EXPERIMENTAL DIABETES IN GUINEAPIGS BY ALLOXAN

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Experimental diabetes was produced first by total or partial pancreatectomy in dogs by Von Mering and Minkowski in 1889. Another method of inducing diabetes was by the administration of anterior pituitary extract (Young, 1937), third form of experimental diabetes was by the administration of alloxan, after its discovery by Dunn, Sheehan and McLetchie (1943). This substance was shown to have selective destructive action on the beta cells of islets of Langerhans of the pancreas. Apart from these action permanent forms of experimental diabetes, there are other diabetogenic substances, nearly ninety in number (Lukens, 1959) which can cause varying temporary hyperglycaemia.

In the course of the production of experimental diabetes by various methods, a search for animals that would survive with diabetes was made. The effect of alloxan on beta cells of islets of Langerhens' was found to vary with species apart from the variability in dose. In some animals the effect is not only instantaneous but also permanent, e. g., rats, rabbits, (Lukens, 1948), but in some it is not so, e. g., sheep, pigeon, dogs (Lukens, 1948). It may be very difficult to produce alloxan diabetes in ducks as it was reported that the islets in duck's pancreas did not contain beta cells. Toads do not develop diabetes after alloxan, may be, the beta cells in them are resistant to the drug (Lukens, 1948).

METHOD

Guineapig was chosen, as the experimental animal in the present work, and alloxan as the chemical diabetogenic agent. Guineapig was chosen as it was not reported to have been tried as an experimental animal by earlier workers, for inducing alloxan diabetes (Lukens, 1948).

Twenty two adult guineapigs (of both sexes) weighing between 500 and 700g were chosen for the experiment. They were isolated from the other animals one week before the experiment. The animals were kept on a standard normal diet prescribed by the Nutritional Laboratories, Hyderabad. Each animal was given 30g of the standard diet per day.

The initial blood sugar levels in all the animals were determined according to Folin and Wu's photometric method, using Beckman's Spectrophotometer, blood being obtained by direct puncture of the heart.

Urinary output was measured daily and routine examination conducted in all the above animals using the normal clinical laboratory methods.

Before alloxan was given, the animals were divided into controls and experimental groups. The control group consisted of 4 guineapigs. The experimental group consisted of 18 guineapigs.

Alloxan monohydrate 500 mg/kg in distilled water was given by intraperitoneal route to each of the experimental animals. In many, a single dose was found to be adequate to produce a sustained hyperglycaemia; while in a few it had to be repeated after an interval of 3 days. All animals showed signs of toxicity such as convulsions and lethargy, during the first few hours after the injection.

The animals were kept on the same diet and urine analysis was done after 48 hrs for the total output and detection of sugar, acetone, albumin etc. Estimations of blood sugar in all the experimental animals was done after 72 hrs. Confirmation of the establishment of permanent hyperglycaemia was done by repeated examination of urine for the presence of sugar and estimation of blood sugar every 10th day, upto 60 days after which, the animals were sacrificed, for histophysiologic examination of islets of pancreas.

The tissues were fixed in Bouins and Gomori's fixatives and sections, 4 m thick, were taken on a Spencer's Rotary Microtome and stained with Gomori's chrome haematoxylin phloxin-B method. Microphotogrophy of the sections were taken with the help of a camera and the microphotograph enlarged four times.

RESULTS

A. *Biochemical* :

Control Group

Avg. initial blood sugar mg%	Avg. blood sugar after 60 days mg%	Avg. urinary output/day (ml)	Urine analysis
70 to 110	70 to 110	30	no albumin no sugar no acetone

Experimental Group (Alloxanized)

Before alloxan			After alloxan			
Avg. initial blood sugar mg	Avg. urinary output/ day (ml)	Urine analysis	Avg. blood sugar mg% after 3rd day	Avg. blood sugar mg% after 60th day	Avg. urine output (ml)	Urine analysis
70 to 110	30	no albumin no sugar no acetone	170 to 190	160 to 190	60	no albumin sugar + no acetone

B. *Histophysiological Results*:—

Normals (Control) : Showed well defined islets of pancreas, the alpha cells stand out at the periphery, while beta cells are clearly seen in the centre (Fig. 1, 2, 3).

