Acute- and Late-Phase Insulin Secretion and Glucose Tolerance in Mild Alloxan Diabetes in Dogs

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SUMMARY

The acute and late phases of insulin secretion were studied in mongrel dogs before and after the induction of mild alloxan diabetes. Fasting glucose and insulin levels were unchanged from pretreatment values. The alloxan-diabetic dogs had significantly decreased early-phase insulin responses to glucose pulses (0.5 gm./kg.) and slower plasma glucose disappearance rates. In contrast, these mildly diabetic dogs achieved comparable insulin levels and higher glucose levels during a four-hour 40 mg./min. glucose infusion than pre-alloxan control values. Similar findings in human congenital mild diabetes have been interpreted as beta cell insensitivity or impedance to efficiency of plasma glucose uptake. The present observations in alloxan-induced mild diabetes in dogs suggest that reduced early-phase secretion and intact later phase of insulin secretion are not dependent on genetic determinants and may be induced in a model of acquired diabetes. DIABETES 25:161-66, March, 1976.

The mechanism of glucose intolerance in diabetes remains unresolved. It is currently accepted that juvenile and severe adult-onset diabetes in man is a consequence of insulin deficiency. Nevertheless, insulin responses in the early states of mild adult-onset diabetes are quite variable.

To explain the finding of a normal or even high insulin levels in mild diabetes, Berson and Yalow[1] and Reaven et al.[2-5] have proposed the concept of insulin resistance or decreased insulin effectiveness. This resistance, which may be inherited or acquired, is reflected by the increased impedance to plasma glucose removal in diabetic subjects.

Lerner and Porte,[6] Seltzer et al.,[7,8] and Perley and Kipnis,[9] studying mild diabetes in man, presented evidence supporting a consistently diminished initial rapid release of insulin secretion followed by a normal, slow, or late response. Cerasi and Luft[10] suggest that the defective acute insulin release in diabetes is due to a decreased sensitivity of the pancreatic beta-cell glucose receptor to an elevated plasma glucose level. However, the finding is also compatible with a failure of adequate hormone output predominantly from preformed, stored insulin available for rapid release.

In an attempt to add some data that could help in the resolution of this controversy, insulin responses to acute and long-term intravenous infusions of glucose in dogs with mild, alloxan-induced diabetes were examined. The major intent of this study was to determine the effects of prolonged glucose infusion in a model of diabetes in which insulin responses are unquestionably deficient.

MATERIAL AND METHODS

Male adult mongrel dogs used in this study were fed for 15 days before and throughout the experimental period a diet containing 30 per cent of calories derived from fat, 20 per cent from protein, and 50 per cent from carbohydrate. The dogs, weighing between 13 and 17

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kg., maintained their body weight during experimental period.

All tests were performed after a 12-hour overnight fast. Under pentobarbital anesthesia (20 mg./kg. body weight intravenously), polyethylene catheters were inserted in the radial and saphenous veins and kept patent with slow administration of isotonic saline. A 15-minute interval elapsed after insertion of the catheter before control samples were obtained.

Development of Mild Alloxan Diabetes

Alloxan doses ranging from 20 to 60 mg./kg.[11,12] were employed, and the results indicated that dogs given 25 mg./kg. of alloxan intravenously developed a diabetic state with normal or slightly elevated fasting blood sugar and glucose intolerance as judged by the intravenous glucose disappearance rates (KG).[13] KG values were estimated from the decline of plasma glucose levels after infusion of glucose (0.5 gm./kg.). Calculation of the disappearance rate was based on samples obtained from the saphenous vein before and 5, 10, 15, 20, 30, 40, 50, and 60 minutes after the glucose injection.[11] Repeated intravenous glucose loads at three-day intervals from the 5th to the 15th day after alloxan demonstrated stable values. In seven dogs, the mean KG before alloxan was 8.45±4.66 per cent/minute (mean±S.D.) and after the drug was administered, 1.36±0.35 per cent/minute ($p < 0.005$).

Determination of the Acute and Late Phase Insulin Release

After establishing the appropriate alloxan dose to develop mild diabetes, acute and late phase insulin secretion was studied in another group of six dogs in two separate tests three days apart. Both tests were performed prior to alloxan and between the 7th and the 10th day after alloxan injection. Thus, each dog served as its own control.

