PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 75-79 (1975)

Development of Response to Glucose of Fetal Rat Islet in Organ Culture[1] (38479)

HUE-LEE C. KAUNG, ORION D. HEGRE, AND ARNOLD LAZAROW

Department of Anatomy, University of Minnesota, Minneapolis, Minnesota 55455

The beta cells in the islets of the fetus are functioning during late gestation; in fact, the levels of plasma insulin as well as the levels of insulin released *in vitro* from pancreatic explants of late gestation rat and rabbit fetal pancreases are higher than insulin levels measured for either neonatal or adult pancreas (1, 2). Further, such fetal pancreases respond to chronic glucose stimulation of maternal diabetes by increased plasma levels of insulin (3), by islet hypertrophy (4) and by beta cell degranulation (5, 6). However, fetal islets do not appear to be capable of responding to acute glucose stimulation with insulin release and develop the insulin secretory responsiveness only in the neonatal period (7–9).

Alpha cell maturity and function may be involved since unresponsive fetal pancreatic slices are capable of releasing insulin in response to glucose stimulation in the presence of glucagon (7, 10). Dietary intake in the immediate neonatal period has been suggested to play an essential role in the development of pancreatic glucose responsiveness (9) although other data suggests that while feeding appears to increase the insulinogenic responsiveness to glucose it is not essential. The present investigation was undertaken to study the insulin secretory behavior of fetal and neonatal rat islet tissue to glucose stimulation in organ culture, and to study the effect of organ culture on the course of development of insulin secretory responsiveness to glucose. It was found that fetal rat islet tissue will develop responsiveness to glucose *in vitro*.

Materials and Methods. Pancreases of 18-day fetal (17.5 days after witnessed mating) and 3-day neonatal (4 days after parturition) rats of the Sprague-Dawley strain were used. Details of the method of organ culture have been described (11). The pancreas was cut into fragments approximately 1 mm^3. Each explant was supported on a millipore grid (8 μm pore size) at the gas-liquid interface in 0.5 ml of medium at 37° in an atmosphere of 95% O_2 and 5% CO_2. The medium consisted of equal portions of rooster serum and 12-day chick embryo extract (6). The standard medium contained 150 mg/100 ml of glucose, the high glucose medium contained 500 mg/100 ml of glucose. Cultures were transferred to fresh medium every 48 hr over a 12-day period. In one series of experiments, explants were cultured on standard glucose medium for 8 days and then transferred to high glucose medium for the succeeding 4 days.

The amount of insulin in the medium was assayed using the two antibody method of Morgan and Lazarow (12). Crystalline rat insulin standards were used (*Novo* 25 units/mg). In the short term incubation studies, aliquots (0.025 ml) of medium were removed at 4 hr intervals between 8 and 24 hr of incubation. For long-term studies, aliquots (0.025 ml) of medium were analyzed at the time the medium was changed.

Tissues were fixed for histological examination after 0, 6, and 12 days of organ culture. Embedded tissues were sectioned serially at 4 μm and stained with aldehyde fuchsin-Ponceau de xylidine. The quantity of the islet tissue and other components were measured by the linear scan method (11, 13).

Results and Discussion. The continued presence of insulin in the medium (Fig. 1) and granulated beta cells in the explants (Figs. 2 and 3) indicate that both fetal and neonatal pancreatic islets synthesize and release insulin when grown in organ culture. However, the response to glucose stimulation is quite different in fetal and neonatal tissues.

After the initial 48 hr of organ culture, the insulin content in the medium of 18-day fetal explants is relatively low (600 μunits/ml) compared to that of 3-day neonatal

[1] These studies were supported by U.S.P.H.S. Grant No. AM5127 and HD00412. An abstract of this work was presented at the American Diabetes Association Meetings, Chicago, 1974. Diabetes 23 (Suppl. 1) 375 (1974).

