# FORMATION OF PANCREATIC INSULAR FUNCTION DURING EMBRYONIC DEVELOPMENT

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It has now been established that glucose, transmitted from mother to fetus through the placenta, is the principal physiological stimulator of development of the B-cells of the fetal pancreas [9].

It has been shown experimentally that maternal hyperglycemia induces hyperplasia of the fetal islet tissue [12]. Clinical data, in agreement with the results of the study of the action of experimental hyperglycemia, demonstrate the presence of hyperplasia of B-cells, an increase in the total weight of the islets [11], and elevation of the insulin level in the islets of Langerhans and in the umbilical blood [4] in fetuses of diabetic mothers. However, it is not yet clear what is the mechanism of the stimulating action of glucose on the fetal insular apparatus or how the reactivity of the B-cells of the developing pancreas changes.

The important role of the hypothalamic-hypophyseal system in the formation of the functions of many endocrine organs is well known [2]. As regards the pancreas, this question remains unresolved. For instance, no differences have been found in the insulin concentration in the pancreas of normal and decapitated rat fetuses [5]. However, the insulin level in the blood of newborn infants of diabetic mothers is higher than in anencephalic infants of such mothers [6].

The object of the present investigation was to study the secretory ability of the fetal pancreas developing under conditions of experimental maternal diabetes and also in the absence of the higher regulating centers of the fetus (as a result of decapitation in utero).

## EXPERIMENTAL METHOD

Fetuses of Wistar albino rats were used. Experimental diabetes was induced in the sexually mature animals before the beginning of pregnancy by intraperitoneal injection of alloxan hydrate in a dose of 180 mg/kg body weight. The maternal blood glucose level was monitored by the glucose oxidase method [1].

The fetuses were decapitated in utero at the 17.5-day stage of pregnancy and the pancreas of the fetuses was investigated at the 21.5-day stage. Intact fetuses of the same litter served as the control. The reactivity of the fetal insular system was determined by measuring changes in the rate of insulin secretion into the incubation medium in response to injection of stimulating agents (glucose – 3 mg/ml and theophylline – 10 mM). Fragments of fetal pancreas were incubated by the method described previously [3]. Immunoreactive insulin was determined in the incubation medium by radioimmunologic assay [8]. The results were processed by the "Multi-Mat" system and their significance assessed by Student's t-test.

TABLE 1. Concentration of Immunoreactive Insulin (IRI) in Incubation Medium before (basal level, A) and after (B and C) Addition of Stimulating Agent (fetuses of normal rats)

| Age of fetus, days | Weight of fetus, g | IRI, microunits/ml | | | | |
|---|---|---|---|---|---|---|
| | | A (basal level) | P | B (glucose – 3 mg/ml) | P | C (theophylline – 10 mM) |
| 19,5 | 3,2±0,08 | 78,2±12,0 (7) | >0,05 | 64,7±21,9 (7) | >0,5 | 69,3±10,0 |
| 20,5 | 4,3±0,12 | 96,2±17,2 (9) | >0,05 | 92,0±25,1 (9) | <0,05 | 152,0±20,1 |
| 21,5 | 5,6±0,05 | 91,3±6,2 (6) | <0,05 | 147,4±13,2 (6) | | |

Legend. Here and in Tables 2 and 3, number of fetuses given in parentheses.

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TABLE 2. IRI Concentration in Incubation Medium before and after Addition of Stimulating Agent (fetuses of rats with alloxan diabetes)

| Age of fetus, days | Weight of fetus, g | Blood sugar, mg% | IRI, microunits/ml | | |
|---|---|---|---|---|---|
| | | | A (basal level) | P | B (glucose – 3 mg/ ml) |
| 18,5 | 1,7±0,09 | 127,5 | 31,9±7,6 (6) | <0,05 | 63,7±10,2 (6) |
| 19,5 | 3,7±0,1 | 119,3 | 32,5±7,9 (8) | <0,01 | 144,8±36,4 (8) |
| 19,5 | 2,7±0,09 | 502,0 | 106,0±26,7 (4) | <0,05 | 192,0±31,1 (4) |
| 20,5 | 4,9±0,07 | 120,0 | 125,4±14,5 (6) | <0,05 | 198,8±13,7 (6) |
| 21,5 | 6,1±0,04 | 131,1 | 28,5±7,43 (9) | <0,05 | 52,5±6,9 (9) |
| 21,5 | 4,9±0,06 | 500,0 | 60,8±10,8 (5) | >0,05 | 78,6±17,4 (5) |

Legend. Here and in Table 3, P represents level of significance of differences between basal and stimulated levels.

