10. C. W. Distelhorst and B. M. Benutto, J. Biol. Chem., **260**, No. 4, 2153 (1985).
11. K. J. Howard and C. W. Distelhorst, Biochim. Biophys. Acta, **151**, No. 3, 1226 (1988).
12. W. G. Luttge, M. E. Rupp, and S. M. Emadian, Endocrinology, **124**, No. 4, 1813 (1989).

EXPERIMENTAL PROTEIN-DEFICIENCY DIABETES AND ITS CORRECTION

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KEY WORDS: protein deficiency; diabetes mellitus; implantation; pancreas

In 1985, in addition to insulin-dependent and insulin-independent types of diabetes mellitus, a new type of this disease was introduced into its classification by a WHO expert group, namely diabetes mellitus connected with protein deficiency (PDD) [3]. The reason for introducing this clinical class of diabetes was the recognition of the precise clinical features of this disease, its severity, and its high incidence in some tropical countries. This new category of diabetes comprises two subclasses: fibrocalculous pancreatic diabetes and pancreatic diabetes linked with protein deficiency [3]. This last type of diabetes is characterized by resistance to the development of ketosis, partial resistance to the action of insulin, and a high degree of emaciation. Information on the new type of diabetes [3] and data relating to the successful use of transplantation of fetal pancreas in recent years to correct the disturbance of carbohydrate metabolism in type I diabetes [2] were the basis for the present investigation.

Its aim was to create a model of diabetes associated with protein deficiency and to determine the effect of transplantation of the fetal pancreas in diabetic rats.

EXPERIMENTAL METHOD

Experiments were carried out on 79 noninbred albino rats: 49 males and 30 females. The rats were deprived of animal proteins (cheese, meat, milk) for 68-224 days. At the end of this period of protein deprivation (PD) the rats were put back on a more balanced diet including animal proteins. There were nine series of experiments, which differed in the duration of PD and the age composition of the rats at the beginning of PD (Table 1). Implantation of the fetal pancreas (IFP) subcutaneously into the ear was carried out on 22 rats. In series I-VI, IFP was carried out on the day when PD ended, in series VII it was carried out 2.5 months after the end of PD, and in series VIII and IX, IPA was not performed. The control consisted of two series (X and XI) of experiments with short-term PD. The state of the rats during the experiments was determined by regular weighing (once a week), observation of changes in carbohydrate metabolism (blood sugar level, glycosuria), and also by biochemical tests of the urine by rapid methods using strips: 1) Glucoprofile, 2) Glucofan, 3) Albufan. At the end of the observations the internal organs and remains of the transplanted pancreas were subjected to pathological and histological investigation. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

As Table 1 shows, 12 of the 79 protein-deprived rats developed diabetes (15.19%). Diabetes did not develop in all series of experiments. However, in those series (II-IV) in which diabetes was not observed, a larger number of the animals

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TABLE 1. Frequency of Development of Diabetes

Series of experiments	Number of animals	Duration of protein deprivation, days	Original weight, g	Original age, days	Number with diabetes	Period of observation, months
I experiment	4	123	44±3	30	1	7
Control	2		45±0,3			
II experiment	4	103	109±3	43	0	6,5
Control	2		107±1,5			
III experiment	4	103	46±6	31	0	
	6		59±5		0	6,5
Control	2		71±7			
	3		63±4			
IV experiment	6	98	87±4	27	0	7
	3		86±0,5			
V experiment	10	68	73±9	31	1	6
	9		62±8		2	
VI experiment	6	78	65,5±5,5	17	2	11
	4		at 1,5 months		2	
VII experiment	4	174	85±7	31	2	13,5
	2		61±1*		2	
VIII experiment	10	224	192±10	60	0	9
IX experiment	5	180	—	3	0	9
	2			Before birth		
X experiment	5	157	73,5±16	31	0	4
Control	5		70±13			
XI experiment	10	45	90±15	31	0	5
Control						

TABLE 2

Duration of disease, days	Incidence of disease, %
174	70
123	25
76	40
68	15

Legend. Asterisk indicates that initial weight of rats ≥100 g.

died during starvation and after its end (six of 23, or 26.08%). In series I and V-VII the incidence of diabetes among the rats was 30.7%.

