Effect of Streptozotocin-induced Diabetes on Pancreatic Insulin Content of the Fetus

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SUMMARY

Diabetes was produced in five-day pregnant Sprague-Dawley rats by intravenous injection of 40 mg./kg. body weight of streptozotocin. Normal pregnant rats were used as controls. Pancreases of the mothers and their offsprings were extracted by acid-alcohol and the insulin measured by immunoassay using rat insulin as a standard.

Nonfasting blood sugar of the streptozotocin-treated pregnant rats was 285 ± 18 mg./100 ml. and their pancreatic insulin concentration was 5.9 ± 1 μg./gm. compared to 103 ± 3 mg./100 ml. and 44.2 μg./gm., respectively, in the normal rats.

Fetal pancreases of streptozotocin-induced diabetic pregnant rats and normal animals were obtained on Days 18, 19, and 21 of gestation, and on Day 4 after birth. The pancreatic insulin concentration of fetuses of diabetic mothers rapidly and progressively increased from 8.0 ± 0.9 μg./gm. on Day 18 to a peak of 63.0 ± 9.0 μg./gm. on Day 21. On Day 4 after birth, the value was 312 ± 109 μg./gm. The total pancreatic insulin content also progressively increased to a prenatal peak of 0.84 ± 0.15 μg. on Day 21. The pancreatic insulin concentration of fetuses of normal mothers was not significantly different from those of diabetic mothers. There was no correlation between the elevation of maternal blood sugar and the insulin concentration of the fetal pancreas. The total pancreatic insulin content of fetuses of diabetic rats was less than those offspring of the normal rats on Day 18 and Day 4 after birth, but was not significantly different on Days 19 and 21. The lower insulin content found in four-day-old rats born of diabetic animals was probably related to their inability to obtain adequate nourishment from their very sick mothers. DIABETES *19*:610-13, September, 1970.

In the past it has been difficult to study experimental diabetes in the pregnant animal because of the disastrous effect upon the pregnancy of either of the two principal means of inducing diabetes, i.e., subtotal pancreatectomy[1,2] and alloxanization.[3] Recently, streptozotocin, an antibiotic complex obtained from *Streptomyces achromogenes,* has been shown to produce diabetes in rats.[4] In a previous study we demonstrated the suitability of streptozotocin as a method of inducing diabetes in pregnant rats.[5] In the present investigation, we have induced streptozotocin diabetes in pregnant rats and have measured the pancreatic insulin concentration and content of their fetuses and newborns, and of the corresponding offspring of normal rats. The insulin was measured by immunoassay, employing rat crystalline insulin as a standard.

MATERIAL AND METHODS

Female Sprague-Dawley rats (Dublin Labs., Va.) weighing 160 to 190 gm. were used in the study. The animals were caged individually, and rat chow (Wayne Lab.) and water were given ad libitum. During estrus, the females were caged for a single night with the males. The presence of spermatozoa in the vagina was confirmed in the morning, this being taken as the Day 0 of gestation. Forty mg./kg. of body weight of a freshly prepared 4 per cent solution of streptozotocin (No. NCS-85598) in citrate buffer at pH 4.6 was injected into the tail vein on Day 5 of pregnancy after an overnight fast.* The presence of diabetes was confirmed by the demonstration of an elevated postprandial blood sugar. Blood sugar was measured by a modification of the Nelson-Somogyi method.[6,7] The maternal blood for sugar determination was obtained from the tail vein on Day 10 and Day 14 ± 1 of pregnancy.

Two hundred and sixty-three fetuses or newborns of twenty-seven normal maternal rats, and 201 fetuses

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or newborns of twenty-four diabetic rats were available for study. Groups of animals were sacrificed by neck dislocation at different times of gestation after sixteen hours of an overnight fast. The days of sacrifice were Days 18, 19, 21, and Day 4 postpartum. The maternal pancreases were immediately dissected free as completely as possible, weighed, minced, and extracted by an acid-alcohol procedure previously described.[8] After removing the fetuses and weighing them individually, their pancreases were dissected and frozen immediately on dry ice. All pancreases of each individual litter were then pooled, weighed, and extracted for their insulin content.