The rapid insulin release and glucose tolerance was determined through a rapid intravenous glucose injection (0.5 gm./kg. of a 50 per cent solution in five seconds) and blood samples drawn five minutes before (−5), immediately before (0), and at 3, 4, 5, 7, 10, 15, 20, 30, 40, 50, and 60 minutes after the injection.

The late-phase insulin release was studied throughout a constant, four-hour glucose (40 mg./min. of a 4 per cent solution) infusion. Blood samples were collected five minutes before (−5), immediately before (0), and at 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes after starting the infusion.

Each blood sample was divided into two tubes. In the first tube blood was allowed to clot and after centrifugation serum was removed for future determination of insulin by a double-antibody radioimmunoassay.[14] The second tube, containing heparin, was placed in an ice bath and centrifuged at 4° C. for glucose determination.[15] All serum and plasma samples were frozen and stored at −20°C. until the day when insulin or glucose assay was performed.

The insulin determinations for a given dog, before and after alloxan, were made simultaneously in order to

TABLE 1

Plasma glucose concentration (mg./100 ml.) and glucose disappearance rate (KG) following a rapid glucose injection (0.5 gm./kg. of body weight) before and after alloxan-induced diabetes in dogs

		Time after glucose loading (min.)												
Dog		0	3	4	5	7	10	15	20	30	40	50	60	KG
1	Before	74	236	265	252	200	222	232	174	196	153	126	80	4.21
	After	87	364	342	328	262	278	264	256	230	214	212	196	1.42
2	Before	76	312	310	312	278	258	230	210	164	103	32	75	8.60
	After	100	390	362	370	326	306	276	246	226	202	172	170	1.80
3	Before	81	302	312	300	232	258	222	208	162	113	106	78	8.38
	After	109	514	516	474	470	420	394	384	314	312	288	244	1.80
4	Before	99.3	467	489	317	325	318	244	222	149	129.5	102.5	87.5	11.96
	After	95.5	440	380	422	354	368	338	321	303	267	229	225	1.61
5	Before	97	414	378	368	368	346	292	252	282	213	178	125	3.41
	After	97	426	372	366	332	322	318	298	228	218	234	206	1.76
6	Before	111.5	382	328	426	394	324	258	252	284	288	191	183	3.14
	After	120	374	528	464	478	298	286	358	316	324	290	264	1.24
Before	$\bar{X}$	89.8	352.2	345.5	329.2	299.5	287.7	246.3	219.7	206.2	166.6	130.9	104.8	6.45
	S.D.	15.0	84.3	80.4	60.2	76.4	48.4	25.7	29.7	61.5	71.2	44.0	42.5	3.57
After	$\bar{X}$	101.4	419.7	416.7	404.0	370.3	332.0	312.7	310.5	269.5	256.2	237.5	217.5	1.61
	S.D.	11.6	59.0	82.7	58.7	86.0	52.7	48.4	54.9	69.0	113.3	103.8	60.7	0.20

*Statistically significant ($p < 0.05$). ($\bar{X}$ = mean and S.D. = standard deviation.)

avoid interassay variations. The insulin assay in our laboratory has an intra-assay coefficient of variation of 3.6 per cent and an interassay coefficient of variation of 5.6 per cent.

Glucose and insulin during the four-hour glucose infusions were assessed in terms of the total area between the curve and abscissa, calculated in arbitrary units.

The significance for statistical analysis was previously set at the 5 per cent level. Paired *t* test[16] was utilized for the comparisons before and after alloxan administration.

RESULTS

No significant difference was found between fasting plasma glucose levels (table 1) before and after alloxan (89.8±15 and 101.4±11.6 mg./100 ml., respectively). Mean fasting serum insulin levels were 8.0±5.3 μU./ml. before and 8.8±2.4 μU./ml. after alloxan (table 2).

The mean serum insulin response was clearly diminished ($p < 0.05$) in the diabetic group for the 4-, 5-, 7-, 10-, 15-, and 20-minute samples, indicating failure of the initial phase of insulin release (table 1, figure 1). The mean glucose disappearance rate (KG) was significantly decreased after alloxan (prealloxan: 6.56±3.57, postalloxan: 1.61±0.20, $p < 0.05$) and paralleled the decreased acute insulin response (tables 1 and 2, figure 1).

During the prolonged glucose infusion, insulin responses were increased (table 4, figure 2). The insulin responses during the infusion were significantly increased above prealloxan values ($p < 0.05$, table 4). In spite of these higher insulin levels, the diabetic group showed a significantly increased glucose area ($p < 0.05$, table 3) over the values found in the same experiment before alloxan administration (table 4).

