Effects of Gestational Age, Birth and Feeding on the Insulinogenic Response to Glucose and Tolbutamide by Fetal and Newborn Rat Pancreas

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SUMMARY

Perinatal maturation of the pancreatic beta cells was studied in the rat. Insulin synthesis proceeded rapidly between the twentieth gestational day and the third postnatal day. The insulin concentration in the pancreas and the amount of insulin available per gram of body weight reached a maximum sixty hours after birth and then decreased slowly toward the values observed in the adult rat. The pancreatic beta cells became responsive to glucose and to tolbutamide during the twenty-third day after fertilization. This phenomenon seemed to be related to the gestational age and not to act of birth or to feeding, although feeding appeared to increase the insulinogenic response to glucose. DIABETES *20*:586-91, September, 1971.

The fetal pancreas synthesizes proinsulin and converts it into insulin.[1,2] The embryologic age at which insulin is found, however, depends upon the sensitivity of the methods used for its detection. Thus, the presence of the hormone has been demonstrated in the pancreas of seventeen or eighteen-day-old rat fetus by histologic and histochemical means,[3,4] in that of fourteen-day-old fetus by means of a bioassay[5] and in that of eleven-day-old fetus by electron microscopy[6,7] and by radioimmunoassay.[8] Immunoreactive insulin (IRI) has been found in the pancreas of the eleven-week-old human fetus (80 mm. crown-rump length).[9-11]

Although the presence of insulin early in intrauterine life is well documented, little information is available regarding the development of mechanisms which control its secretion. Most investigators agree that the fetal pancreas of man and animals responds poorly or not at all to an increase of serum glucose concentration.[12-19] Studies in vitro led to similar conclusions. Thus, it has been reported that rat pancreatic tissue responds to glucose only twenty-four to forty-eight hours after birth[20] and that pancreatic tissue removed from the eighteen-day-old rat fetus and cultured in vitro for four days is insensitive to glucose.[21] However, the interpretation of these results is difficult. Studies in vivo are handicapped by the lack of precise knowledge regarding the placental transfer of insulin. Studies in vitro using whole pancreas are complicated by the release of proteolytic enzymes which tend to destroy insulin, while tissue cultures per se may alter the rate of maturation of the beta cells. Some of these difficulties have been avoided in a recent study showing that islets isolated from human fetal pancreas by collagenase digestion do not respond to glucose or to tolbutamide.[22]

This paper describes the pancreatic insulin content and its secretion in rats from the twentieth day of gestation to the twelfth day after birth; and the influence of birth itself and of feeding on the insulinogenic response to glucose and to tolbutamide added in vitro, singly or in combination. Insulin was protected against pancreatic digestion by the addition of guinea pig anti-insulin serum (GPAIS).[23]

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MATERIALS AND METHODS

Virgin female albino rats weighing 250 to 300 gm. were caged individually and given free access to food (Purina rat chow) and water. Pregnancy was ascertained by abdominal palpation and by the increase in body weight, ten to fourteen days after an overnight stay with a male. Thus, the gestational age was determined with an error of twelve hours. When zero to eighteen-hour-old pups were needed, the pregnant rats were brought to the laboratory on the twenty-second day of gestation and kept under constant watch to determine the exact time of delivery. In other experiments, pregnant rats were killed by cervical dislocation twenty or twenty-one days after fertilization, the premature pups were rapidly removed by cesarean section, cleaned, and kept at 30° C. in O_2-enriched atmosphere until they were killed by cervical dislocation. Their pancreases were removed, transferred to a Petri dish containing ice-cold Hanks solution,[24] trimmed of extraneous tissues with the aid of a dissecting microscope, blotted on filter paper, weighed and placed in an Erlenmeyer flask with 2.5 ml. of Krebs-Ringer bicarbonate buffer containing albumin (1 per cent) and glucose (50 mg./100 ml.). After equilibration for five minutes with a mixture of 95 per cent O_2 and 5 per cent CO_2, the stoppered flasks were preincubated for thirty minutes at 37° C. The pancreases were then transferred to another Erlenmeyer flask containing 3, 5, or 10 ml. of the same buffer enriched with albumin (1 per cent), an amount of GPAIS (lot No. 469*) sufficient to bind twice as much insulin as was expected to be secreted, glucose (50 or 300 mg./100 ml.) and, in some experiments, tolbutamide (200 μgm./ml.). After equilibration with a mixture of 95 per cent O_2 and 5 per cent CO_2 for five minutes, the stoppered flasks were incubated for one hour at 37° C., in a Dubnoff metabolic shaker. At the end of the incubation, the tissue samples were removed from the medium, blotted on filter paper, ground individually in glass homogenizers with 2 or 5 ml. of acid alcohol, and allowed to stand at 4° C. for twenty-four hours. The mixture was then spun at 2,000 rpm for twenty minutes and the insulin was measured in the supernatant fluid as well as in the incubation medium using the method of Malaisse and collaborators.[23]

In another group of experiments, the influence of feeding on the secretion of insulin using pancreatic tissue obtained one day before term and at term was observed. For this purpose, immediately after delivery (spontaneous or by cesarean section), each litter was divided into two groups: One remained unfed and the other was allowed to nurse. Three to twelve hours later, the pups were killed, the presence of milk in the gastrointestinal tract was noted and the secretion of insulin was measured as described above. Since beef insulin was used for the preparation of the standards and of the labeled material, the results must be understood as beef-insulin equivalents.

