Note

Glucose Tolerance Tests in the Perinatal Rats

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Synopsis

Glucose tolerances *in vivo* during perinatal days in the rat were studied. Glucose was injected intraperitoneally, and blood sugar levels were determined 0, 0.5, 1, 2, and 3 hr after the glucose challenge.

The blood sugar level reached the peak at 0.5 hr in all series of animals. In both the premature newborn rats delivered by Caesarean section on the 22nd day of gestation and 2-day-old rats, the levels declined thereafter, but the decline was relatively slower particularly 1 hr after the glucose challenge than that observed in the adult. Glucagon added to glucose did not enhance the glucose tolerance in the young. In pancreatectomized 2-day-old rats and premature newborn rats born to alloxan-treated mothers, the blood sugar level remained high even 3 hr after the challenge.

The results suggest the capacity of the late fetal and early postnatal pancreatic islets for releasing insulin in response to a stimulus of hyperglycemia *in vivo*.

In the development of the pancreatic islets in the rat, insulin-like activity has been demonstrated during the last third period of fetal life (Grillo, 1964; Dixit *et al.*, 1964). The glucose tolerance of a pancreatectomized pregnant rat shifts toward normal during the late period of gestation (Frye, 1959), a finding suggesting compensatory activities of the fetal pancreases, though non-permeability of the rat placenta to insulin has been pointed out (Seller, 1964). In addition, under maternal diabetic conditions, it is said that the fetal pancreatic islets would work hyperactively to show the degranulation and hydropic changes of β cells (Frye, 1957; Angervall, 1959; Kozma-de Bokay *et al.*, 1961). Therefore, it is of much interest because of expanding

knowledge concerning physiology of the mammalian fetus to know whether or not a developing pancreas *in vivo* is capable of releasing insulin.

As regards the insulin secretion during fetal life, conflicting conclusions have so far been induced about the role of glucose: the fetal pancreatic islets can or can not release insulin in response to glucose injected to the fetus. Also, about the role of glucagon in insulin response to glucose, there are some controversies in the literature.

The present study was designed to estimate whether the perinatal pancreas could release insulin in response to hyperglycemia and whether glucagon could potentiate this response, by examining glucose tolerances of perinatal rats. In addition, tolerances of the young of alloxan-diabetic pregnant rats were examined. The glucose tolerance tests in such young rats are the first of their kind heretofore reported.

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Materials and Methods

Rats of the Wistar strain were used. They were given a commercial diet (Oriental pellets NMF) and water, both *ad libitum*. Days of gestation were counted from the day following an overnight mating as the 1st day of gestation.

For glucose tolerance tests, premature newborn rats obtained by Caesarean section on the 22nd day of fetal life and 2-day-old newborn rats were used one hour after separation from their mothers. The glucose challenge was made by an intraperitoneal injection of 25% glucose in saline at a dose of 2.5 mg/g body weight. Five littermates served as an experimental series for the test. Animals in each series were sacrificed 0, 0.5, 1, 2, and 3 hr after the challenge for the determination of blood sugar levels by the method of Hagedorn-Jensen.

The intraperitoneal injection of glucose to young rats was made as follows. A threaded needle was passed under the ventral skin in 2 places so as to tie 2 ends into a loose knot. Through the knot, the injection was made. As the injection needle was withdrawn, the knot was tightened in order to prevent the injected material from leaking out.

Some newborn rats delivered by Caesarean section were given glucagon (Eli Lilly Co.) intraperitoneally at a dose of 0.1 μ g/g body weight together with the glucose challenge. Some 2-day-old rats were pancreatectomized subtotally by the following method. After left-ventral laparotomy under ether anesthesia, the tail of the pancreas was removed from the spleen and then the pancreatic tissue was torn off in small fragments with forceps while clamping blood vessels coming into the pancreas from the celiac artery with a second pair of forceps. Portions of pancreatic tissue adhering to the transverse colon were left *in situ* in order to avoid damaging the trunk of the celiac artery. The incision in the ventral abdominal wall was closed with sutures. After complete recovery from anesthesia, the animals were subjected to the glucose tolerance tests.

