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Fetal Rat Islet Insulin Deficiency following Maternal Administration of Streptozotocin[1] (42165)

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Abstract. Pancreatic islets were isolated from the fetuses of normal rats and rats made diabetic by the iv administration of streptozotocin (STZ) on either Day 3 or 5 of pregnancy. Of the rats made diabetic on Day 3, one group also received insulin injections at the appearance of glucosuria. Maternal blood glucose on Day 20 of gestation was significantly different in the diabetic rats (405 ± 27 mg/dl) from the normal (97 ± 1 mg/dl) and insulin-treated diabetic rats (69 ± 9 mg/dl). While fetal weight was significantly decreased in the STZ-treated rats (2.64 ± 0.13 g vs 3.52 ± 0.05 g for the control group, $P < 0.005$), fetal glucose was significantly higher in the STZ-treated than in normal pups (342 ± 11 vs 35 ± 1 mg/dl, $P < 0.005$). Both fetal weight and glucose were normalized by insulin treatment: 3.16 ± 0.18 g and 31 ± 7 mg/dl, respectively. Insulin release from fetal islets of diabetic dams was blunted after a week in culture both in basal and stimulated conditions. After 2 weeks in culture, there was partial recovery in the insulin response to glucose but it did not equal to that measured in fetal islets from the normal and insulin-treated diabetic rats. These data suggest (a) maternal hyperglycemia severely impairs fetal weight and insulin release from fetal rat islets *in vitro*, and (b) correction of the hyperglycemia by insulin treatment not only improves fetal weight and glucose concentrations, but it also normalizes insulin release from fetal rat islets *in vitro*.

Infants of mothers with poorly controlled diabetes are, at birth, generally macrosomic, hyperinsulinemic, and hypoglycemic (1). Unfortunately, in experimental animal models of maternal diabetes, it has not been possible to reproduce these clinical and pathological characteristics (2). Recent reports (3–5), however, suggest that the role of insulin in growth and development may be clarified by using the model of the rat fetus made insulin-deficient by streptozotocin (STZ)-induced diabetes in the mother.

When severe diabetes in female rats is induced by STZ administration before conception, their offspring are hypoinsulinemic, hyperglycemic, and of small weight (3, 6, 7). Other authors have reported that mild diabetes (7) and diabetes caused by STZ administered postconception (8) apparently cause increased fetal body weight and hyperinsulinemia. Because of these conflicting results, we investigated in rats the effects on fetal weight and fetal pancreatic islet function of treated and untreated STZ diabetes induced early in gestation.

Methods. Sprague–Dawley female rats, 180–240*g*, were housed in a room with a 1630–0730 dark cycle. Females were mated 1:1 with males, and each morning vaginal smears were examined for the presence of sperm. The date of sperm detection was considered Day 0 of pregnancy and males were removed. One group of rats on Day 3 and another on Day 5 of pregnancy were anesthetized with ether and then given STZ (50 mg/kg in sodium citrate buffer, pH 4.5) in the jugular vein. Control animals received the buffer alone. Animals were housed in individual cages for the remainder of pregnancy. Glucosuria was first noted 2–3 days after injection in the animals given STZ. Their diabetes was left untreated during pregnancy except for a group of dams which had received STZ on Day 3; at the first appearance of glucosuria, these rats were given sc 2–5 units once daily, between 8 and 9 AM, of ultralente insulin in efforts to maintain glucosuria at less than 1/10% (by Diastixs, Miles Laboratories, Elkhart, Ind.). Glucosuria was monitored daily and the dose of insulin adjusted to 2 units if less than

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1%, and 4 to 5 units if between 2 and 5%. The last insulin injection was given 24 hr before sacrifice. On Day 20 of gestation, after maternal cervical dislocation, blood for glucose measurements was obtained by cardiac puncture; the fetuses, delivered by cesaerian section, were weighed and decapitated, and a blood sample was obtained for glucose measurements. Then the pancreas was immediately removed for islet isolation. Pregnancy was terminated on Day 20 because the islet cell population doubles during the last 2 days of gestation and gestation is prolonged in severely diabetic rats (5).

Only the dorsal portion of each pancreas was used; the pooled pancreatic fragments were digested by collagenase following the method of Hellerstrom *et al.* (9), slightly modified by us (10). They were then incubated in petri dishes at 37°C in a humidified atmosphere of 5% CO_2 and air after suspension in medium RPMI 1640 containing 10% heat inactivated fetal calf serum and antibiotics: (penicillin 100 U/ml and streptomycin 0.1 mg/ml). After 1 week in culture, islets were hand-picked with the aid of a braking-pipet under direct vision with a stereomicroscope. Qualitatively, a marked decrease in the yield of islets from the fetal pancreas of diabetic dams was noted. Groups of five islets of equal diameters to compensate for islet size were transferred to tissue culture plates (Multiwell, Falcon), and incubated first in modified RPMI 1640 for 60 min at basal conditions (glucose 5.6 m*M*) and then for 60 min at stimulated conditions (glucose 16.7 m*M* plus aminophylline 10 m*M*). Samples from the media were taken at the end of each 60-min incubation period and frozen for later simultaneous radioimmunoassay of insulin content (11). After an additional week in the described culture conditions, different groups of islets obtained from the same control and treated fetuses were handled in the same way.