DISCUSSION

The induction of mild alloxan diabetes in dogs is clearly associated with a reduction in acute insulin response to rapid glucose administration, which appears to parallel the diminished glucose tolerance. In contrast, the constant glucose infusion elicited significantly higher serum insulin levels, which were associated with higher plasma glucose levels.

The diminished insulin response to glucose stimulation, which appears to be selective only for the initial phase of insulin release, is remarkably similar to that reported in human maturity-onset diabetes.[6-8] Moreover, the high glucose levels despite increased late-phase insulin secretion found in the dogs with alloxan-acquired mild diabetes have been observed also in human genetic mild diabetes by Lerner and Porte,[6] Reaven and Farquhar,[17] and Graber et al.[18]

Thus, it appears that the pattern of blunted early-

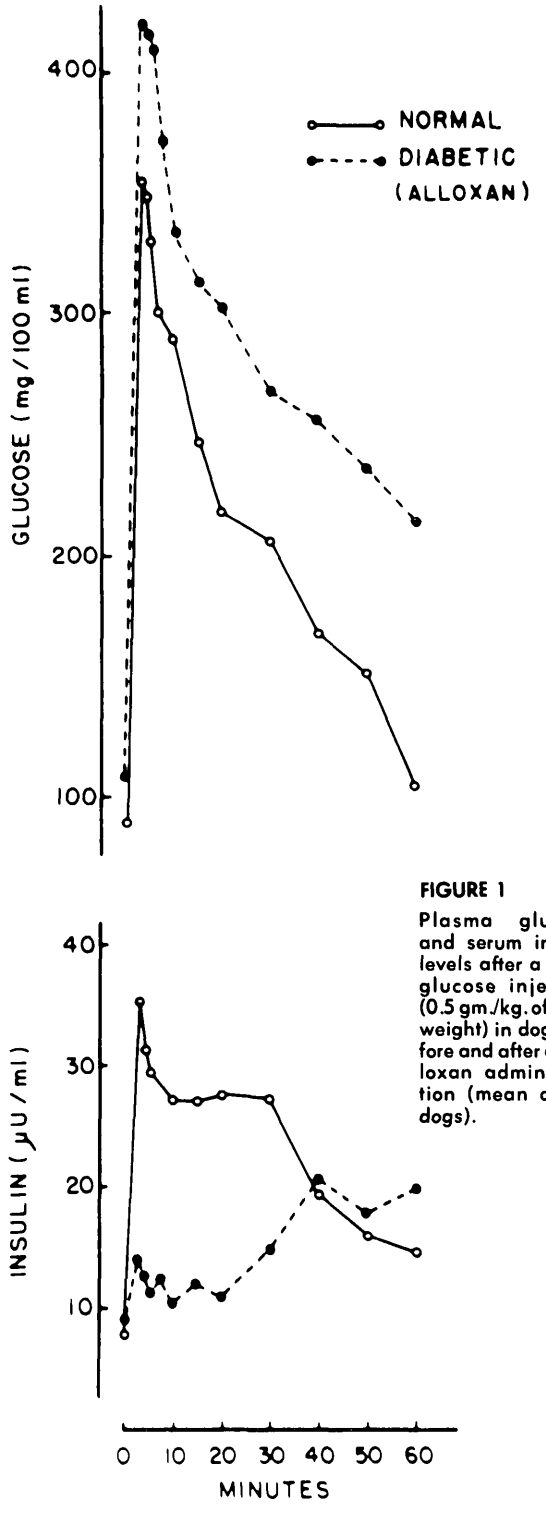

FIGURE 1

Plasma glucose and serum insulin levels after a rapid glucose injection (0.5 gm./kg. of body weight) in dogs before and after an alloxan administration (mean of six dogs).