Alloxanized group.—Selective destruction of beta cells was observed in some islets, where as alpha cells were intact. In some islets, degenerated or shrunken beta cells are seen. All these are suggestive of beta cell destruction by alloxan. (Fig. 4, 5, 6, 7).

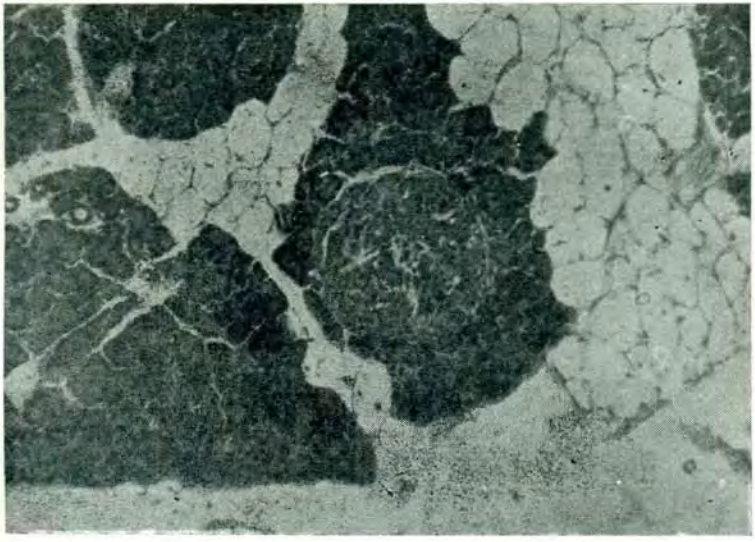

Fig. I Pancreas guineapig-control, Gomori's chrome haematoxylin phloxin. Islets well demarcated-α-cells stand out peripherally with β-cells in between x 100

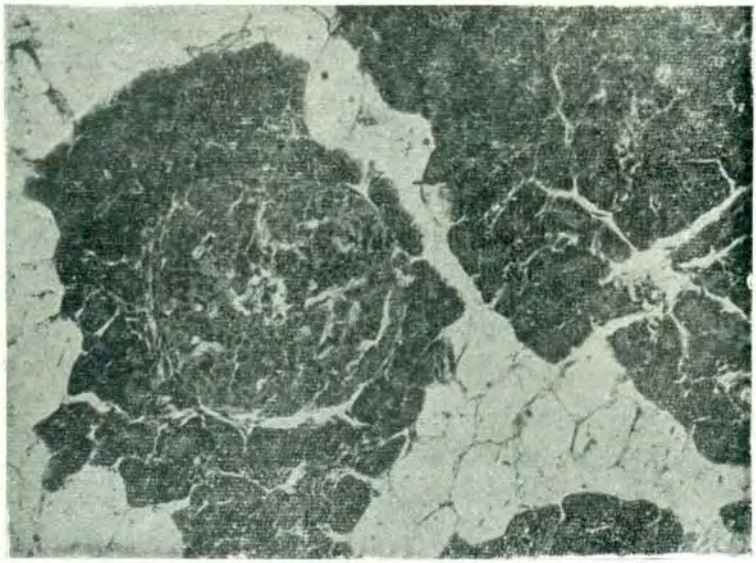

Fig. 2. Pancreas guineapig-control, Gomori's chrome haematoxylin phloxine. Islets well demarcated, α-cells stand out peripherally with β-cells in between x 150

