Effects of Insulin on the Complications of Diabetes and Pregnancy in the Rat

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The administration of insulin to pregnant diabetic women has largely solved the problem of fertility and maternal mortality.[1-4] On the other hand, the fetal mortality has remained higher than that in nondiabetic women.[5,6]

Previous work from this laboratory has indicated that in untreated diabetic rats the growth of the fetuses is retarded.[7,8] The length of the gestation period is significantly prolonged. If the prolongation is sufficiently great, the diabetic rats may show increased birth weights at the time of spontaneous delivery.

In the present study, insulin was administered to pregnant diabetic rats in order to determine whether it would correct these abnormalities.

METHODS

The animals used in this study were a subline of the Sprague-Dawley strain (Holtzman). They were virgin females whose age varied between 120 and 150 days and whose weight ranged between 230 and 280 gm. Alloxan was administered intravenously in doses of 40 mg./kg. body weight, in accordance with the method previously described (Lazarow and Palay[9]). All the diabetic rats were placed in metabolism cages, and fed a Purina fox chow diet. Food was supplied ad libitum; water was limited to 160 ml. per day. Diabetic animals were injected with 5 or 10 units of Protamine Zinc Insulin per kilogram of body weight per day, beginning on the third day after alloxan injection and continuing throughout the period of the experiment. No attempt was made to control the diabetes; insulin was administered primarily to increase fertility and the likelihood of pregnancy's going to term.

Blood samples were drawn in the morning at least twice each week and the blood sugar levels were determined by the Folin-Malmros micro blood sugar method.[10] The twenty-four-hour urinary glucose excretion was measured twice each week. The urine sugar was determined by the caramelization method of Somogyi,[11] and the twenty-four-hour glucose excretion was calculated.

A vaginal smear was made daily in each of the diabetic females and those animals showing estrus were placed with males of the same strain and watched until copulation took place. The time was recorded, and the mated females were caged separately.

The length of gestation between the time of witnessed mating and the time of witnessed delivery of the first young was measured. Beginning at twenty-one days and fifteen hours postcoitum, i.e., one hour prior to the earliest time of delivery in normal pregnancies (Wells[12]), the rats were watched at fifteen- to sixty-minute intervals. Fetuses or newborns were weighed individually. The newborns were weighed promptly after parturition and before they had nursed. The chainomatic balance was read to the closest milligram.

RESULTS

The sixteen treated diabetic rats were subdivided into three groups according to the amount of sugar excreted during twenty-four hours (table 1). It should be noted that the doses of insulin, arbitrarily selected, were suboptimal; they did not completely control the symptoms of diabetes. Four of sixteen animals excreted 0 to 1 gm. of glucose per day; seven of sixteen, 1 to 2 gm.; five of sixteen, 2 to 5 gm. The average blood sugar levels in these groups were 162, 254, and 343 mg. per 100 ml., respectively. Occasional rats showed hypoglycemia on a particular day, in which case the insulin dose was omitted that day.

In the sixteen treated diabetic rats, both the average length of the gestation period and the average birth weights were not significantly different from those in the normal group (table 1).

In those animals which showed a glycosuria of 2 gm. per day or less (Grades 1 and 2), there appeared to be a positive correlation between the birth weight and the insulin dose administered during the last one third of pregnancy (figure 1). Figure 2 shows that

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TABLE 1

The severity of diabetes versus birth weight, gestation age and litter size

Characterization of the diabetes	Mothers: Number	Mothers: Average urine sugar (gm./day)	Mothers: Average blood sugar (mg. per 100 ml.)	Mothers: Average daily dose of insulin (units/kg.)	Newborns: Number	Newborns: Average birth weight (gm.)	Newborns: Average gestation age (hr.)	Newborns: Average litter size
Grade 1	4	0-1	162	8.35	27	6.102	538	6
Grade 2	7	1-2	254	8.73	63	6.055	545	9
Grade 3	5	2-5	343	9.40	51	6.290	539	10
Normal rats	39		109*	0	289	6.224	542	10

*Observations on ten rats.

seven out of the eight treated diabetic rats which had one to four days of hypoglycemia (blood sugar less than 70 mg. per 100 ml.) had subnormal birth weights. The one of the eight with a birth weight above normal was a litter consisting of a single newborn (rightmost bar); this may have contributed to the overweight.