In addition, each of some pregnant rats was given a subcutaneous injection of alloxan at a dose of 125 mg/kg body weight on the 18th day of gestation. On the 22nd day of gestation, fetuses of these rats were exteriorized from the uterus and subjected to the glucose tolerance test. Separately, 2 alloxan-treated pregnant rats were examined for their glucose tolerances on the 21st day of gestation. For comparison, some adult females were also subjected to the test. The tests for adults were performed after deprivation of food for one day. Glucose was given intraperitoneally at a dose level of 250 mg/100 g body weight. The blood

of adults was obtained from the venous plexus in the orbit of the eye by means of a glass pipet (Schermer, 1967).

Results

Detailed data for glucose tolerance tests are presented in Table 1. The blood sugar level reached the peak at 0.5 hour in all series of animals. Therefore, the data are also expressed as percent depressions from the value at 0.5 hr (Frye, 1959) in Figure 1.

Both premature newborn rats obtained by Caesarean section (a in Fig. 1A) and 2-day-old newborn rats (b in Fig. 1A) showed almost the same tolerance curves. When compared with the curve of the adult (f in Fig. 1A), those of young were relatively slow in dropping, particularly one hour after the glucose challenge. In pancreatectomized 2-day-old rats (e in Fig. 1A), the curve did not drop appreciably even 3 hr after the challenge.

Glucagon added to the glucose challenge did not enhance the glucose tolerance in the premature newborn rats delivered by Caesarean section (d in Fig. 1B). Alloxan given to pregnant rats caused the high increase of blood sugar level both in the pregnant rats and in the young born to such rats, as shown in the "0 hour" column in Table 1. After the glucose challenge in both alloxan-treated pregnant rats (g in Fig. 1C) and young (c in Fig. 1B), the tolerance curves did not decline during the period of observation.

Discussion

Our observations suggest that the fetal pancreatic islets at least near term in the rat may be able to release insulin in response to a stimulus of hyperglycemia. The tolerance curve in premature newborn rats delivered by a Caesarean section dropped one or 2 hr after the glucose challenge, as was observed in 2-day-old rats. The fact that such a drop was not observed in pancreatectomized young argues against the possibilities of spontaneous

Fig. 1. Glucose tolerances of (a) newborn rats delivered by Caesarean section on the 22nd day of gestation, (b) 2-day-old rats, (c) newborn rats delivered by Caesarean section of alloxan-treated pregnant rats, (d) newborn rats delivered by section Caesarean and given glucagon, (e) pancreatectomized 2-day-old rats, (f) adult female rats and (g) alloxan-treated pregnant rats, expressed as curves of percent blood sugar depression from the value at 0.5 hour. 1A shows comparisons of curves among a, b, e and f. 1B shows comparisons of curves among a, c, and d. 1C shows the curve of g alone.

Table 1. Glucose tolerances of premature newborn rats delivered by Caesarean section, 2-day-old rats and adult rats under various conditions

Condition of animals	No. of* series (young) or animals (adults)	Blood sugar levels at hr after the glucose challenge				
		0 mg% $\pm$ S. E.	0.5 mg% $\pm$ S. E.	1 mg% $\pm$ S. E.	2 mg% $\pm$ S. E.	3 (hr) mg% $\pm$ S. E.
Premature newborn rats by Caesarean section						
Normal	10	100 $\pm$ 6	279 $\pm$ 26	240 $\pm$ 13	138 $\pm$ 15	76 $\pm$ 3
+ Glucagon	5	117 $\pm$ 11	307 $\pm$ 6	265 $\pm$ 23	201 $\pm$ 23	100 $\pm$ 8
Mat. Alloxan	9	291 $\pm$ 20	341 $\pm$ 3	341 $\pm$ 3	331 $\pm$ 9	326 $\pm$ 26
2-day-old rats						
Normal	10	123 $\pm$ 10	324 $\pm$ 24	293 $\pm$ 30	118 $\pm$ 8	112 $\pm$ 5
Pancreatectomy	11	168 $\pm$ 12	341 $\pm$ 4	321 $\pm$ 12	306 $\pm$ 16	294 $\pm$ 21
Adult female rats						
Normal	6	94 $\pm$ 6	335 $\pm$ 28	228 $\pm$ 32	141 $\pm$ 13	121 $\pm$ 10
Pregnant rats on the 21st day of gestation						
Alloxan	2	303—	354—	346—	344—	341—

* In young rats, each series consisted of 5 littermates for blood sugar determinations at different times.

utilization of the sugar and action of insulin-like agents, if any, from tissues other than the pancreas.