From each litter, pancreatic specimens were also obtained for immunocytochemical studies after fixation in Bouin's solution. Some of the sections were stained with hematoxylin and eosin, and most were used for the localization of insulin and glucagon by the peroxidase–antiperoxidase method utilizing guinea pig anti-insulin and rabbit antiglucagon serum, respectively (12).

Statistics were done by paired and unpaired Student's *t* test.

Results. On Day 20 of gestation all the STZ-treated dams were diabetic with most individual blood glucose concentrations exceeding 300 mg/dl (405 ± 27 mg/dl mean ± SEM). By contrast, the control and insulin-treated animals had serum glucose concentrations in the normal range of 97 ± 1 and 69 ± 9 mg/dl, respectively ($P < 0.005$ diabetic vs control and STZ + insulin).

The effects of STZ-treatment on fetal weight and serum glucose concentrations are shown in Table I. Separate from the effects on fetal weight, early STZ treatment caused a marked increase in spontaneous termination of pregnancy. When conception was documented by the presence of sperm on vaginal smear, 30% of STZ-treated animals on Day 20, were not pregnant. Similarly, 5% of controls were not pregnant. Maternal mortality following STZ treatment was 5%; none of the control animals died. Thus, when STZ is given early in pregnancy 30–35% of the animals are not useful for study on Day 20.

The results of the functional studies carried out on the islets isolated from the different groups of fetal pancreases are shown in Fig. 1. After a week in culture, insulin release, both at basal and stimulated conditions, was greater in the fetal islets obtained from normal and insulin-treated dams than in fetal islets of the dams given only STZ (P, < 0.01 at basal and <0.005 at stimulated conditions). In addition, following stimulation, insulin release in the fetal islets of the dams treated with both STZ and insulin did not reach statistical significance ($P < 0.10$), in contrast to the response measured in the control islets ($P < 0.005$).

TABLE I. EFFECTS OF MATERNAL STZ TREATMENT ON FETAL WEIGHT AND SERUM GLUCOSE CONCENTRATION

Group	Fetal weight (g)	Glucose (mg/dl)
Control (4)	3.52 ± 0.05	35 ± 1
STZ (12)	2.64 ± 0.05*	342 ± 11*
STZ + insulin (4)	3.16 ± 0.18	31 ± 7

Note. Number of pregnant animals are in parentheses. Mean ± SEM.

* $P < 0.005$.

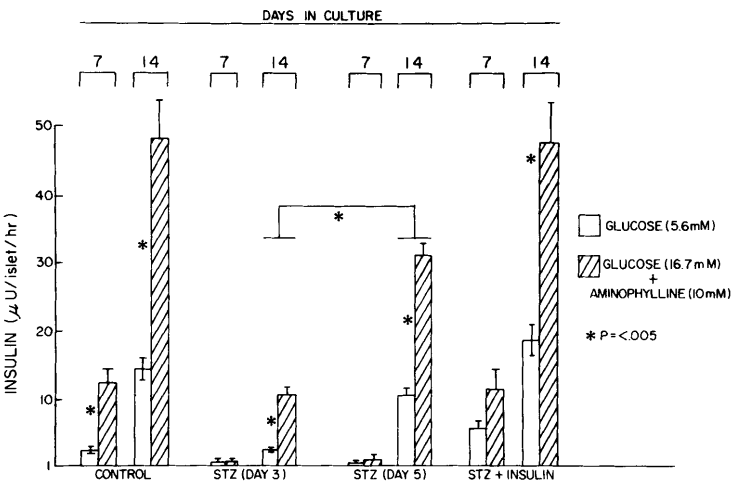

FIG. 1. Insulin release from isolated fetal islets maintained in culture for 1 and 2 weeks. Each bar represents mean ± SEM of 10 experiments. Islets obtained from fetuses whose mothers were treated (left to right) as follows: control, buffer injected; STZ given on Day 3, STZ given on Day 5 of pregnancy, and STZ given on Day 3 or pregnancy followed by insulin treatment.

After a second week of incubation, insulin release was significantly higher (baseline vs stimulation) in all four groups of islets ($P < 0.005$). However, for both basal and stimulated conditions, the fetal islets from control and insulin-treated diabetic rats released significantly more insulin than those obtained from the fetuses of overtly diabetic dams given only STZ ($P < 0.001$ basal and <0.025 stimulated conditions). In addition, the fetal islets of rats made diabetic by injection of STZ at 3 days of gestation released less insulin than those from rats injected with STZ at 5 days ($P < 0.005$).

There were other differences: (a) extensive disorganization of islets in fetuses from rats made diabetic at 3 or 5 days of gestation was shown when the fetal pancreases were stained with hematoxylin and eosin; in these preparations by light microscopy it was difficult to separate exocrine from endocrine tissue. In distinct contrast, the islets in the fetuses of control and insulin-treated rats were easily identified and remained spherical and well-preserved, (b) only minimal evidence of pancreatic insulin in fetuses of diabetic rats was shown by immunocytochemical staining, whereas the same staining displayed an abundance of insulin in fetuses from normal dams.