The radioimmunoassay of insulin was performed by the method of Berson and Yalow.[9] Anti-pork insulin guinea pig serum was used. Radioactive insulin was obtained from Abbott Laboratories (Inusay—131-H, catalog #7791). Crystalline rat insulin was used as a standard.* Pancreatic extracts were assayed in dilutions which ranged from 1/25 to 1/3,200. The insulin concentration was expressed as μg./gm. of wet pancreatic tissue, and total insulin content was expressed as μg./pancreas. Each maternal pancreas was separately assayed, and the pancreases of the fetuses from the same litter were pooled, each litter being assayed separately. A standard curve of immunoassay is shown in figure 1.

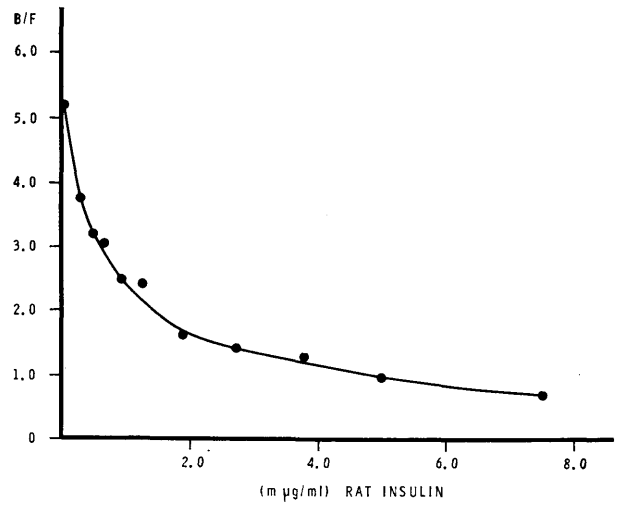

FIG. 1. Standard curve of immunoassay.

RESULTS

Maternal rats

The mean nonfasting blood sugar of the normal pregnant rats was 103 ± 3 mg./100 ml. (range 78 to 141 mg./100 ml.). The mean nonfasting blood sugar of the diabetic pregnant rats was 285 ± 18 mg./100 ml. (range 155 to 450 mg./100 ml.).

The mean insulin concentration of the pancreas of normal pregnant rats between Days 18 and 21 was 44.2 μg./gm. (range 22 to 81 μg./gm.). The streptozotocin-treated pregnant rats were found to have a mean pancreatic insulin concentration of 5.9 ± 1.1 μg./gm. (range 0.6 to 20 μg./gm.). The difference between the pancreatic insulin concentration of the pregnant streptozotocin-diabetic rats and that of the normal pregnant rats is highly significant ($p < 0.001$).

Offspring of diabetic and normal rats

In table 1 are shown the data pertaining to pancreatic weight, pancreatic insulin concentration, and total pancreatic insulin content of the fetuses and newborns of diabetic and normal rats. The mean pancreatic weight of the fetuses of the diabetic rats on Day 18 was 2.8 ± 0.1 mg. This increased to 13.0 ± 0.9 mg. by Day 21, there being no further significant change on Day 4 postpartum. The pancreatic weights of the fetuses of normal mothers on Day 19 and Day 21 were not significantly different from the pancreatic weights of corresponding diabetic fetuses, but were significantly higher ($p < 0.02$ on Day 18 and Day 26).

The fetal pancreatic insulin concentration of the diabetic group was 8.0 ± 0.9 μg./gm. on Day 18, 63.0 ± 9.0 μg./gm. on Day 21, and 312 ± 109 μg./gm. on Day 4 after birth. The corresponding values for pancreatic insulin concentration of the normal group were not significantly different.

The total pancreatic insulin content in the fetuses of diabetic rats on Day 18 was 0.02 ± 0.002 μg. On Day 21 this value reached the prenatal maximum of 0.84 ± 0.15 μg. On Day 4 after birth, the total pancreatic insulin had increased to 3.5 ± 1.15 μg. There was no significant difference in the total pancreatic insulin between the fetuses of diabetic and normal rats on Days 19 and 21. On Day 18 and on Day 4 after birth, the total pancreatic insulin of the offspring of the diabetic rats was significantly less, however, than that of the offspring of the normal rats (0.07 ± 0.001 μg.; $p < .02$ and 9.56 ± 1.94 μg.; $p < .05$ respectively).

DISCUSSION

There have been several studies describing the morphologic changes in pancreatic islets of fetuses and/or newborns of diabetic mothers.[10-16] There is very little information regarding the effect of clinical or experi-

*We are grateful to Dr. I. Arthur Mirsky for the crystalline rat insulin.