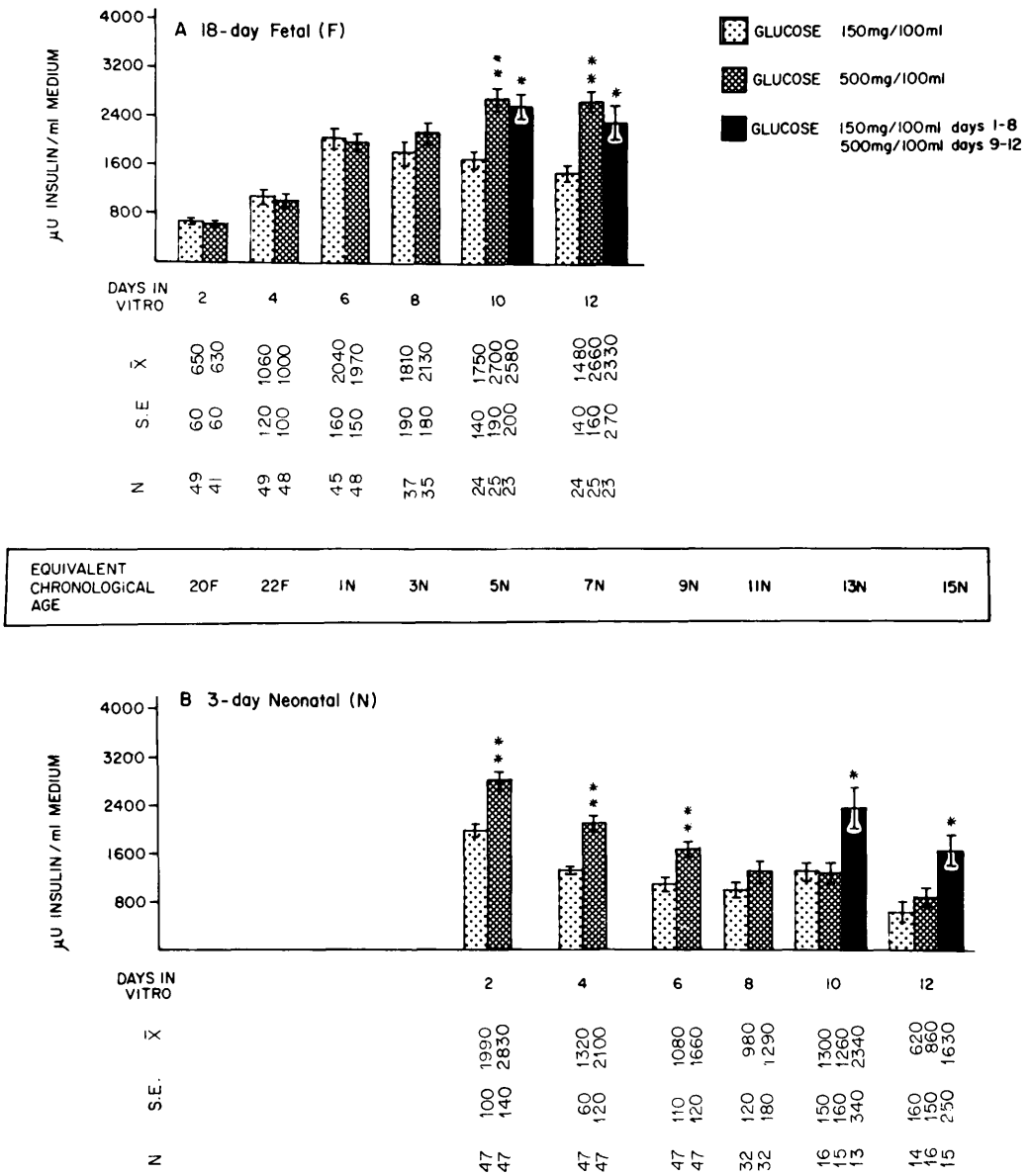

FIG. 1. Insulin content of media at various times during organ culture: effect of high glucose levels (A) 18-day fetal pancreas cultured for 12 days, (B) 3-day neonatal pancreas cultured for 12 days. F = Fetal; N = Neonatal. ** Significantly different from standard glucose controls ($P < 0.001$). * ($P < 0.01$.)

explants (2000 μunits/ml) (Fig. 1). In the fetal cultures, the amount of insulin increases during the first 6 days of organ culture, reaching 2000 μunits/ml after 6 days; by 12th day *in vitro* the level reaches 1500 μunits/ml (Fig. 1A). The increasing level of insulin in the medium of fetal organ cultures can probably be accounted for by a corresponding increase in islet mass as reported earlier (11). In the neonatal cultures, by contrast, more insulin was detected in the medium during the initial 48 hr (2000 μunits/ml), but the amount of insulin decreases progressively as the culture period increases (Fig. 1B).