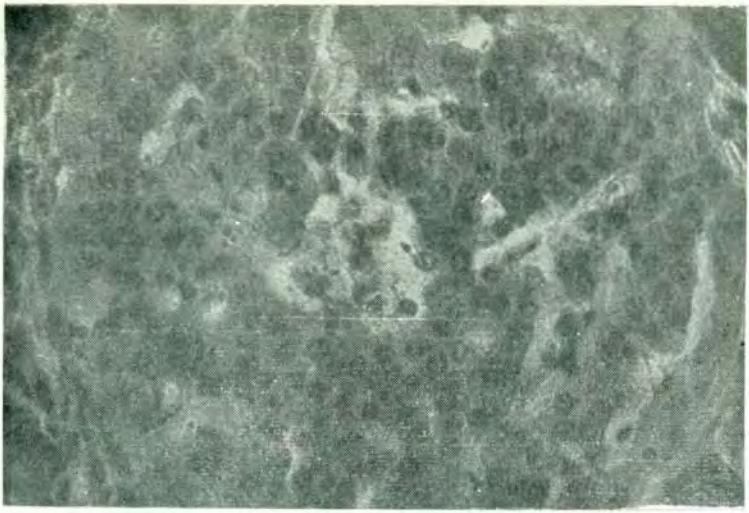

Fig. 3. Pancreas guineapig-control, Gomori's islets well demarcated. α-cells are well seen at the periphery with β-cells in between. In the center few sinusoidal spaces are seen x 600

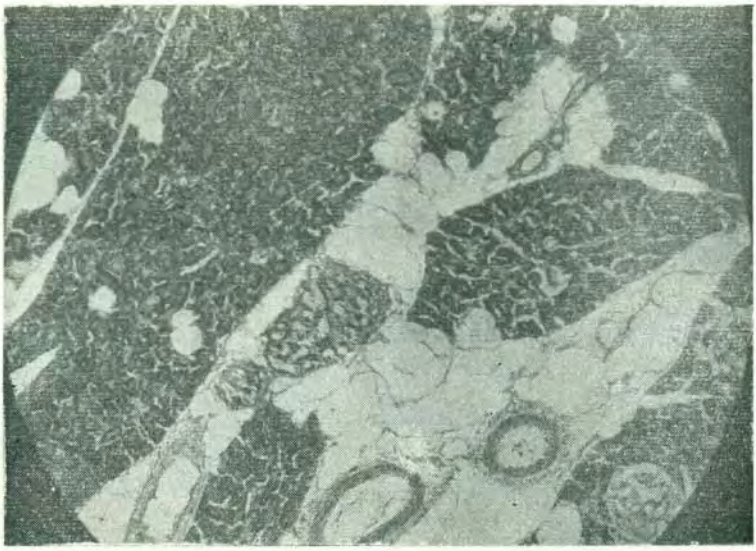

Fig. 4. Pancreas guineapig - alloxanized, Gomori's islets showing empty spaces, suggestive of β-cell destruction x 100

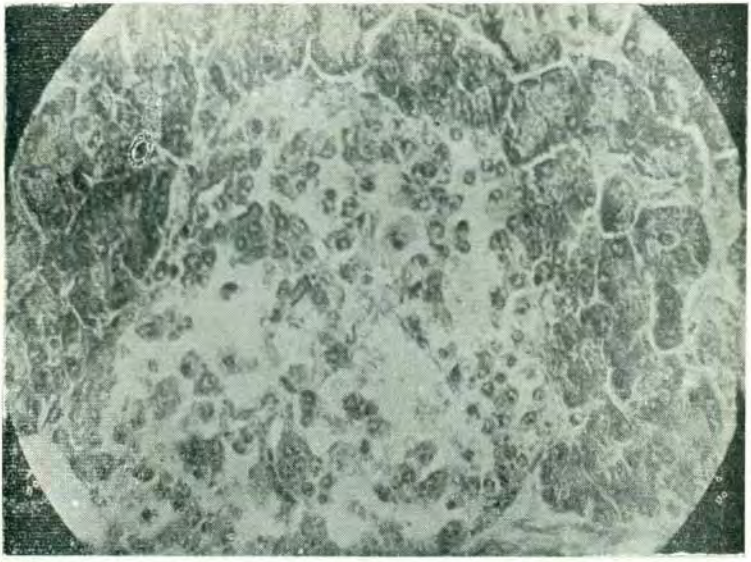

Fig. 5. Pancreas guineapig - alloxanized, Gomori's islets showing empty spaces islets distorted α-cells are seen in the center x 150.

