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Degranulation of Beta Cells of Rat's Pancreas by Glucose Correlated with Alterations in Glucose Tolerance.*

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After the relationship of the pancreas to diabetes had been established by Von Mering and Minkowski,[1] and before the discovery of insulin, Allen[2] and Homans,[3] working independently, demonstrated changes in the beta cells of the islands of Langerhans in partially depancreatized diabetic dogs. Allen postulated that these observed alterations would be reversible, and Copp and Barclay,[4] after the discovery of insulin, proved this hypothesis to be correct by observing the recovery of the beta cells in partially depancreatized diabetic dogs treated with insulin. Allen explained this phenomenon by postulating that the beta cells were the source of the antidiabetic hormone and that an excessive functional demand caused an exhaustion of these cells and produced the histological changes he observed—degranulation and vacuolization. These early observations, verified and amplified by later research, were made using the Lane Bensley technic for staining the beta cells. If one can judge from the photomicrographs included in these early reports this method gave very inadequate results and the staining would not compare in quality or accuracy with the results produced by the Gomori stain[5] which was used exclusively in these experiments.

More recently diabetes has been produced experimentally by methods other than pancreatectomy; by the injection of the hormones of the anterior pituitary[6] and thyroid,[7] by the injection of alloxan,[8] and by the injection of large amounts of glucose.[9] And when a diabetic state has been established alterations in the beta cells of the pancreas, degranulation, vacuolization, and necrosis, are invariably found to be present.

In other experimental methods the changes are limited to degranulation of the beta cells and diabetes does not develop. Starving a white rat or feeding it an exclusively fat diet will completely degranulate the beta cells of the pancreas.[10] Repeated daily injections of protamine zinc insulin in the rat will produce degranulation of the beta cells.[11,12] Gomori[13] and associates demonstrated variable degranulation of the beta cells in the guinea pig following a single intraperitoneal injection of glucose.

The present investigation is concerned with the changes produced in the beta cells in the pancreas of the white rat and the alterations in the glucose tolerance following a single intra-cardiac injection of glucose.

Materials and methods. White rats of the Sprague-Dawley strain were used exclusively. Rats of various ages and weights and of both sexes were used. Glucose was given in 20%

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TABLE I.
Results of One Injection of Glucose into Blood Stream of White Rat.

Rat No.	Sex	Wt, g	G glucose/kg	Hr inj. to autop.	Beta gran.	Rat No.	Sex	Wt, g	G glucose/kg	Time inj. to autop.	Beta gran.
20	M	200	0.25	1	3	307	M	300	3	5 min.	3
X	M	230	0.5	2	3	308	M	340	3	10 min.	1
E	F	130	1	¼	3	R	M	280	3	15 min.	0
D	F	160	1	½	3	Q	M	420	3	30 min.	0
C	M	120	1	¾	3	P	F	310	3	45 min.	0
B	F	185	1	1	2	303	M	330	3	12 hr	0
A	M	150	1	1½	2	310	F	300	3	24 hr	0
K	M	220	2	1	1—	T	M	280	3	24 hr	1
L	F	190	3	1	0	U	M	400	3	48 hr	2
M	M	250	4	1	0	311	F	320	3	48 hr	3
N	M	210	6	1	0	V	M	400	3	72 hr	3
O*	M	360	3	1	0	Z*	F	230	3	1 hr	0

* 3 units of crystalline zinc insulin per 1.0 g glucose added to syringe.

solution in distilled water via needle and syringe directly into the left ventricle of the heart of the rat previously anesthetized with ether. Blood for glucose determinations was withdrawn by the same method and the Folin-Wu micro-method was used for the determinations. The rats were sacrificed at various intervals by injecting air into the left ventricle of the heart. All pancreases were fixed in Bouin's fixative and stained with Gomori's stain. A pancreas from a normal rat was included with each staining and all estimations of partial degranulation were made with reference to this control tissue. The degree of granulation was graded from 3, representing a pancreas with all the beta cells fully granulated, to 0, a totally degranulated pancreas. The designation 1 indicates a pancreas with very marked degranulation of the beta cells while 2 indicates a definite partial degranulation but not as marked as 1. The glucose tolerances were determined by injecting 0.25 g per kilo body weight of 20% glucose into the left ventricle of the heart.

