

# Response to Alloxan Modified by Partial Pancreatectomy

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When alloxan is injected into animals, one of the effects is a hypoglycemia preceded by a transient elevation of blood sugar and followed by hyperglycemia that is often permanent. Some time before the diabetogenic capacity of alloxan had been discovered, its more immediate hypoglycemic effect was observed by Jacobs<sup>1</sup> in rabbits. The precipitate drop in blood sugar that simulates insulin action, since it occurs at the time of extensive islet necrosis, led many<sup>2-5</sup> to the conclusion that the cause is the liberation of the final remnant of insulin from the dying beta cells of the pancreas. Studies on the alloxan reaction after pancreatectomy in several species have generally supported this postulate, though they have sometimes been equivocal.<sup>6</sup> Furthermore, the albino rat, though usually the first choice for animal experimentation, has been notably absent from this problem because of the difficulties attendant upon pancreatectomy in the species. It was to fill that deficiency in alloxan experimental work that the present study was undertaken.

## EXPERIMENTAL

Albino rats were partially depancreatized by a technic essentially as described by Ingle and Griffith.<sup>7</sup> Pre- and postoperative care described by Treadwell and Roe<sup>8</sup> improved survival, which in our hands varied from 55 to 95 per cent depending on the strain of animal and the age at operation. All surgery was performed on rats under Evipal anesthesia and was done throughout the study by one operator (P.J.B.) to avoid variations in operative skill. About 250 partial pancreatectomies were required to obtain enough survivors for the projected tests.

Throughout the study, males and females were used in equal numbers to avoid any confusion of results by the sex factor, which is of major effect in experimental diabetes.<sup>9</sup> The two age groups employed for partial pancreatectomy were thirty- to forty-day-old immature animals and 250- to 350-day-old adults.

From the Biochemical Laboratory, Metropolitan Life Insurance Company, New York City.

The young animals after operation were reared to adulthood and in the sixth postoperative month were given alloxan after an assessment of the damage that surgery had made in their glucose metabolism. In the adult groups the effect of partial pancreatectomy was evaluated at the third or fourth postoperative week, and the alloxan experiments followed shortly thereafter. Albino rats of both the Wistar and the Osborne-Mendel strains were used to permit further comparison of these two hereditary lines, which are known to differ in glucose economy,<sup>10</sup> in their pattern of reaction to alloxan when unoperated,<sup>11</sup> and in the ease with which diabetes may be produced through surgery.<sup>12</sup>

Alloxan monohydrate was administered subcutaneously, following a four-day fast, at a level of 20 mg. per 100 gm. body weight. Fifteen minutes thereafter the animals were returned to their usual Purina Laboratory Chow diet fed ad libitum. To follow the blood sugar changes tail blood was obtained at intervals during the first forty-eight hours after alloxan. Subsequently the diabetic state was assessed by a fasting oral glucose tolerance test two weeks after injection and, at three weeks, by examination of the urine for glucose and acetone bodies and of the postprandial blood sugar. Blood sugars were estimated by the method of Folin and Malmros,<sup>13</sup> urine glucose by Somogyi's quantitative procedure<sup>14</sup> and ketonuria was evaluated qualitatively by a nitroprusside spot test.\*

## RESULTS

Partial pancreatectomy was found in this study to have essentially the same effect on the blood sugar response to alloxan whether the operation was done before maturity or in adulthood. Furthermore, both sexes were similarly affected, and the presence or absence of pancreatectomy diabetes was of little influence. For these reasons it has been possible to simplify the presentation of results merely to a demonstration of the difference in

\*Several companies market this type of simple test. The one used here is manufactured by the Denver Chemical Company, New York, and is sold as Acetone Test-Denco.

blood sugar response between intact and operated animals and between the two animal strains.