Eighteen-day fetal islets showed only a limited response to high glucose stimulation after 20–24-hr in organ culture (Fig. 4A);

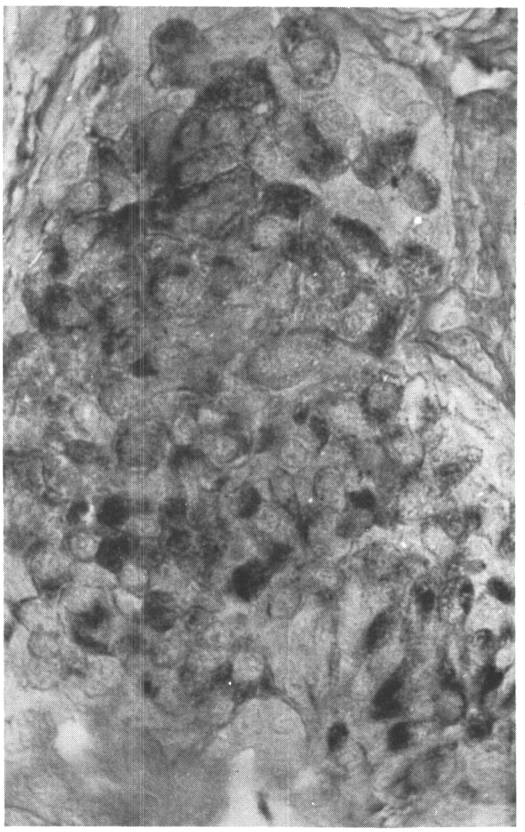

FIG. 2. Eighteen-day fetal pancreas after 12 days in organ culture. Note the well developed beta cells. (×325) Aldehyde fuchsin and ponceau de xylidine.

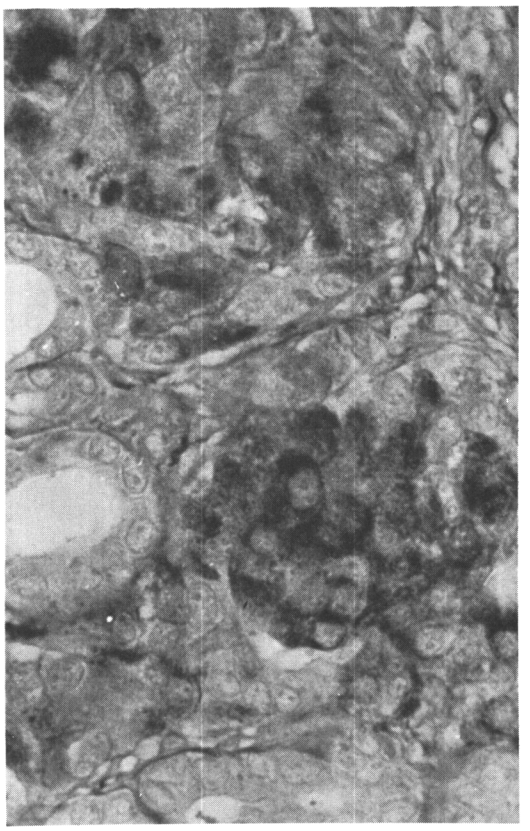

FIG. 3. Three-day neonatal pancreas after 12 days in organ culture. Note the well developed beta cells. (×325) Aldehyde fuchsin and ponceau de xylidine.

however, the amount of insulin present in the medium of the fetal cultures during the first 8 days of explantation is the same in both high glucose and standard glucose media (Fig. 1A). However, by the tenth day of organ culture the islets show a responsiveness to glucose.

After 18-day fetal tissue had been grown in organ culture for 8 days, (either in high glucose or in standard glucose media), the islets show a responsiveness to glucose. This is observed both in a short-term experiment (shown in Fig. 4B) where the media was changed to high glucose and sampled at 4–12 hr intervals and in a long-term experiment (shown in Fig. 1A) where the response was measured after 10 and 12 days of organ culture. These studies indicate that an insulin secretory response to high glucose levels develops between the eighth and tenth day of organ culture in 18-day fetal explants.

By contrast, 3-day neonatal islets responded to high glucose as early as 12 hr after explantation (Fig. 4C) and they remained responsive even after 12 days in organ culture (Fig. 1B). The amount of insulin in high glucose medium of neonatal cultures is significantly higher ($P < 0.001$) than that in standard glucose medium during the first 6 days *in vitro* (Fig. 1B).