RESULTS

Figure 1 illustrates the rate of growth of the pancreas and of the pups between the twentieth and thirty-fourth day after conception. Figure 2 shows that the rise of total extractable insulin with age is described by an

*Prepared by Dr. Peter Wright, Department of Pharmacology, University of Indiana.

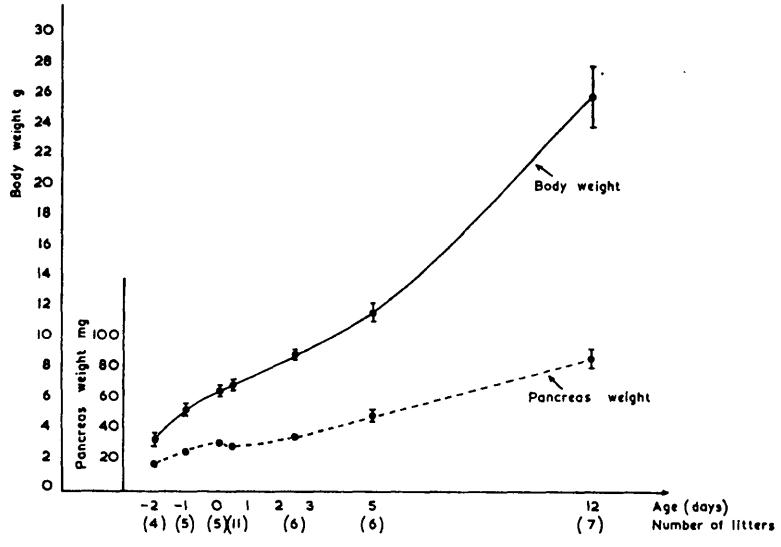


FIG. 1.

Body and pancreas weights of fetal and neonatal rats.

