and McLetchie produced diabetes in rats by repeated subcutaneous injections of alloxan; glycosuria usually became apparent after a number of injections had been given. In the present experiments a single intraperitoneal injection of alloxan was promptly followed by symptoms of diabetes. The histologic changes of the pancreas, as far as it is possible to compare the results obtained with different staining techniques, seem to be identical in the 2 experiments.

The remarkable resistance of hooded rats to alloxan may be due to the exceptionally large amount of islet tissue this strain possesses (Richardson and Young).[10]

Summary. A single intraperitoneal injection of 200 mg/kg of alloxan into white rats causes typical diabetes mellitus. The histologic changes are necrosis and complete disappearance of the beta cells from the pancreatic islets.

[10] Richardson, K. C., and Young, F. G., *J. Physiol.*, 1937, **91**, 352.