Continuous exposure of neonatal islets to high glucose for 8–12 days seemed to result in an adaptation in which the islet response to high glucose is diminished or absent (Fig. 1B). However, when the 3-day neonatal tissue was transferred to high glucose medium after 8 days in standard medium, a significantly greater ($P < 0.005$) amount of insulin was detected in the high glucose medium than in standard glucose medium (Fig. 1B). Thus, the response of neonatal islets to high glucose is not lost during this period of

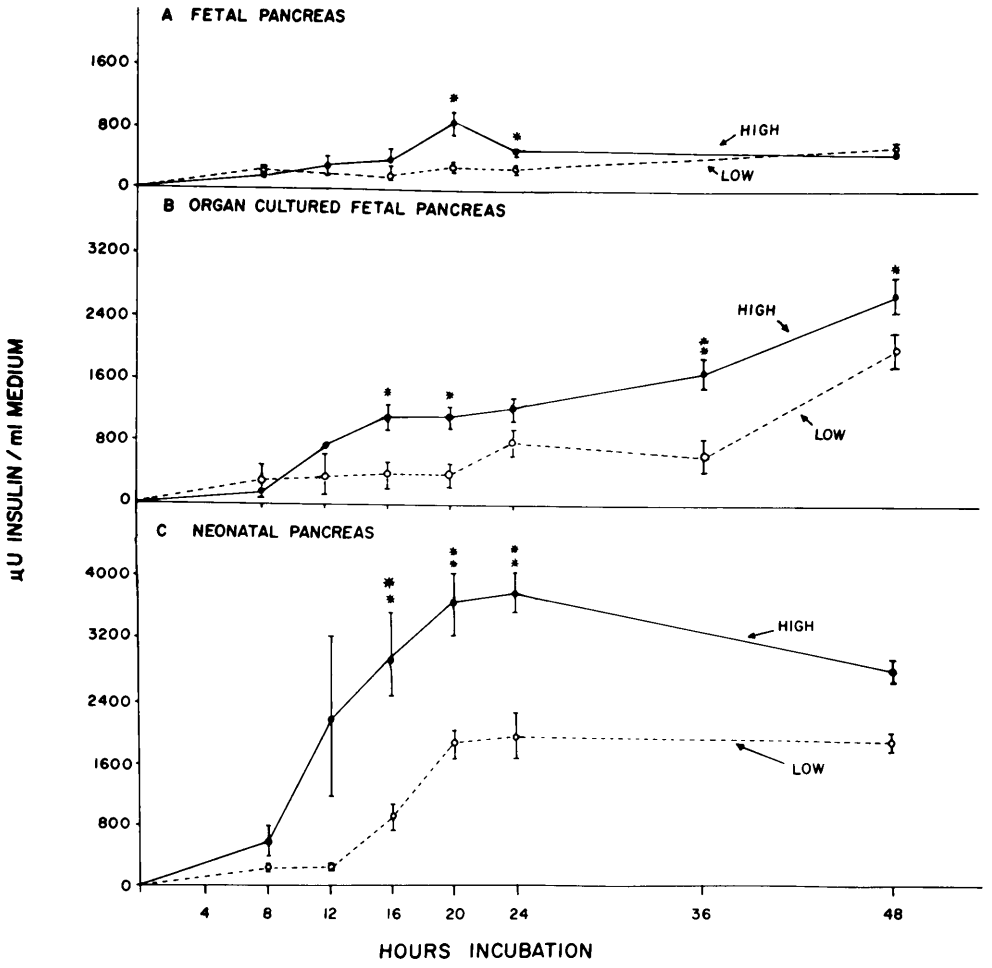

FIG. 4. Response of pancreas to high glucose during 48 hr of incubation. (A) Eighteen day fetal explant. (B) Eighteen day fetal explant after 8 days *in vitro*. (C) Three day neonatal explant. ** Significantly different from standard glucose controls ($P < 0.01$). *($P < 0.05$.)

organ culture, although it is obliterated by continuous exposure to glucose.

These studies show that 3-day neonatal islets are responsive to glucose stimulation immediately after explantation, whereas the 18-day fetal islets are not responsive at this time. But they become responsive to glucose after 8–10 days of organ culture, i.e. at a chronologic age corresponding to the 3-day neonatal rat (Fig. 1).[2]

[2] Eighteen days *in utero* + 10 days *in vitro* is equivalent to 23 days *in utero* (time of spontaneous delivery in our colony usually occurs on the 23rd day after witnessed mating) + 3 days postnatal (4th day after delivery) + 2 days *in vitro*.

It has been suggested that the development of islet responsiveness to glucose is related to extra pancreatic factors such as dietary intake of the neonatal rat (9). Since our results indicate that the fetal pancreas can become responsive to glucose stimulation during organ culture, this maturation of islet response is probably inherent in the islet itself.