TABLE 3. Insulin Release into Incubation Medium from Pancreas of Decapitated Fetuses of Normal Rats and of Rats with Alloxan Diabetes

| Mother | Fetuses | IRI, microunits/ml | | |
|---|---|---|---|---|
| | | A (basal level) | P | B (after addition of glucose – 3 mg/ ml) |
| Normal | Decapitated | 53,2±6,7 (10) | >0,05 | 42,0±8,7 (10) |
| | Control | 43,1±6,5 (10) | <0,05 | 68,5±12,3 (10) |
| With alloxan diabetes | Decapitated | 49,9±8,4 (8) | >0,05 | 47,8±5,5 (8) |
| | Control | 36,4±3,4 (10) | <0,05 | 51,6±6,1 (10) |

## EXPERIMENTAL RESULTS

Incubation of fragments of pancreas showed that the insular system of rat fetuses is refractory to glucose until the 21st day (Table 1). However, addition of theophylline to the medium together with glucose caused increased liberation of insulin after the 20th day. This is evidence in support of the view that a subthreshold level of cyclic AMP is maintained in the B-cells during fetal life [10].

The problem of maturity of the developing fetal insular system is important in the light of changes which may arise in response to a disturbance of the maternal blood sugar level. For instance, the pancreas of rat fetuses from mothers with alloxan diabetes was found to respond to glucose loading after the 18th day (Table 2). However, if maternal diabetes was severe (blood glucose above 300 mg%), the fetal pancreas was less reactive and the body weight of the fetuses was lower than if maternal diabetes was mild. Similar results were obtained by Eriksson et al. [7], who showed that in severe streptozotocin-induced diabetes most of the fetal B-cells were degranulated, and insulin biosynthesis in the gland and the body weight of the fetuses were reduced.

We now know that neural and neuroendocrine centers can influence the morphological and functional maturation of islet cells in the fetus. Decapitation of rat fetuses does not disturb histological differentiation of the pancreas [12]. The present results are evidence (Table 3) that after decapitation the fetal pancreas does not respond to a change in the glucose concentration in the incubation medium, even if the fetus develops in the presence of an increased blood glucose concentration (decapitated fetuses from animals with diabetes). In decapitated fetuses from diabetic mothers, incidentally, hyperplasia of the islets or B-cells is not observed [12].

The results of the present investigation showed that maternal hyperglycemia induces a premature increase in reactivity of the B-cells of the rat fetus, and that for normal development of the secretory activity of the fetal pancreas and regulation of the blood sugar level the presence of higher regulatory centers (in the pituitary and, perhaps, in the hypothalamus) is essential.

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# PARTICULAR FEATURES OF PORPHOBILINOGEN SYNTHESIS FROM δ-AMINOLEVULINIC ACID IN VISCERAL TISSUES OF RATS

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The biosynthesis of porphobilinogen, a precursor of heme, begins with a reaction of condensation of two δ-aminolevulinic acid (ALA) molecules, catalyzed by ALA dehydratase. The most intensive formation of the porphyrin takes place in bone marrow cells, followed by liver and, at a lower level, the remaining organs of animals [2, 9].

Activity of ALA dehydratase is inhibited by lead by a noncompetitive mechanism [13]. ALA dehydratase of erythrocytes is the most sensitive to the inhibitory action of lead [10]; the enzyme of the liver and kidneys [7, 14] and of other viscera [11] is less sensitive.

This paper describes a comparative study of porphobilinogen biosynthesis from ALA by homogenates of rat visceral tissues, using administration of lead as a method of revealing the particular features of porphyrin formation in different organs.

## EXPERIMENTAL METHOD

Experiments were carried out on 145 noninbred male albino rats weighing 260-310 g, of which 77 animals were controls and 68 were poisoned with lead acetate by subcutaneous injection in a dose of 25 mg lead/kg body weight. The poisoning continued for 5-6 weeks, with three injections a week. Control rats were given injections of distilled water.

The development of lead poisoning was judged from the general condition of the animals, changes in the red blood picture, and disturbance of porphyrin metabolism. The hemoglobin level and reticulocyte count were determined in the usual way, and the number of erythrocytes with punctate basophilia was counted after "enrichment" in a moist chamber. The ALA concentration in the urine was determined by the method in [12] in the writers' own modification [3]. Iron in the plasma was determined by the orthophenanthroline method. The rats were killed by decapitation.

ALA dehydratase activity was estimated from the quantity of porphobilinogen (in μg/mg protein/2 h of incubation) synthesized by visceral homogenates from a 0.1 M solution of ALA added [1, 4]. Protein was determined by Lowry's method. Squash preparations from the liver, kidneys, and spleen for cytological investigation were stained with azure-eosin.

## EXPERIMENTAL RESULTS

It will be clear from Table 1 that in the rats receiving lead acetate there was a tendency for body weight to fall and anemia to develop, accompanied by an increase in the number of reticulocytes and stippled erythrocytes and a marked increase in the ALA concentration in the urine. These observations indicated the development of severe lead poisoning in the rats. The plasma iron level showed no significant change under these circumstances.

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