In analyzing the data on birth weight, it was noted that the variance in the treated diabetic series was much larger than that in the normal series. Overweight newborns were observed 3.6 times more frequently in the treated diabetic group than in the normal group (3.6 per cent versus 1 per cent, see table 2). On the other hand, underweight newborns were found forty-one times more often in the treated diabetic group than in the normal (12.3 per cent versus 0.3 per cent). All seventeen underweight newborns in the treated

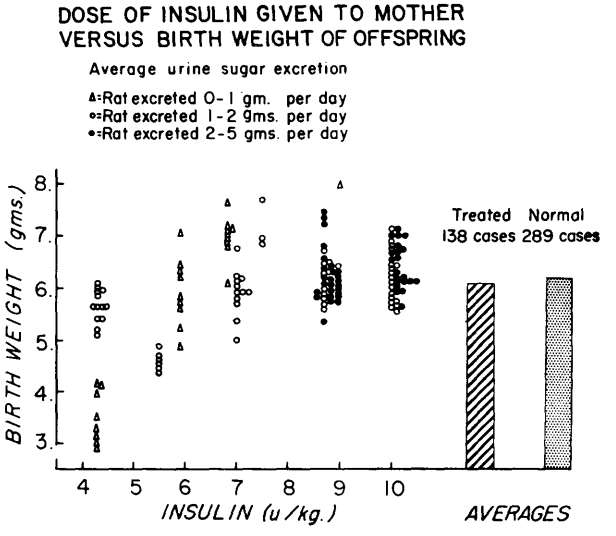

FIG. 1. The abscissa represents the average of the daily dose of Protamine Zinc Insulin administered during the last one third of pregnancy. The two bars show the average birth weights of all newborns of the two groups, these averages having been calculated from the mean weights of the litters.

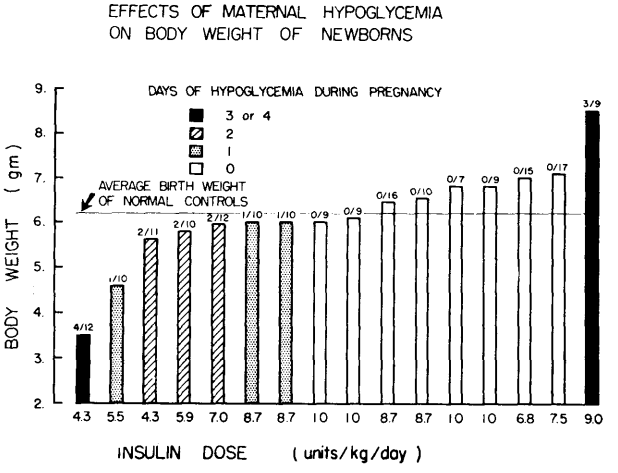

FIG. 2. The numbers above each bar compare the number of days hypoglycemia was observed with the number of times the blood sugar levels were determined during pregnancy. The abscissa represents the average of the daily dose of Protamine Zinc Insulin administered during the last one third of pregnancy.

TABLE 2

Overweight and underweight newborns in normal and diabetic groups*

Groups	Total number of newborns	Overweight, + 2 S.D. (7.384 gm. or more): Number	Overweight: Per cent	Underweight, — 2 S.D. (5.164 gm. or less): Number	Underweight: Per cent
Normal	289	3	1.0	1	0.3
Diabetic, O-term; insulin, O-term	138	5	3.6	17	12.3

*Overweight newborns are those whose weights exceed + 2 standard deviations from the mean for normal controls; underweight newborns are those whose weights are smaller than — 2 standard deviations from this mean.

diabetic group were from rats which had one or more episodes of hypoglycemia during the pregnancies.