Quantitative analysis of the islet mass in explants of fetal and neonatal pancreas grown on high and standard glucose media for 6 days is shown in Table I. There is no statistical difference ($P > 0.3$) in islet mass (mm islet) between fetal and neonatal pancreases. Nevertheless, the amount of insulin in medium is almost twice as great with fetal

TABLE I. ISLET MASS[a] AND PERCENT ISLET IN PANCREASES CULTURED FOR 6 DAYS.[b]

Age of pancreas at explanation	150 mg glucose/100 ml medium			500 mg glucose/100 ml medium		
	Mass total explant	Mass islet	% Islet	Mass total explant	Mass islet	% Islet
18 day fetal	55.3 ± 6.2 (7)	3.3 ± 0.6 (7)	6.6 ± 1.7 (7)	54.2 ± 5.6 (6)	3.3 ± 0.5 (6)	6.6 ± 1.3 (6)
3 day neonatal	61.3 ± 3.8 (11)	2.6 ± 0.4 (11)	4.4 ± 0.6 (11)	63.5 ± 4.3 (11)	3.0 ± 0.5 (11)	4.9 ± 0.7 (11)

[a] mm scan is used to express the mass of explant or islet (11).
[b] Values represent mean (N) ± S.E.M.

explants (2040 μunits/ml), as compared to neonatal cultures (1080 μunits/ml) (Fig. 1). In both fetal and neonatal groups, the islet mass is not significantly ($P > 0.5$) different in high glucose or standard glucose cultures. Although hyperglycemia is reported to cause beta cell hyperplasia in monolayer (14) and organ cultures (10), we found no evidence of such glucose induced hyperplasia in fetal or neonatal explants under the culture conditions used. Thus, the increase in insulin content observed in high glucose medium in neonatal tissue at 6 days (1660 vs. 1080 μunits/ml) cannot be attributed to the difference in the amount of islet tissue mass.

Summary. Pancreases of 18-day fetal and 3-day neonatal rat were grown in organ culture in both standard glucose (150 mg/100 ml) and high glucose (500 mg/100 ml) media. Insulin content of medium was measured by radioimmunoassay at time of transfer.

In fetal cultures, standard and high glucose media contained a similar level of insulin through 6 days of culture. In neonatal cultures, high glucose medium contained 40–60% more insulin than did standard medium. However, after 8 days of organ culture fetal pancreas developed responsiveness to high glucose; a greater amount of insulin was present in the high glucose medium (50% by 10 days and 70% by 12 days) than in standard glucose medium. The time at which this responsiveness develops *in vitro* approximates the chronologic age which corresponds to 3–5 days postnatal period. The maturation of this responsiveness appears to be inherent to the pancreas and is independent of other organ systems.

When neonatal explants, grown in standard medium for 8 days, were transferred to high glucose medium, 80–160% more insulin was detected in the high glucose medium than in standard medium during the next 4 days of culture. These results indicate that once glucose responsiveness has developed, it is maintained in organ culture for at least 12 days.

1. Blazquez, E., Montoya, E., and Lopez-Quijada, C., J. Endocrinol. **48**, 553 (1970).
2. Milner, R. D. G., J. Endocrinol. **44**, 267 (1969).
3. Clark, C. M., Cahill, G. F., and Soeldner, J. S., Diabetes **17**, 362 (1968).
4. Kim, J. N., Runge, W., Wells, L. J., and Lazarow, A., Anat. Rec. **138**, 239 (1960).
5. Wells, L. J., and Lazarow, A., Diabetes **16**, 846 (1967).
6. Hegre, O. D., Wells, L. J., and Lazarow, A., Diabetes **19**, 906 (1970).
7. Milner, R. D. G., Leach, F. N., and Ashworth, M. A., Biochem. Biophys. Acta **304**, 225 (1973).
8. Sodoyez-Goffaux, F., Sodoyez, J. C., and Foa, P. P., Diabetes **20**, 586 (1971).
9. Asplund, K., Diabetologia **8**, 153 (1972).
10. Lambert, A., Vecchio, D., Gonet, A., Jeanrenaud, B., and Renold, A. E., *in* "Tolbutamide—After Ten Years" (W. J. H. Butterfield and W. Van Westering, eds.), p. 61. Excerpta Medica Foundation, Amsterdam (1967).
11. Hegre, O. D., McEvoy, R. C., Bachelder, V., and Lazarow, A., *In Vitro* **7**, 366 (1972).
12. Morgan, C. R., and Lazarow, A., Diabetes **12**, 115 (1963).
13. Carpenter, A.-M., and Lazarow, A., J. Histochem. Cytochem. **10**, 329 (1962).
14. Chick, W. L., Diabetes **22**, 687 (1973).

Received May 7, 1974. P.S.E.B.M. 1975, Vol. 148.