Fetuses of alloxan-treated pregnant rats did not show any consistent tolerance to

glucose. The fetal blood sugar was leveled up as high as maternal blood sugar by alloxan treatment, as described by other investigators (Kim *et al.*, 1960; Kozma-de Bokay *et al.*, 1961).

It has been reported that in babies born to diabetic women, blood sugar levels abruptly decline just after birth due to the increased release of insulin in response to hyperglycemia resulted from maternal diabetes (Pedersen and Osler, 1961; Jorgensen *et al.*, 1966). In the young rats born to alloxan-diabetic rats, hyperplasia of pancreatic islets and degranulation and hydropic changes of β cells are significant alterations as described in the introduction. Therefore, failure to respond to glucose in the premature young born to alloxan-treated rats which we observed would suggest that the fetal islets under such a condition have been exhausted to be unable to secrete any more insulin in response to further glucose shock. It is also possible that the fetal islets have been destroyed directly by transplacental alloxan. However, according to the report of Angervall (1963) that alloxan injected into fetal rats caused no histologic change in fetal islets, the direct destruction of fetal islets by transplacental alloxan seems less possible.

Our observation of responsiveness of the islets at the end of fetal life to hyperglycemia *in vivo* is out of harmony with reported non-responsiveness of the fetal islets *in vitro* to a high glucose medium (Wells *et al.*, 1967; Vecchio *et al.*, 1967; Milner, 1969; Asplund *et al.*, 1969). Alpha cells may be involved in the mechanism of insulin release in view of the studies of Vecchio *et al.* (1967) and Milner (1969) who found that glucagon was an effective stimulus of insulin release from fetal pancreas which had been cultured *in vitro*. In our studies, however, glucagon did not enhance glucose tolerances of premature newborn rats. Although light microscopy has so far revealed definite α cells only just after birth (Hard, 1944; Nerenberg, 1954), the electron microscopic observation by Orci *et al.* (1969) showed the presence of α cells loaded with α granules already in the late fetal days in the rat. If glucagon from α cells is an initiative agent for the insulin release, the fetal pancreas *in vivo* would be able to secrete glucagon from

its own islets and would not need any more additional glucagon. Also possibly, there would be some other agents to initiate insulin response to hyperglycemia *in vivo*. Our results are almost in line with those of Basset *et al.* (1971), who reported that an intravenous infusion of glucose or fructose into fetal sheep increased the plasma insulin concentrations in the fetus and that glucagon did not potentiate the insulin response to glucose, assuming that the immaturity of cyclic-3',5'-AMP synthesizing systems might limit the action of glucagon.

As regards fetal insulin secretion, there are considerable controversies in the literature. Some authors reported that the fetal pancreatic islets were able to secrete insulin in response to a high glucose level *in vitro* (in calf fetus, by Correa *et al.*, 1967; in rat fetuses, by Lambert *et al.*, 1967). Davis *et al.* (1969) reported that fructose infusion into chronically cannulated fetal sheep increased plasma insulin concentrations in the fetus. Alexander *et al.* (1969) showed that glucose injected intravenously into exteriorized fetal sheep increased the concentrations of insulin in plasma. In contrast, Willes *et al.* (1969) showed that neither fructose nor glucose given to cannulated fetal sheep evoked insulin secretion from the fetal pancreas. Regarding human subjects, Paterson *et al.* (1968) reported that an intravenous glucose load to a pregnant woman in labor did not cause any appreciable rise in plasma insulin concentrations in the fetus in spite of the secondary rise of the fetal blood glucose level by maternal temporary hyperglycemia. Espinosa de los Monteros *et al.* (1970) showed that glucose failed to enhance secretion of insulin from isolated human fetal islets without glucagon. On the other hand, Bossart *et al.* (1969) reported that a glucose load to a pregnant woman caused some rise of insulin concentrations in fetal plasma, though less than the rise in maternal plasma. Although neither any observation nor any notion has been warranted to resolve such conflicting results, it might be possible that

long exposure of fetal islets to high glucose circumstances causes insulin secretion from the islets both *in vivo* and *in vitro*. In our studies, glucose was given peritoneally in relatively so large amount that a considerable amount of glucose still persisted in the abdominal cavity at autopsy. This perhaps stimulated young islets.

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