Several experiments were attempted: Glucose in concentrations from 0.25 g per kilo body weight to 6.0 g per kilo was injected. Rats were then sacrificed at intervals from 5 minutes to several days. Glucose tolerances, blood glucose levels, and urine sugars were determined at varying intervals. All determinations of glycemia were made in the fasting state; all urine sugar recordings were determined on overnight specimens. In 2 rats 3 units of crystalline zinc insulin per 1.0 g of glucose were added to the syringe and injected simultaneously with the glucose. One rat was given 12 single injections of glucose, 3.0 g per kilo, over a period of 2 weeks.

Results. The beta cells of the islands of Langerhans were invariably totally degranulated within 15 minutes after the injection if 3.0 g per kilo, or higher concentrations of glucose were used. Lesser concentrations gave variable results: Less than 1.0 g per kilo resulted in no perceptible degranulation; 1.0 g per kilo reduced the granulation to the level of 2 in one hour; 2.0 g per kilo reduced the granulation to 1 or 1— at the end of one hour.

This process of degranulation was found to be reversible; regranulation of the beta cells followed degranulation. However, a delay of about 48 hours was observed. Twenty-four hours after the injection the granulation was found to remain markedly reduced (0-1); but in 48 hours the granulation appeared to be almost complete (2-3).

Glucose tolerance studies on rats 24 hours after the single injection of glucose (3.0 g per kilo) revealed curves of the diabetic or potential diabetic type (Fig. 1a). One week after the injection the glucose tolerance had returned to normal.

When 3 units of crystalline zinc insulin per 1.0 g of glucose were added to the syringe a marked reduction in the hyperglycemia followed, but not for approximately 30 minutes during which interval degranulation of the beta cells took place (Fig. 1b).

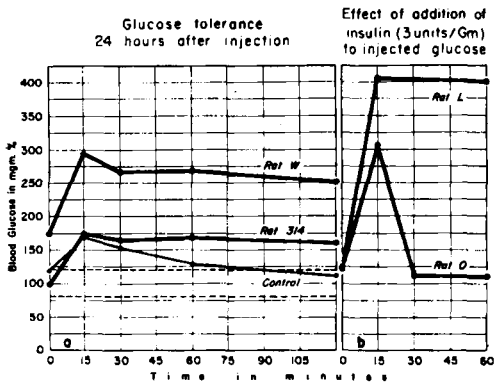

FIG. 1a.

Rats W and 314 received injection of glucose, 3.0 g/kg, 24 hr previous. The curves represent upper and lower extremes of glucose tolerance. Most other determinations fell in between.

FIG. 1b.

Rats L and O were both given glucose 3.0 g/kg. Zinc crystalline insulin, 3 units per g of glucose, was added to the syringe in injecting rat O.

Fasting blood sugars, determined 24 hours after the injection, were found to be elevated in almost every instance. One rat, No. 314, which showed a fasting blood sugar within the normal range, was found to have a glucose tolerance curve of a potential diabetic type (Fig. 1a). As would be expected most rats also showed a marked glycosuria 24 hours after the injection. This usually disappeared by 48 hours.

From these observations it became apparent that a correlation existed between the degree of beta cell granulation, the level of glycemia, the type of glucose tolerance curve, and the degree of glycosuria.

Attention is again directed to rat 314, glucose tolerance of which is illustrated in Fig. 1a. Following this determination, which as previously noted consists of the injection of 0.25 g of glucose per kilo, this rat showed a daily glycosuria of 2+ for one week. The possible significance of this observation is discussed below.

The one rat, No. Y, which was given 12 daily single injections of glucose, 3.0 g per kilo, showed a persistent elevation of fasting blood sugars and marked glycosuria for 8 days after cessation of the injections—a temporary diabetic state.

It should be noted that all control animals, treated exactly as the above animals with the exception that no glucose was injected, showed no degranulation, hyperglycemia, glycosuria, or abnormal glucose tolerances.