Figure 1, which is the pattern blood sugar follows after alloxan in Wistar animals, is marked, in the unoperated subject, by a strong hyperglycemia reaching a peak at twenty-one hours followed by a steep decline to relatively low levels at thirty hours. Animals having been subjected to partial pancreatectomy present, in contrast, a greatly flattened response curve. The initial blood sugar rise is decreased, and the intervening relative hypoglycemia is of less intensity. Each point plotted in the charts represents an average on at least thirty animals. As in earlier studies, wide individual variability was found. However, statistical evaluation confirmed the high significance of this difference, in the Wistar strain, between animals partly deprived of pancreas and their intact controls.

Figure 2 presents a study entirely similar to that in figure 1, except that Osborne-Mendel strain rats were used. Because of a shortage of operated animals, mortality in this strain being particularly severe, observations on blood sugar levels were largely confined to those hours in which significant changes would be most likely to occur. A comparison of the control subjects in the two figures demonstrates the difference in response behavior of the two strains. The Osborne-Mendel animals show a nearly complete lack of triphasic response, and it is, therefore, not surprising that pancreatectomy in this strain is without further influence.

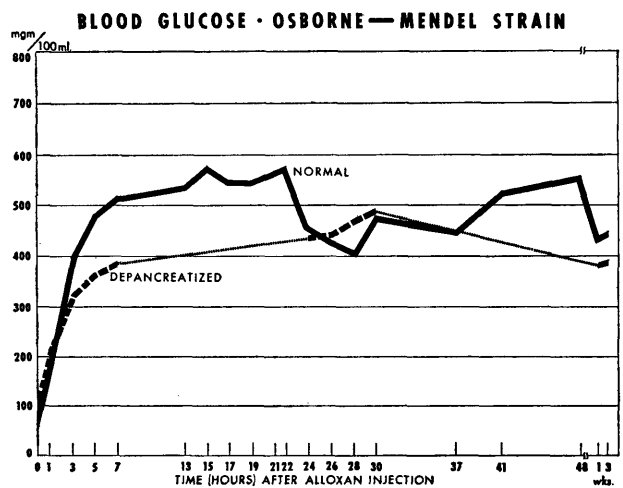
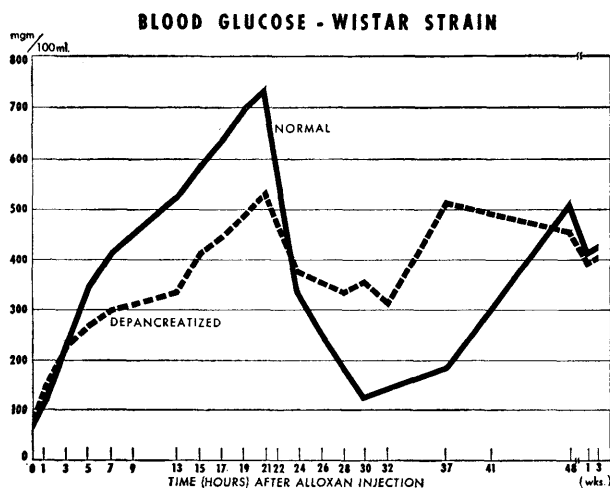
The diabetic condition of the alloxan-treated animals

was further assessed by determining the oral glucose tolerance at two weeks and the urinary excretion of glucose and ketone bodies at three weeks after injection. These measurements together with postprandial blood sugar are presented in table 1 for the partially depancreatized animal and its unoperated control in two strains.

Fasting blood sugar values obtained in the glucose tolerance test emphasize the outstanding strain difference in alloxan diabetes. Severely diabetic animals of the Osborne-Mendel strain have a characteristic high fasting blood sugar whereas the Wistar animals are inconsistent in this regard, many developing more or less severe hypoglycemia in fasting. Postprandial blood sugars in the two strains are similar and, of course, elevated.

In the Wistar strain partial pancreatectomy prior to alloxan had an effect on the diabetes induced by alloxan only among the small group depancreatized as adults. Among these animals the fasting blood sugar is greatly depressed so that, as shown by standard deviation, severe hypoglycemia may occur. Subsequent one-half-hour and two-hour values after glucose are correspondingly low. These animals were free of ketonuria when fed. In contrast, the partial pancreatectomy before maturity appears to be without effect.