TABLE 2

Serum insulin concentration (μU./ml.) following a rapid glucose injection (0.5 gm./kg. of body weight) before and after alloxan diabetes in dogs

		Time after glucose loading (minutes)											
Dog		0	3	4	5	7	10	15	20	30	40	50	60
1	Before	5.3	21.5	19.5	16.5	17	16	18	12	10	18.5	12.5	13
	After	6.8	8.5	5.5	4	5	1	1.5	2	4.5	11	11.5	10
2	Before	5	16	17	12.3	19.5	22.5	31	18.5	44	19.5	3.5	5.5
	After	8.3	17	10.5	11	14.5	3	14	4	13	27	9	16
3	Before	4.5	40.8	25.8	23.3	24.5	20.5	20.5	42.3	25	14.3	14.3	11.8
	After	8.4	9.3	7.8	8	8	8.5	8.8	7	9.3	9.8	8.8	7.8
4	Before	5.3	72	65	56.5	55	48	46	44.5	40.5	21.5	19	10
	After	6.8	8	9.5	10	9	8.5	10.5	10.5	14	16.5	18	21.5
5	Before	18	41	41.5	39.5	37.5	32	29	23	21.5	21	22	22.5
	After	13.3	27	28.5	26	21.5	24	21	23.5	31.5	42	41.5	49
6	Before	9.8	19	20.5	22.5	22.5	27.5	19	25	22.5	21	24.5	23
	After	9.3	12	11.5	8	14.5	16.5	15	17	15	16	16.5	15
Before	$\bar{X}$	8.0	35.0	31.5	28.4	29.3	27.8	27.3	27.5	27.3	19.3	16.0	14.3
	S.D.	5.3	21.2	18.6	16.6	14.5	11.4	10.7	13.1	12.8	2.7	7.6	7.0
After	$\bar{X}$	8.8	13.6	12.2	11.2	12.1	10.3	11.8	10.7	14.5	20.4	17.5	19.9
	S.D.	2.4	7.4	8.3	7.7	5.9	8.6	6.6	8.2	9.2	12.2	12.4	15.1

*Statistically significant ($p < 0.05$). ($\bar{X}$ = mean and S.D. = standard deviation.)

TABLE 3

Plasma glucose concentrations (mg./100 ml.) and glucose areas during glucose infusions (40 mg./kg. of body weight) before and after alloxan-induced diabetes

	Time after starting the infusion (minutes)															
Dog	0	5	10	15	30	45	60	75	90	105	120	150	180	210	240	Glucose areas
1 Before	78	84	88	91	101	107	102	97	101	90	92	97	93	98	93	23,040
After	106.5	117	116	126	141	162	176	170	183	176	174	191	184	185	193	41,421.25
2 Before	75	76	91	77	83	72	94	96	87	81	83	83	84	90	91	20,430
After	88.5	106	108	100	110	112	125	117	117.5	124.5	121	122	120.5	117	121	28,203.75
3 Before	85.5	91	101	98	100	95	91	99	100	102	108	106	111	109	108	24,788.75
After	101	111	117	120	140	141	141	144	128	151	146	163	204	202	203	38,667.50
4 Before	95.5	103	107	110	108.5	105.5	97.5	102	100.5	94	106.5	109.5	114.5	118.5	122.5	26,017.5
After	115.5	124	129	127	154	174.5	188	200	204.5	208.5	202.5	211	199	184	188	44,944.5
5 Before	86	98	109	106	106	115	98	99	97	112	112	94	84	89	90	23,592
After	101.5	100	102	122	101	129	89	116	119	104	138	138	137	109	120	28,778.75
6 Before	83.5	93	99	104	104	98	101	91	91	88	93	106	86	102	96	23,156.25
After	107	113	116	102	126	129	117	111	96	110	113	97	92	128	113	26,515
Before $\bar{X}$	83.9	90.8	99.2	96.0	100.4	98.8	97.3	97.3	96.1	94.5	99.1	99.2	95.4	101.1	100.1	23,504.08
S.D.	7.2	9.7	8.4	10.7	9.1	14.9	4.2	3.7	5.8	11.0	11.4	9.9	13.9	11.4	12.8	1,884.77
After $\bar{X}$	103.3	111.8	114.7	116.2	128.7	141.3	139.3	143.0	141.3	145.7	149.1	153.7	156.1	154.2	156.3	34,755.13
S.D.	9.0	8.4	9.2	12.0	20.2	23.2	37.3	35.8	42.5	40.9	33.8	43.0	46.2	40.6	42.4	7,875.33

*Statistically significant ($p < 0.05$). ($\bar{X}$ = mean and S.D. = standard deviation.)

phase insulin secretion and continued late-phase insulin secretion is not necessarily dependent on genetic determinants and may be induced in mild alloxan diabetes, a model in which there is an acquired beta-cell insulin deficiency.[19]