Discussion. The injection of a large amount of glucose into the blood stream of the rat, as described above, undoubtedly causes an excessive functional demand upon the beta cells of the pancreas leading to complete exhaustion of the cells—the degranulated state. The stimulus initiating this exhaustion of the cell is of necessity the injected glucose. And from the data recorded in these experiments and from the work of Gomori and associates,[13] it is evident that the greater the concentration of the stimulating agent in the blood the greater the amount of beta cell secretion.

The beta cell in its completely exhausted state is not organically injured as determined by histologic studies—it shows no alterations other than the degranulation. However, physiologic studies as reported herein would indicate that the cell is functionally impaired. About 48 hours are required for the recovery of the cell as measured by glucose tolerance, levels of glycemia, and degree of glycosuria. Repeated injections, as exemplified by rat Y, apparently cause an accumulative functional impairment upon the beta cells; the beta cells recover within 48 hours after one injection but require one week to recover after 12 injections. Theoretically, continued injections might transform the temporary functional impairment into a permanent deficiency resulting in diabetes. This feature is being investigated.

The sensitivity of the beta cells to overstimulation probably varies somewhat from one animal to another. Rat 314 is an example of marked sensitivity to a slight stimulus following the production of functionally impaired beta cells. The day following the intra-cardiac injection of 3.0 g glucose per kilo, rat 314 had a fasting blood sugar within normal range but showed a glucose tolerance curve of a potential diabetic type, Fig. 2a. In doing the glucose tolerance 0.25 g of glucose per kilo was again injected. For one week following this procedure the rat showed a marked daily glycosuria and an elevated fasting blood sugar. Thus a small extra demand upon a previously functionally impaired

TABLE II.
Correlation of Beta Granulation with Blood Sugar, Glucose Tolerance, and Glycosuria Following One Injection of Glucose (3.0 g/kg).

Hr after injection	0	1	24	48	72	Week
Beta granulation	3	0	0-1	2-3	3	3
Avg blood glucose	118	390	163	122	108	106
Glucose tol. curve	Normal		Diabetic or Pot. diab.			Normal
Glycosuria	0	4+	2+ to 4+	0 to Tr.	0*	0*

* One exception, Rat 314, 2+ daily glycosuria for one week.

beta cell produced the picture of a temporary diabetic state.

The fact that the beta cells eventually regranulate completely is also strong evidence that the functional impairment is only temporary. The final proof, of course, for the theory of functional impairment due to overstimulation is the work of Lukens and Dohan,[9] in which cats were made permanently diabetic by the administration of large amounts of glucose.

The marked correlation between the degree of beta cell granulation with alterations of glucose tolerance, elevation of the fasting blood sugars, and the presence of glycosuria would confirm the widely held opinion that the granules are a form of insulin. The phenomenon of complete degranulation within 15 minutes in response to the stimulus of injected glucose would be further evidence for this opinion.

The addition of crystalline zinc insulin to the injected glucose resulted in an interesting observation. We had anticipated that the exogenous insulin would protect the beta cells from degranulation but interval blood sugars explained the results. The beta cells were apparently degranulated during the initial period of hyperglycemia, as shown in Fig. 1b. An initial lag in the effect of the injected insulin is well demonstrated by the blood sugar curves. The significance of this phenomenon has not yet been determined.

Summary. The beta cells of the rat's pancreas can be completely degranulated within 15 minutes by a single intra-cardiac injection of glucose. Dosage: 3.0 g glucose per kilo body weight.

The phenomenon of degranulation is reversible; regranulation follows degranulation, but requires about 48 hours.

Rats with degranulated beta cells show a reduced glucose tolerance, elevated fasting blood sugars, and glycosuria.

The addition of 3 units of crystalline zinc insulin per 1.0 g of glucose does not prevent the degranulation of the beta cells due to a delay in the effect of the injected insulin and the consequent production of a hyperglycemia.

Repeated injections of glucose cause a functional impairment of the beta cells as evidenced by the production of a temporary diabetic state.