In the experimental rats in which the diabetes was most nearly controlled by the insulin (Grade 1), hypoglycemia occurred frequently (table 3).

The average fetal mortality for the entire diabetic group was 2.5 times greater than that for the normal group (figure 3); the P value for this difference was less than .001. It was greater in rats with diabetes of Grades 1 and 2 than that in those of Grade 3. These

TABLE 3

Maternal blood sugar versus stillborns in sixteen pregnant rats

Diabetes	Individual rats	Blood sugar mg. per 100 ml.	70 mg. per 100 ml. or less*	Stillborns
Grade 1	1	149	4 of 12	5 of 7
	2	152	3 of 9	0 of 1
	3	162	2 of 10	2 of 9
	4	186	0 of 15	0 of 10
			(19.6%)	
Grade 2	5	197	1 of 10	4 of 6
	6	252	2 of 12	2 of 12
	7	256	0 of 7	0 of 5
	8	259	2 of 11	3 of 13
	9	265	0 of 16	3 of 11
	10	266	0 of 9	0 of 13
	11	281	0 of 17	1 of 3
			(6.1%)	
Grade 3	12	303	1 of 10	1 of 9
	13	316	0 of 9	0 of 10
	14	346	1 of 10	0 of 10
	15	368	0 of 10	0 of 11
	16	384	0 of 9	0 of 11
			(4.2%)	

*Number of determinations during the entire pregnancy. Figures in parentheses indicate frequency of episodes of hypoglycemia.

animals of Grades 1 and 2 likewise showed more frequent episodes of hypoglycemia than those of Grade 3 (table 3).

Surprisingly, however, the fetal mortality was highest in the group of treated rats of Grade 1, i.e., those rats which had blood sugars ranging from 149 to 186 mg. per 100 ml. and which excreted 0 to 1 gm. of urine sugar per twenty-four hours. Also, the fetal mortality was lowest in the group of treated rats of Grade 3, i.e., those rats which had blood sugars ranging from 303 to 384 mg. per 100 ml. and which excreted 2 to 5 gm. of urine sugar per twenty-four hours.

DISCUSSION

The fetal mortality of 14.9 per cent in the insulin treated diabetic group was not as high as that found in two previous groups of diabetic rats which were reported by Kim, Runge, Wells and Lazarow.[7] In the

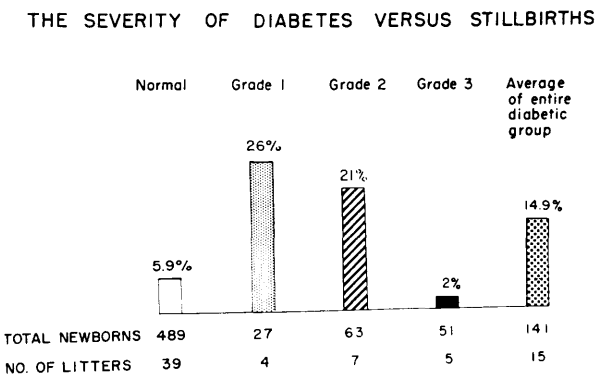

FIG. 3. The height of the bars represents the per cent of stillbirths in the normal and insulin treated diabetic groups. The left and right bars represent averages for the two groups. The three center bars represent subgroups of the treated diabetic group.

first of these two groups, the alloxan diabetes was induced on the twelfth day of pregnancy; the fetal mortality was 22.6 per cent. In the second group, the alloxan diabetes was induced before conception and the insulin treatment was given daily from day 0 to day 12 of pregnancy; the fetal mortality was 47.6 per cent. These observations suggest that in our present insulin treated diabetic rats the insulin reduced the fetal mortality.