The present data do not provide a satisfactory explanation for the abnormally high glucose levels observed during the long-term glucose infusion, despite the increased insulin output. Alloxan may have altered the mechanism by which glucagon is normally suppressed in the presence of rising plasma glucose and insulin levels.[20] This lack of suppression could be due to alloxan per se or to a mechanism entailing release of less biologically active forms of insulin. For example, the effect of alloxan on the beta cells may have resulted in an increased release of proinsulin that, because of its

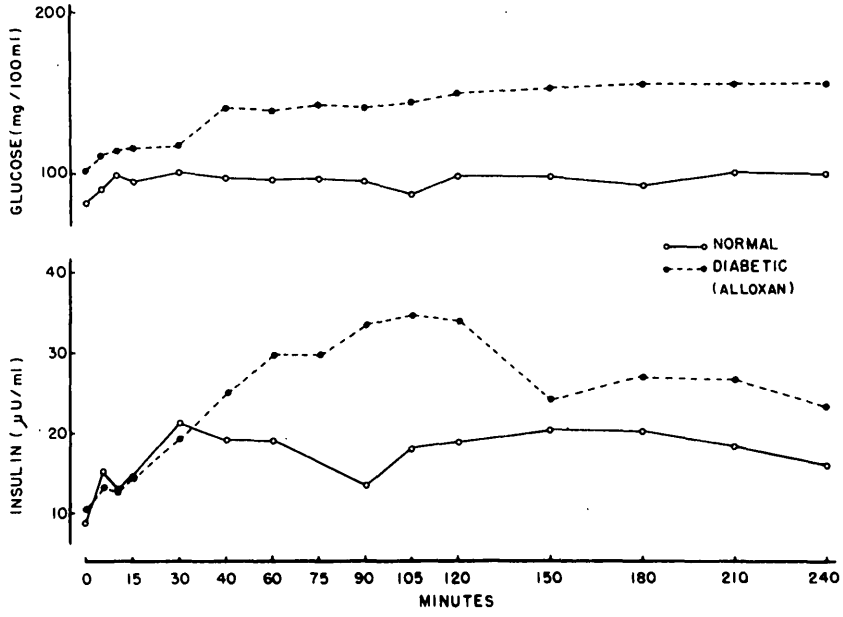

FIGURE 2

Plasma glucose and serum insulin levels during a constant glucose infusion (40 mg./min.) in dogs before and after alloxan-induced diabetes (mean of six dogs).

lesser biologic activity, may have not suppressed glucagon or been effective in diminishing hepatic glucose output. Either mechanism independently or in combination could explain the above data. Lastly, the data do not exclude the possibility that alloxan induces a form of acquired insulin resistance.

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REFERENCES

[1]Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39:1157-75, 1960.

[2]Shen, S. W., Reaven, G. M., and Farquhar, J. W.: Compari-

TABLE 4

Serum insulin concentrations (μU./ml.) and insulin areas during glucose infusion (40 mg./min.) in dogs before and after alloxan-induced diabetes

		Time after starting the infusion (minutes)															
Dog		0	5	10	15	30	45	60	75	90	105	120	150	180	210	240	Insulin areas
1	Before	10	27.5	15.5	10	11	9.5	10	7.5	8.5	17	8.5	11	9	7	10	2,443.75
	After	6.6	4	8	6.5	9.5	17	17	19	28	41	36.5	19	17	20.5	14	4,840.25
2	Before	9.7	22	15	14.5	7	10	24	15	10	8	4.5	8	13	3.5	9.5	2,442.5
	After	2.3	9.5	6.5	3	1	7	11.5	4	11.5	8	10.5	4	7	10	9.5	1,769.50
3	Before	5.9	8.5	8.3	9.3	21	14	9.5	13.3	10	10.5	18.3	17	24.8	34.8	25.8	4,463
	After	7.8	13.3	9.3	10	25	43	51	44.5	43	43	32.5	16.5	22.8	32.5	25	7,235.25
4	Before	6.8	8.5	9	7	19	25	20	11	11.5	9	9	9.5	10	14	9	2,949.5
	After	7.8	8	9	11.5	10.8	16	22	34	37	38	37	34.5	36.5	35	34.5	7,111.5
5	Before	15	20.5	25	32	52	38.5	34	29.5	27	38	48.5	55.5	43.2	28.5	30	9,227.25
	After	16.8	18.5	21.5	34.5	45	37.5	36	32.5	36	42.5	46	48.5	47	37	34	9,549.5
6	Before	7.8	5	10	17.5	16	17	18	17.5	14	19.5	21.5	24	20.5	18.5	10	4,323.25
	After	21	28.5	23	22	24	28.5	42	38	33.5	36.5	41.5	25.5	32	26	22	7,336.25
Before	$\bar{X}$	9.2	15.3	13.8	15.0	21.0	19.0	19.3	15.6	13.5	17.0	18.4	20.8	20.1	17.7	15.7	4,308.21
	S.D.	3.3	9.2	6.3	9.1	16.0	11.1	9.2	7.6	6.9	11.3	16.1	18.0	12.8	12.2	9.5	2,569.99
After	$\bar{X}$	10.4	13.6	12.9	14.7	19.2	24.8	29.9	28.7	31.5	34.8	34.0	27.7	27.0	26.8	23.2	6,307.04
	S.D.	7.0	8.8	7.3	11.7	15.6	13.9	15.5	14.7	11.0	13.4	12.4	15.4	14.4	10.3	10.2	2,676.65

