The results of this investigation are evidence that maturation and differentiation of bone marrow cells are connected with synthesis and isolation of factor VIII antigen. These processes somehow or other lower the level of fibronectin, which plays an extremely important role in hemostasis.

Bone marrow, as regards its functional manifestations, is thus not only the central organ of hematopoiesis, but it is also a central organ of the functional system regulating the state of aggregation of blood [2].

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ROLE OF INTESTINAL HORMONES IN DEVELOPMENT OF PANCREATIC B CELL REACTIVITY OF RAT FETUSES TO GLUCOSE

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Because of its marked ability to respond directly to changes in the blood glucose level, for a long time the pancreas was considered to be self-regulating. However, data obtained on adult animals in recent years indicate that the action of glucose on B cells is modulated by the brain–islet cell system, the entero-insular axis, and paracrine influences in the islets [1, 7, 9].

In previous experiments in vitro and in vivo the writer obtained data to show that the hypothalamus and pituitary are involved in the control of development of B-cell reactivity of the fetal pancreas to glucose [11].

In view of data in the literature on the insulinotropic action of intestinal hormones in adults and neonates [4, 5, 8], on the high level of these hormones in the prenatal period of development [10], and the absence of information on relations of these hormones with insulin in the intrauterine period of development, it was decided to study whether intestinal factors are involved in the development of the insulin-releasing capacity of rat fetuses.

EXPERIMENTAL METHOD

Wistar albino rat fetuses were used. Experimental inactivation of the hypothalamus and pituitary was carried out by performing encephalectomy on the fetuses in utero [2] at 17.5 days of development, and reactivity of the fetal pancreas were studied at the age of 21.5 days.

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The proximal fragment of the duodenum of adult and newborn rats and of 21.5-day rat fetuses was removed for combined incubation with the pancreas. Incubation was carried out in bicarbonate buffer at pH 7.4, containing 2 mg/ml of bovine serum albumin, 1000 KIU/ml of trasylol (proteolytic enzyme inhibitor), and 2.08 mM glucose, in an atmosphere of 95% O_2 and 5% CO_2, at 37°C, with gentle agitation. Before incubation the adult rat duodenum was washed with buffer solution by means of a syringe.

In version 1 of the experiment, after incubation of the fetal pancreas and the fetal, neonatal, or adult rat duodenum separately, the fragments were placed in a common flask with 2 ml of buffer and incubated for 30 min in medium containing a physiological (2 mM) and 30 min in medium with a high (15 mM) glucose concentration.

In version 2 of the experiment fetal duodenum and pancreas were preincubated separately in buffer, then also incubated the first time separately in buffer with a physiological glucose concentration in order to determine the basal insulin secretion. The pancreas and duodenum were then placed together in a flask and incubated for 30 min each in medium containing low and high glucose concentrations.

In version 3 of the experiment preincubation and the first incubation were carried out as described above. The fetal pancreas was then placed in buffer obtained after incubation of the duodenum, and incubated in it for 30 min in medium with 2.08 and 15 mM glucose.

Until required for insulin determination the incubated material was kept on dry ice. Insulin in the incubation medium was determined by radioimmunoassay, using standard kits from the Institute of Isotopes, Hungarian Academy of Sciences.

EXPERIMENTAL RESULTS

The experiments with combined incubation of a fragment of fetal pancreas with duodenum taken from adult, newborn, and fetal rats showed that the intestinal factors can restore in vitro the response of the pancreas to glucose loading in encephalectomized fetuses (Fig. 1). For instance, significant differences were found in the immunoreactive insulin (IRI) level in response to physiological and stimulating glucose concentrations in the medium. It is inter-

TABLE 1. Release of IRI into Medium from Rat Fetal Pancreas during Combined Incubation with Duodenal Fragment

Version of experiment	IRI concentration in incubation medium, μU/mg in 30 min		
	2 mM glucose	15 mM glucose	*P*
Pancreas of anencephalic fetus + adult rat duodenum	117,7±4,2 (10)	188,7±3,7	<0,001
Pancreas of intact fetus + neonatal duodenum	161,9±5,9 (8)	230,5±10,3	<0,001
Pancreas of anencephalic fetus + neonatal duodenum	74,0±3,5 (12)	104,6±3,1	<0,001
Pancreas of intact fetus + neonatal duodenum	126,6±5,2 (8)	191,9±11,7	<0,001
Pancreas of anencephalic fetus + duodenum of intact fetus	110,3±7,5 (16)	150,3±8,9	<0,01
Pancreas of intact fetus + duodenum of intact fetus	77,2±9,3 (9)	139,1±11,0	<0,001
Pancreas of anencephalic fetus + duodenum of anencephalic fetus	96,5±3,2 (8)	118,5±3,9	<0,01
Pancreas of anencephalic fetus + muscle	103,7±6,3 (8)	102,7±6,4	>0,05

Legend. Here and in Table 2, number of fetuses in parentheses. Differences significant at $P < 0.05$ level.

TABLE 2. IRI Release from Rat Fetal Pancreas during Combined Incubation with Intestinal Fragment or in Incubation Medium of Fetal Intestine

Version of experiment	IRI concentration in incubation medium, μU/mg in 30 min				
	2 mM glucose	2 mM glucose + intestinal factors	P	15 mM glucose + intestinal factors	P
Pancreas of intact fetus + intestine	75,8±7,9 (13)	90,6±11,0	>0,05	157,8±13,4	<0,001
Pancreas of anencephalic fetus + intestine	80,8±10,7 (12)	97,6±12,2	>0,05	174,0±18,1	<0,01
Pancreas of anencephalic fetus + incubation medium of intestine	81,5±6,9 (20)	107,4±7,8	>0,05	188,4±16,1	<0,001

esting to note that the intestine not only of the normal fetus, but also of an anencephalic fetus, possesses this kind of ability, from which it follows that the activity of the intestinal insulinotropic agent is not limited to cephalic factors. Combined incubation of the pancreas of anencephalic fetuses with muscle, undertaken as a control, gave no such effect: the gland remained refractory to glucose.

When the basal insulin level was compared with that in the medium with a physiological concentration of glucose no significant differences were found in versions 2 and 3 of the experiment (Table 2); however, with an increase in the glucose concentration in the medium there was a considerable increase in the release of insulin into the medium. This suggests that intestinal hormones have no insulin-stimulating action in fetuses in the presence of a physiological glucose concentration, but potentiate the action of high glucose concentrations on B cells. The discrepancy between the results of the experiments in vivo and in vitro and in the writer's previous investigations [3, 11], when B cells of encephalectomized fetuses were found to be refractory to glucose in vitro, but responded to glucose when injected into fetuses, in that case becomes understandable. Intestinal hormones evidently participate in regulation of the sensitivity of B cells to glucose, and they compensate for the absence of cephalic insulinotropic factors in this particular experiment.

The absence of any differences in the rate of insulin release during incubation in the incubation fluid of the intestine or in the presence of the intestine itself (Table 2) may be evidence of the refractoriness of the endocrine cells of the fetal intestine to glucose. In agreement with data in the literature on neonatal formation of an adequate response of the intestinal endocrine apparatus to the maternal milk [10].

It can be postulated on the basis of these results that intestinal hormones may be modulators of the action of high glucose concentrations on insular B cells as early as in the prenatal period of development. They may perhaps help to maintain hyperinsulinemia of the fetus of a mother with diabetes, which induces exhaustion of the fetal B cells in the presence of severe hyperglycemia.

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