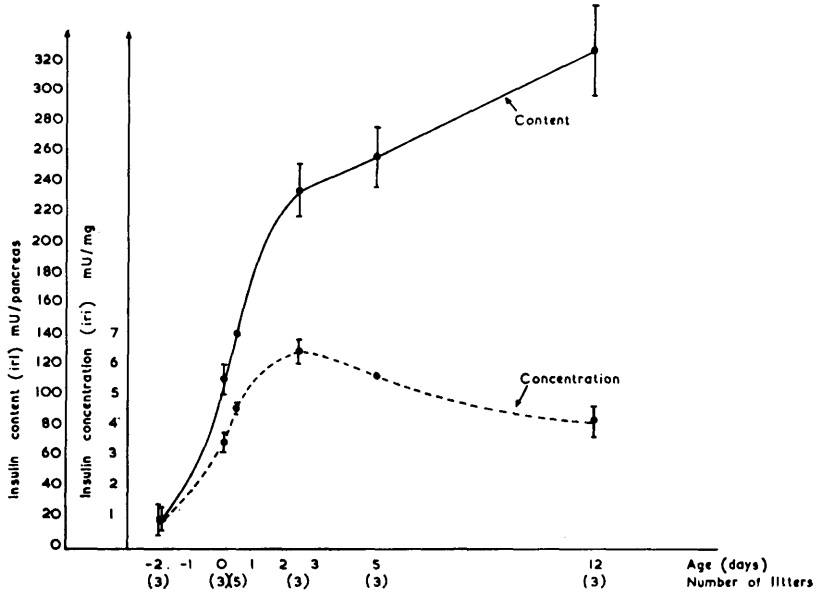


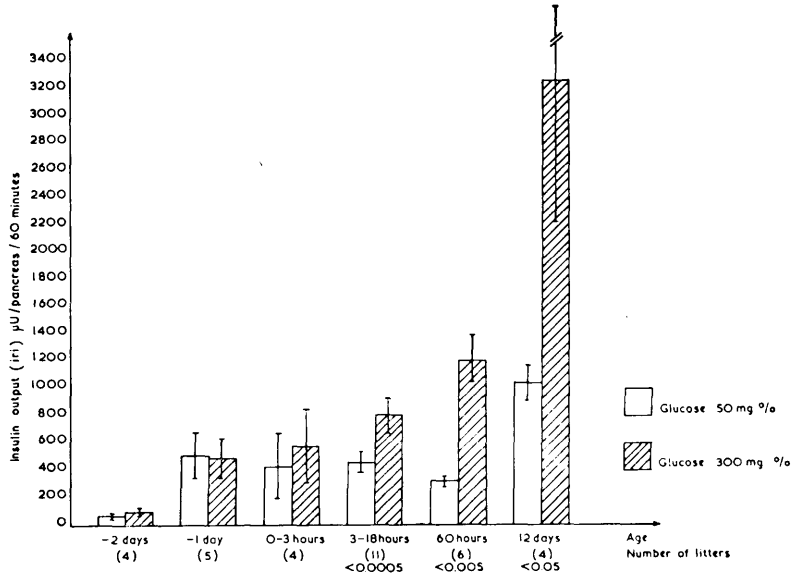
FIG. 2.

Insulin content and concentration of the fetal and neonatal rat pancreas.

S-shaped curve with the greatest slope between the twentieth day of gestation and sixty hours after birth, followed by a slower and almost linear increase. The concentration of insulin in the pancreas also rose sharply from the twentieth day of gestation, reaching a maximum sixty hours after birth and then decreasing slowly towards the value found in the adult rat pancreas. Figure 3 illustrates the secretion of insulin by whole pancreases removed at various ages and incubated for one hour under "basal conditions" (glucose concentration in the medium, 50 mg./100 ml.) or following stimulation by glucose at the concentration of 300 mg./100 ml. It can be seen that, before term and immediately after birth, the β cells did not respond or responded poorly to glucose, but a significant response appeared three to eighteen hours after birth. The large standard error indicates that the secretion of insulin varied widely from one litter to another, although pancreases removed from animals of the same litter and incubated under the same conditions, released almost exactly the same amount of insulin. This biologic variability has already been noted in the rabbit.[25] Figure 4 shows the results obtained when tolbutamide was used as the insulinogenic stimulus: Again, a significant response appeared three to

FIG. 3.

Effect of glucose on the insulin output of fetal and neonatal rat pancreas incubated in vitro.



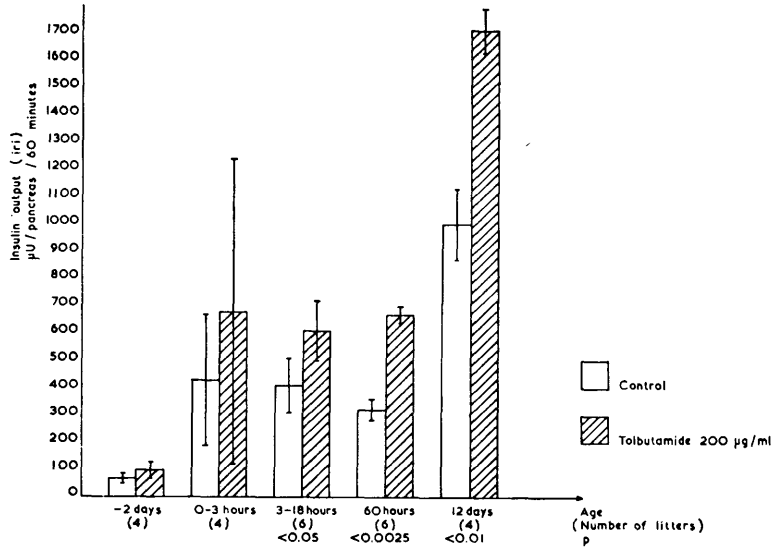


FIG. 4.

Effect of tolbutamide (200 µg./ml.) on the insulin output of fetal and neonatal rat pancreas incubated in vitro. Glucose concentration in the medium: 50 mg. per cent.

eighteen hours after birth. Figure 5 shows that the pancreas of premature pups remained insensitive to glucose even after feeding, whereas the pancreas of full-term animals responded to glucose also when the animals had not been fed. In the latter group, feeding somewhat improved the response to glucose. Figure 6 shows that the amount of insulin available per gram of body weight increased rapidly in the perinatal period reaching a maximum between the second and the third day after birth.

DISCUSSION

Between the twentieth gestational day and the second day after birth we observed a twofold increase of pancreatic weight and a twelvefold increase of pancreatic insulin content. During this phase of rapid hormone synthesis, the pancreatic insulin content rose following a curve similar to that which describes the accumulation of digestive enzymes in the maturing exocrine pancreas.[8] After the third postnatal day, the pancreatic weight increased more rapidly than the insulin content. Accordingly, the concentration of insulin rose at first, reaching a peak between the second and the third day of postnatal life. This perinatal peak of insulin concentration has been noted also by other investigators in the rat[26] and in the lamb.[27] The variations of insulin concentration in the pancreas can be explained, at least in part, by the fact that during the prenatal period, the endocrine tissue of the pancreas develops faster than the exocrine tissue whereas, after birth, the mass of exocrine tissue increases dramatically. Thus, the endocrine tissue represents 3 to 5 per cent of the total pancreatic weight on the eighteenth day of gestation and approximately 1 per cent in the adult animal.[28] In addition, while in the adult animal the insulin content of the pancreas has been found to be proportional to body weight,[29,30] during the first days of extrauterine life, the amount of insulin available per gram of body weight was found to be very large.