Glucagon was clearly stained in the fetal islets of all rats used in the experiments.

Discussion. The studies reported here indicate that hyperglycemia induced by STZ administration to rats during early pregnancy is deleterious to the fetus and the fetal islet. Also, it seems that the earlier the metabolic insult, the more severe at least *in vitro,* is the reduced release of insulin from islets. This is shown in Fig. 1 by the difference in insulin release when STZ is given on Day 3 or Day 5 of pregnancy. From previous articles on this subject it seems clear that STZ administered to female rats before conception in doses sufficient to cause severe hyperglycemia produces decreased fetal weight and hypoinsulinemia (3, 7, 13). Our data shown in Table I also indicate that when STZ is given early in pregnancy, the effects on fetal weight and serum glucose concentrations are similar.

What is not clear from the literature on this subject, however, is the effect of STZ on the total fetus and on the fetal endocrine pancreas in particular, when the STZ is administered in low doses sufficient to cause only mild diabetes, or in high doses followed by injections of insulin. Some authors have reported increased fetal weight and hyperinsulinemia in rats (6, 7, 13), but more recent data, in accord with that presented here, have shown reduced weight, low pancreatic insulin concentration, and poor growth of the fetal pancreatic B cell (3, 5). According to Cuezva *et al.* (8), when

STZ was administered to a pregnant rat on Day 5 of gestation, the fetuses suffered hyperinsulinemia at birth regardless of any treatment of the STZ-diabetic dams with insulin. In contrast, our experiments *in vitro* show that while there is impairment of islet function in fetuses of rats given only STZ, fetal islets from rats made diabetic by STZ and then given insulin behave *in vitro* like fetal islets of normal dams. The difficulty in maintaining normal glucose concentration in diabetic animals has been well documented (14); thus, although at 20 days of gestation mean maternal blood glucose was normal in the insulin-treated dams, one cannot assume that normoglycemia was present throughout pregnancy. Assuming that mild glucose intolerance existed during pregnancy, such a situation did not cause exaggerated insulin release since the fetuses were normoglycemic at 20-day gestation.

It has been demonstrated that depending upon the route of administration and the dosage of STZ, the clinical onset of diabetes can be altered (15–17). The diabetogenic properties of STZ are related to selective destruction of pancreatic B cells, a process which is readily demonstrable by electron microscopy within hours of injection (18). Thus, when STZ is given to adult animals, pancreatic B cells are selectively damaged and subsequently phagocytized by macrophages (18–20). In the experimental model described here, the fetal pancreatic tissue had not yet developed B cells at Day 3 or Day 5 of pregnancy the dates when we administered the STZ. Therefore, the mechanism causing decreased islet insulin release in the fetus must be different from that in the adult. Moreover, while some of the features of insulin deficiency, such as the lack of immunoperoxidase staining, could be attributed to extensive B-cell degranulation in response to maternal and fetal hyperglycemia, the altered fetal pancreatic architecture cannot be so easily explained. Recently, Swenne and Eriksson (21) demonstrated decreased islet cell replication in rats made diabetic by STZ before mating, corroborating their previous observations (5) of decreased B-cell mass in the offspring of diabetic rats. Our data are in complete agreement with their findings. Although we also show that islet functional recovery *in vitro* was not complete after 2 weeks in culture, these data do not imply that the situation would be the same *in vivo*. The normal islets, on the other hand, behaved similarly to those from other rat strains (10): early refractoriness to the stimulatory effects of glucose and aminophylline, but full response after 2 weeks in culture.

At a gestational age of 3 days, the growing rat ovum is in early blastocyst state (22, 23). Direct effects of STZ on the differentiating blastocyst have not been examined, but studies in rabbits have shown that the preimplanted blastocyst will take up drugs from the maternal circulation (24). Thus, although it may appear that STZ affects the pluripotential blastocyst cells early in gestation, resulting in a selective reduction of pancreatic B cells in the newborn fetus, as suggested by Helgason *et al.* (25), it is not possible to separate a single cause from the combined effects of high glucose, fetal insulin deficiency, and STZ on the developing pancreas. Since in our experiments improvement in maternal hyperglycemia by insulin treatment restored the altered fetal islet development and islet function *in vitro*, it seems most likely that hyperglycemia per se caused these problems.

In conclusion, STZ administration to pregnant rats on or before Day 5 of pregnancy retards fetal weight, causes fetal and maternal hyperglycemia, and profoundly alters the functional and morphological development of the fetal islet. Correction of maternal hyperglycemia by insulin treatment leads to normalization of fetal weight and insulin release by islets *in vitro*. Such treatment does not cause exaggerated insulin responses to known secretagogues. Consequently although the effects of maternal diabetes on the offspring of the rat are different from those observed in humans, the rat offers a useful model to study fetal islet development under conditions of altered metabolism.

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