With the Osborne-Mendel strain the position is reversed. Here adult pancreatectomy made but little change from the control. But partial pancreatectomy at an early age affected the diabetic state after alloxan, so that fasting hyperglycemia and sugar levels during the tolerance test were significantly depressed, postprandial blood



FIGS. 1 & 2. Blood sugar changes in partially depancreatized and in intact animals following alloxan. Alloxan was given to adult rats only but the operation was performed on half the animals in infancy and half of the animals in adulthood. The time of operation had no effect on the subsequent alloxan response. Only animals severely diabetic following alloxan are included in the study. The operation produced no diabetes in the Wistar strain (figure 1); in the Osborne-Mendel strain (figure 2) about 50 per cent of the subjects were already diabetic due to operation before the alloxan was given.

TABLE 1

Blood sugar, glucose tolerance and urine studies on alloxan diabetic rats, with and without preliminary partial pancreatectomy

Animal strain	Time after glucose, hour	Oral Glucose Tolerance*			Postprandial† blood sugar mg. per cent	Urine‡	
		0	0.5	2		Glucose per cent	Per cent of rats with ketonuria
Wistar	Control, not depancreatized (61)§	134  ± 62	266 ± 69	162 ± 72	425 ± 72	6.4 ± 1.8	32
	Adult pancreatectomy (12)	¶76 ± 33	177 ± 66	82 ± 43	427 ± 188	8.0 ± 3.3	none
	Immature pancreatectomy (26)	154 ± 79	269 ± 91	196 ± 97	406 ± 116	6.5 ± 1.5	50
Osborne-Mendel	Control, not depancreatized (57)	264 ± 92	394 ± 143	340 ± 100	446 ± 80	7.2 ± 2.8	75
	Adult pancreatectomy (18)	253 ± 89	377 ± 118	339 ± 111	415 ± 37	5.3 ± 0.9	50
	Immature pancreatectomy (33)	183 ± 83	318 ± 77	285 ± 98	384 ± 60	5.4 ± 1.7	15

\*Tests made two weeks after alloxan injection; 100 mg. glucose per 100 gm. body weight in 20 per cent solution given by mouth after twenty-four-hour fast.

†Postprandial blood taken three weeks after alloxan injection.

‡The urine was tested three weeks after alloxan. During the test the animals were placed in metabolism cages for an eighteen-hour period for urine collection; 60 ml. of whole milk was presented to the subject and upon finishing this, only water ad libitum was permitted for the rest of the period.

§Figures in parenthesis show number of animals observed.

||± indicates standard deviation.

¶Figures that are underscored show a statistically significant difference from control. P values less than 0.01.

sugars were significantly below control levels, urinary glucose excretion was reduced and the incidence of ketonuria greatly decreased.

#### DISCUSSION

The study demonstrates that an animal strain that normally gives a marked triphasic response to alloxan will no longer do so if deprived of a substantial portion of the pancreas. The finding in the Wistar group that both the initial hyperglycemia and intermediate hypoglycemia are thus curtailed emphasizes the role of the pancreas in producing them. The fact that the Osborne-Mendel strain, from which the triphasic response is normally absent, remains unchanged in this respect after pancreatectomy is not contrary to this view. The conclusion from albino rat experiments supports similar findings in other species. Goldner and Gomori<sup>15</sup> reported that pancreatectomy in dogs effectively eliminates alloxan hypoglycemia.

If, therefore, it is true that insulin release from the pancreas is the basis for alloxan hypoglycemia, the virtual absence of this response in the Osborne-Mendel strain suggests either a deficiency in insulin production by the pancreas or a resistance to insulin action. This is consistent with the known diabetic tendencies of the strain.