Although the average gestation period and the average birth weight in the treated diabetic group were essentially normal, the average fetal mortality was nevertheless 2.5 times greater than that in the control group. The fetal mortality was greatest in those members of the insulin treated diabetic group which had an average blood sugar of 162 mg. per 100 ml., which excreted 1 gm. or less of urine sugar per twenty-four hours, and which had the greatest frequency of episodes of hypoglycemia (Grade 1). These episodes of hypoglycemia were consequences of the insulin treatment, and they might be a factor which contributed to the increased fetal mortality and to the seven subnormal birth weights which are shown in figure 2 (bars 1 to 7, reading from left to right).

SUMMARY AND CONCLUSIONS

Rats were rendered diabetic by alloxan injection prior to conception and treated with insulin throughout pregnancy. Although insulin treatment decreased fetal mortality, these treated rats nevertheless showed a significantly greater fetal mortality than the normal controls.

The treated diabetic animals with the greatest fetal mortality showed the least hyperglycemia and glycosuria. It is suggested that occasional hypoglycemia, occurring secondary to insulin administration, may be a factor in causing an increased fetal mortality and a

decreased fetal weight. The insulin treated group likewise showed increased frequencies both of overweight and of underweight newborns.

SUMMARIO IN INTERLINGUA

Le Effectos de Insulina Super le Complicationes de Diabete e de Pregnantia in le Ratto

Rattos esseva rendite diabetic per injectiones de alloxano ante le pregnantia e alora tractate con insulina usque al parturition. Ben que le tractamento con insulina reduceva le mortalitate fetal, iste tractate rattos monstrava nonobstante un significativemente plus alte mortalitate que normal animales de controlo.

Le tractate rattos diabetic con le plus grande mortalitate fetal manifestava le plus basse hyperglycemia e glycosuria. Es speculate que le hypoglycemia que occurre sporadicamente secundari al administration de insulina es possibilemente un factor in le causation del augmento del mortalitate fetal e del reduction del peso fetal.

Le gruppo tractate a insulina manifestava etiam augmentate frequentias de tanto neonatos a peso excessive como de neonatos a peso infranormal.

ACKNOWLEDGMENT

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An alteration in the physical state of cortisol in the blood during pregnancy has been described by W. R. Slaunwhite and A. A. Sandberg (*J. Clin. Investigation* *38*:384, 1959). Under certain conditions, plasma from pregnant women was shown by dialysis equilibrium to bind significantly more cortisol than plasma from nonpregnant women. The authors suggest that there is in pregnancy an increase in the plasma protein which normally is responsible for the binding of nearly all the cortisol of plasma.

The metabolism of aldosterone in pregnancy has attracted investigative interest because of the possibility that increased secretion of this hormone might be responsible in part for the salt retention which occurs in toxemia. Although the excretion of aldosterone in pregnancy has been measured by a number of methods, the most definitive study appears to be that of K. M. Jones, et al. (*Acta Endocrinologica* *30*:321, 1959).

Tritiated aldosterone was injected into six pregnant women and a similar number of women who were not pregnant to permit an estimation of the secretion rate of aldosterone by measuring the specific activities of aldosterone and its metabolites in the urine. The mean total secretion rate for aldosterone in the normal woman so measured was 192 μg. per twenty-four hours. Most of the pregnant women were found to be producing larger amounts of aldosterone; the mean adrenal secretory rate was estimated to be 582 μg. per twenty-four hours. In the urine of normal women there was only about 0.44 μg. of unconjugated aldosterone; a modest increase to 1.7 μg. was present in the urine from pregnant women. In contrast, there was an eight- to ninefold increase in the aldosterone which is excreted in the urine as a labile conjugate which is hydrolyzed at room temperature at *p*H 1. This relative increase in the labile conjugate does not occur in the hyperaldosteronism which follows sodium restriction and may indicate an increase in this pathway of aldosterone inactivation.

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