Although the rat pancreas contained insulin long before birth, the β cells did not respond to glucose and tolbutamide until the first day after birth when the rate

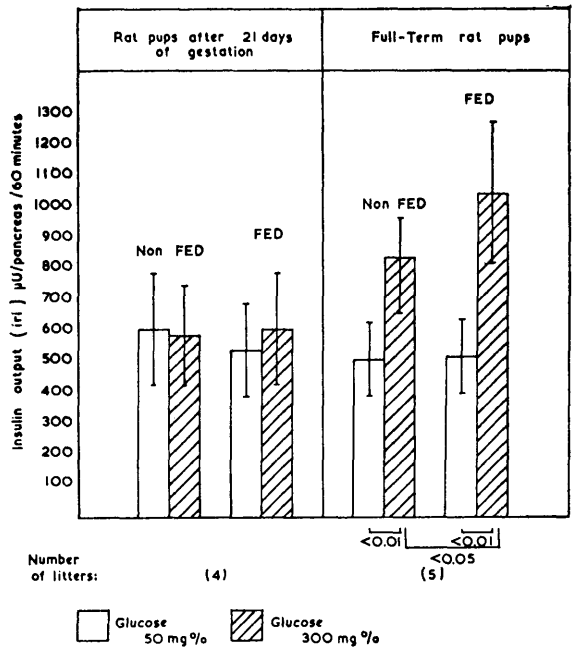


FIG. 5. Influence of feeding on β-cell's reactivity to glucose.

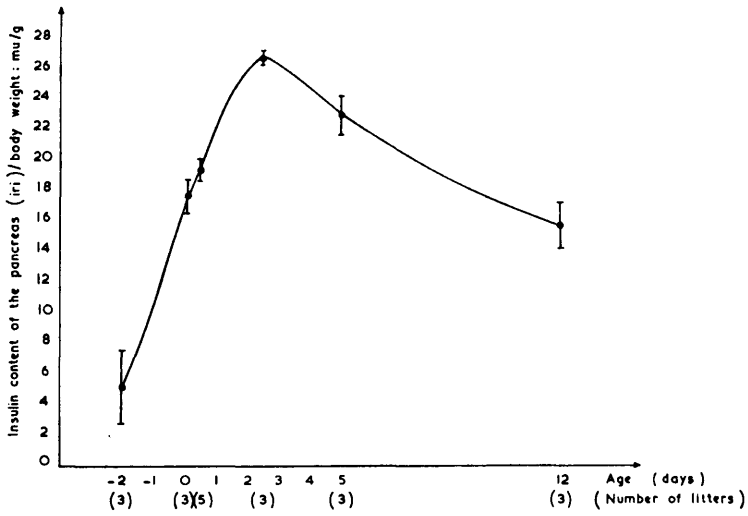


FIG. 6.

Pancreatic insulin available per gram of body weight in fetal and newborn rats.

of insulin accumulation in the pancreas was the highest. This is also the time when the normal newborn rat begins to nurse. Nevertheless, feeding or the act of birth do not seem to have been the initiating factors in the ability of the β cell to respond to glucose, since no response was noted in the pancreas of fed animals delivered one day before term, whereas a good response was noted in the pancreas of full-term pups, even when they had not been fed. This finding is in agreement with the observations that glucose causes little or no increase of plasma IRI in premature human babies[31,32] and in newborn puppies[33] and a delayed response in normal-term babies[34] and that glucose-stimulated insulin release in fetal explants of rat pancreas does not occur, unless an inhibitor of phosphodiesterase activity is added to the incubation media.[28] The enhancement of β-cell reactivity by feeding may be due to a direct effect on the β cells since nutrients promote the rate of islet growth in tissue culture[26] or to the action of gastrointestinal factors, such as secretin, which potentiate the insulinogenic effect of glucose.[35] On the other hand, fasting is known to depress β-cell function and cause islet atrophy.[36,37] Thus, the appearance of β-cell sensitivity to glucose seems to be related primarily to maturation, although the nutritional state may serve to modulate the magnitude of the insulin response. Birth itself does not seem to play any important role.

ADDENDUM

After this paper was submitted for publication, we read two reports showing that fetal rabbit[38] and rat[39] pancreases, in the latter part of pregnancy, release significant amounts of insulin in response to glucose stimulation.

ACKNOWLEDGMENT

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