That the character of the diabetes produced by alloxan action can also be modified by preliminary partial pancreatectomy has been demonstrated. We are at a loss to explain why this modification was apparent in the Wistar strain only in the animals depancreatized as adults and in the Osborne-Mendel strain in the animals depancreatized in infancy. The effect was to lower the fasting

blood sugar in alloxan diabetes and correspondingly to lower the subsequent glucose tolerance curve. In the Wistar strain, which is normally subject to hypoglycemia of fasting in alloxan diabetes, the further lowering of the blood sugar by pancreatectomy made fasting an even more precarious experience. Among the Osborne-Mendel rats, with their strain characteristics of fasting hyperglycemia in alloxan diabetes, the lowering of the blood sugar by pancreatectomy seemed to benefit the diabetic condition. This benefit is further substantiated by a decrease in postprandial blood sugar, glucose concentration in the urine and reduced incidence of ketonuria.

Pancreatectomy has been widely demonstrated to produce a decrease in insulin requirement in diabetes,<sup>16-19</sup> and this benefit has been largely interpreted as being due to the removal of the source of glucagon or the hyperglycemic factor. Mirsky and others<sup>20</sup> have shown in the dog that total pancreatectomy, resulting in loss of the external secretion of the pancreas, so curtails intestinal absorption of carbohydrate as to cause a drop in insulin requirement without reference to any glucagon function. The current experiments are not subject to this criticism, however, because the pancreatectomy was only partial and some secreting pancreas always remained. Where lowering of the fasting blood sugar was obtained, as in the highly significant series of immature-operated Osborne-Mendel rats, the findings are at least not inconsistent with the thought that the hyperglycemic principle of the pancreas may be a factor in determining the character of the diabetic symptoms in an animal.

The study as a whole points to a need for further work on the Osborne-Mendel rat to determine whether

the strain is so overendowed with glucagon or so deficient in insulin supply as studies on this unique hereditary line have thus far suggested.

#### SUMMARY

Albino rats of the Wistar and the Osborne-Mendel strains were partially depancreatized. Some groups were operated on at thirty to forty days of age while others were operated on at 250 to 350 days of age. Both groups were given alloxan in adulthood to determine the effect of partial pancreatectomy upon the triphasic blood glucose response to alloxan as well as upon the character of the subsequent diabetes. The study reveals:

1. In the Wistar strain, in which a marked triphasic response occurs normally, partial pancreatectomy significantly reduces the initial hyperglycemia and the intermediate hypoglycemia.

2. In the Osborne-Mendel strain, partial pancreatectomy produced no change from the normal response, from which the triphasic feature is characteristically absent.

3. The diabetic state produced by alloxan may be modified by preliminary partial pancreatectomy. Thus in the Wistar strain animals operated on in adulthood and in the Osborne-Mendel strain animals operated on before maturity, the diabetes following alloxan was characterized by a lower fasting blood sugar and subsequent tolerance curve than in uncomplicated alloxan diabetes. Furthermore, the incidence of ketonuria was decreased and the concentration of urine glucose and postprandial blood sugar was diminished in the Osborne-Mendel rats.

#### SUMMARIO IN INTERLINGUA

##### *Responsa A Alloxano, Modificate Per Pancreatectomia Partial*

Rattos albin del racias Wistar e Osborne-Mendel esseva partialmente pancreatectomisate. Certe gruppos esseva operate a etates de inter trenta e quaranta dies, alteres a etates de inter 250 e 360 dies. Omne le gruppos recipeva alloxano post attinger etates adulte con le objectivo de determinar le effecto de pancreatectomia partial super le responsa triphasic de glucosa sanguinee a alloxano e etiam super le character del subsequente diabete. Le studio ha resultate in le sequente constatationes.

1. In le racia Wistar—in que un marcate responsa triphasic occurre normalmente—pancreatectomia partial reduce significativamente le hyperglycemia initial e le hypoglycemia intermediari.

2. In le racia Osborne-Mendel, pancreatectomia partial resulta in nulle alteration del responsa normal, incluse le absentia characteristic del triade de phases.

3. Le stato diabetic que es producite per alloxano pote esser modificate per un antecedente pancreatectomia

partial. Assi, in le racia Wistar, animales operate a etates adulte e in le racia Osborne-Mendel, animales operate ante lor maturitate esseva characterisate per un plus basse contento de sucro sanguinee in stato jejun e subsequentemente per un plus basse curva de tolerantia, comparate con animales con non-complicate diabete a alloxano.

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