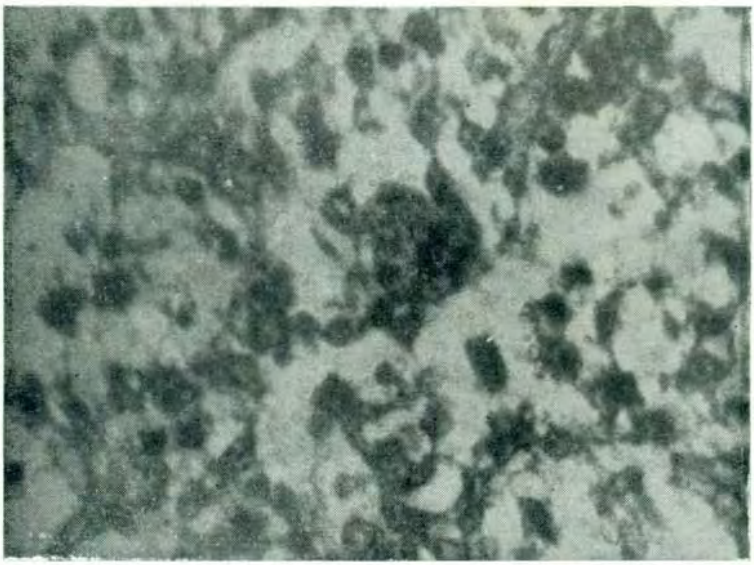

Fig. 6. Pancreas guineapig-alloxanized, Gomori's islets showing empty spaces here and there α-cells, some of the β-cells showing degenrative changes x 600

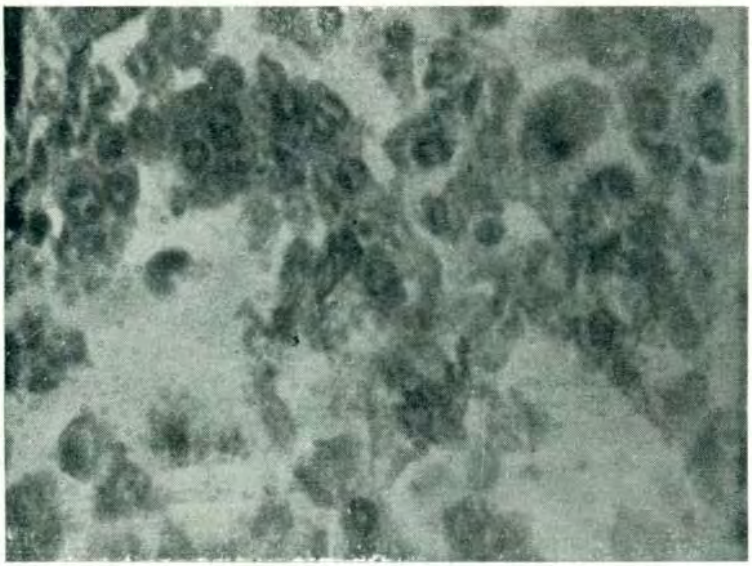

Fig. 7. Pancreas guineapig-alloxanized, Gomori's big empty spaces previously occupied by β-cells. α-cells stand out clearly x 350

SUMMARY

A brief account of the various methods of experimental diabetes and the species difference in their response to alloxan is given. Guineapigs also are shown to be susceptible to alloxan and sustained hyperglycaemia can be produced in them. Histological examination confirms the selective destruction of beta cells by alloxan in the guineapigs.

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