TABLE 1

Pancreatic weight, pancreatic insulin concentration and total pancreatic insulin content of the fetuses and newborns of normal and diabetic rats. Mean ± S.E.M. and range.

Days of gestation		18	19	21	26
Fetuses of nondiabetic rats	No. of litters (total—27)	9	7	6	5
	No. of fetuses (total—263)	80	77	67	39
	Pancreatic weight (mg.)				
	Mean ± S.E.M.	4.4±0.3	7.0±0.4	15.0±0.5	15.1±0.5
	Range	(3—6.1)	(5.5—9.0)	(13.0—17.0)	(13.6—17.0)
	Pancreatic insulin concentration (μg./gm.)				
	Mean ± S.E.M.	14.0±2.0	25.0±5	80.0±9.0	621.0±103.0
	Range	(7—20.0)	(9.0—42.0)	(58.0—104.0)	(428.0—988.0)
	Total pancreatic insulin (μg./pancreas)				
	Mean ± S.E.M.	0.07±0.001	0.17±0.03	1.23±0.15	9.56±1.94
	Range	(0.03—0.16)	(0.06—0.30)	(0.79—1.68)	(7.13—16.79)
Fetuses of diabetic rats	No. of litters (total—24)	3	9	7	5
	No. of fetuses (total—201)	30	86	63	22
	Pancreatic weight (mg.)				
	Mean ± S.E.M.	2.8±0.1	6.9±0.6	13.0±0.9	11.4±0.8
	Range	(2.5—3.5)	(4.4—9.0)	(10.0—14.3)	(10.0—13.0)
	Pancreatic insulin concentration (μg./gm.)				
	Mean ± S.E.M.	8.0±0.9	26.0±2.0	63.0±9.0	312±109
	Range	(6.0—9.0)	(15.0—44.0)	(29.0—85.0)	(106—480)
	Total pancreatic insulin (μg./pancreas)				
	Mean ± S.E.M.	0.02±0.002	0.19±0.03	0.84±0.15	3.5±1.15
	Range	(0.018—0.027)	(0.09—0.93)	(0.29—1.25)	(1.20—4.80)

mental maternal diabetes on the immunoassayable insulin concentration or content of the fetal or neonatal pancreas, however.

In 1960, Rose,[17] in a study of infants of diabetic mothers, reported increased pancreatic insulin concentration in three out of four infants studied at autopsy. The actual amounts of insulin and the methods used were not mentioned in the paper. Steinke and Driscol,[18] using the epididymal fat pad bioassay, found increased pancreatic insulin concentration in the fetuses and newborns of diabetic women. In 1964, Dixit et al.,[19] using the epididymal fat pad, measured the "insulin-like activity" in microdissected pancreatic islets of fetuses and newborns of alloxan diabetic rats, and reported a marked reduction. Wellman et al.[20] measured the immunoassayable pancreatic insulin of newborns of alloxan diabetic rabbits. These workers did not find a significant difference in the pancreatic insulin concentration of the newborns of diabetic rabbits compared to the control offspring.

In table 1 are given the pancreatic insulin concentrations of the offspring of the diabetic and control rats. At none of the periods of gestation was there a significant difference between the pancreatic insulin concentrations of the fetuses of diabetic and normal rats ($p < .05$).

The mean pancreatic weight of the offspring of diabetic rats was, at all stages of development, less than that of the corresponding offspring of normal rats, and on Day 18 and Day 26 of gestation, this difference was statistically significant ($p < 0.02$). The body weights of the fetuses of streptozotocin rats were also significantly lower than those of control fetuses.[5] The decreased pancreatic weight may be a reflection of delayed growth and differentiation, perhaps accounting for the decreased total pancreatic insulin content on

Day 18. We do not know whether this decrease is due to a direct effect of streptozotocin upon the fetus, or the maternal hyperglycemia.

Although the total pancreatic insulin content was somewhat less in the offspring of the diabetic rats at all stages except Day 19, only on Day 18 and on Day 26 was this difference significant ($p < .05$ and $p < .02$ respectively). It is interesting that on Day 14 the mothers of the Day-18 fetuses also happened to have significantly higher blood sugar than any other diabetic group (mean 431 mg./100 ml.). No intragroup correlation was observed, however, between the level of maternal blood sugars on Day 14 and the subsequent fetal pancreatic insulin concentrations or total insulin contents. The significantly reduced total insulin content of the pancreases of four-day-old rats may have been related to their inability to obtain adequate nourishment from their very sick diabetic mothers.

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