*Statistically significant ($p < 0.05$). ($\bar{X}$ = mean and S.D. = standard deviation.)

son of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. J. Clin. Invest. *49*:2151-60, 1970.

[3]Reaven, G. M., Shen, S. W., Silvers, S. A., and Farquhar, J. W.: Is there a delay in the plasma insulin response of patients with chemical diabetes mellitus? Diabetes *20*:416-23, 1971.

[4]Reaven, G. M., Silvers, A., and Farquhar, J. W.: Study of the relationship between plasma insulin concentration and efficiency of glucose uptake in normal and mildly diabetic subjects. Diabetes *19*:571-78, 1970.

[5]Ginsberg, H., Olefsky, J. M., and Reaven, G. M.: Further evidence that insulin resistance exists in patients with chemical diabetes. Diabetes *23*:674-78, 1974.

[6]Lerner, R. L., and Porte, D., Jr.: Acute and steady-state insulin responses to glucose in nonobese diabetic subjects. J. Clin. Invest. *51*:1624-31, 1972.

[7]Seltzer, H. S., Allen, E. W., Herron, A. L., Jr., and Brennan, M. T.: Insulinic secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. *46*:323-35, 1967.

[8]Fujita, Y., Herron, A. L., Jr., and Seltzer, H. S.: Confirmation of impaired early insulin response to glycemic stimulus in nonobese mild diabetics. Diabetes *24*:17-27, 1975.

[9]Perley, M. J., and Kipnis, D. M.: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J. Clin. Invest. *46*:1954-62, 1967.

[10]Cerasi, E., Luft, R., and Efendic, S.: Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects. Diabetes *21*:224-34, 1972.

[11]Goldner, M. G., and Gomori, G.: Alloxan diabetes in the dog. Endocrinology *33*:297-308, 1943.

[12]Pemberton, L., Beaty, L., and Manax, G. W.: Problems in producing alloxan diabetes. Surgery *68*:375-78, 1970.

[13]Amatuzio, D. S., Stutsman, F. L., Vanderbilt, M. J., and Nesbett, S.: Interpretation of the rapid intravenous glucose tolerance test in normal individuals and mild diabetes mellitus. J. Clin. Invest. *32*:428-35, 1953.

[14]Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system—plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes *12*:115-26, 1963.

[15]Hoffman, W. S.: A rapid photoelectric method for the determination of glucose in blood and urine. J. Biol. Chem. *20*:51-55, 1937.

[16]Dixon, W. J., and Massey, F. L., Jr.: Introduction to Statistical Analysis. Second edit. New York, McGraw-Hill, 1966.

[17]Reaven, G. M., and Farquhar, J. W.: Steady state plasma insulin—response to continuous glucose infusion in normal and diabetic subjects. Diabetes *18*:273-79, 1969.

[18]Graber, A. L., Wood, F. C., and Williams, R. H.: Serum immunoreactive insulin response during prolonged glucose infusions in nondiabetic and diabetic humans. Diabetes *16*:145-49, 1967.

[19]Morgan, C. R., and Lazarow, A.: Immunoassay of pancreatic and plasma insulin following alloxan injection of rats. Diabetes *14*:669-71, 1965.

[20]Braaten, J. T., Faloona, G. R., and Unger, R. H.: The effect of insulin on the alpha-cell response to hyperglycemia in long-standing alloxan diabetes. J. Clin. Invest. *53*:1017-21, 1974.