Analysis of the data in Table 1 shows that diabetes developed when PD began at an early age (the first month after birth) and if its duration exceeded 2 months. Females developed the disease more often than males. The time of appearance of PDD was between the 4th and 6th months after the beginning of PD, regardless of when it ended. The exception was series IX (seven cases), in which deprivation began 3 days before birth, i.e., the mother was deprived, and diabetes did not develop even after 5 months of starvation. The percentage of rats developing the disease showed some degree of dependence on the duration of PD (Table 2).

After IFP, no further cases of diabetes developed among animals which had not developed the disease (18 cases). Among those rats (four cases) which had already developed diabetes at the time of IFP, two were subjected to the operation, two were not. In one of the animals undergoing the operation carbohydrate metabolism returned to normal after 52 days and remained normal until the end of the period of observation (Fig. 1). In the other rat there was a marked decrease in the blood sugar levels and glucosuria. In rats not undergoing the operation, the diabetes remained stable (both rats died before the end of the experiment).

Analysis of the results shows that our model of protein-deficiency diabetes closely resembles the state observed in man, as shown by the times of appearance and the incidence of the disease. For PDD to develop, an important role is played by the original age of the animals (the younger they are the more often they develop PDD) and by the duration of deprivation (as a rule, longer than 2 months). It is interesting to note that when protein deprivation began 3 days before birth, despite its long duration (up to 180 days) PDD did not develop. PDD likewise did not develop when PD began at the

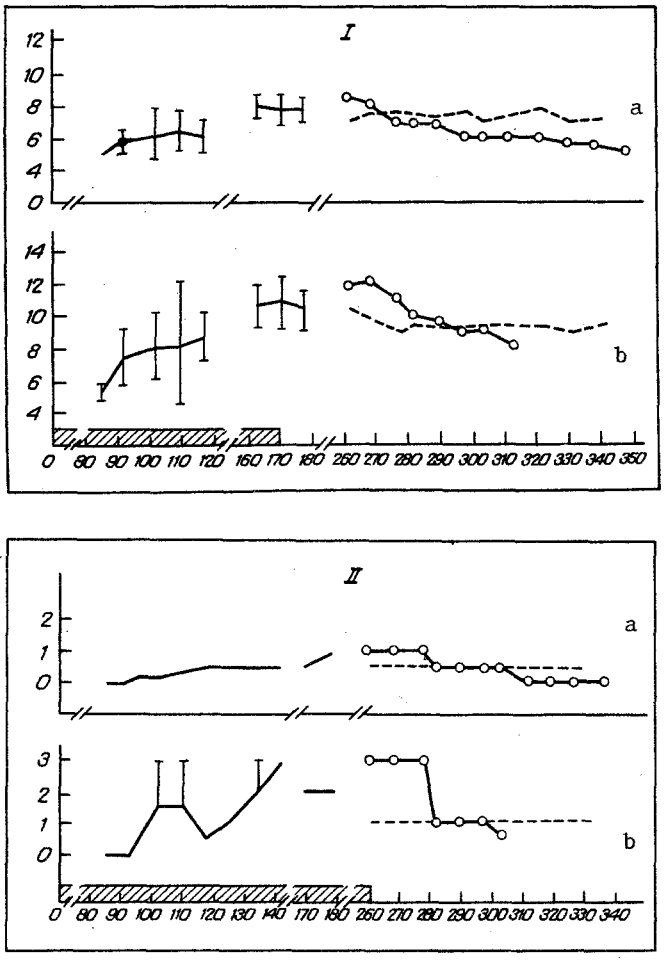

Fig. 1. Time course of blood sugar (I) and glucosuria (II) levels in male (a) and female (b) rats during and after PD. In the case of implantation of the fetal pancreas, the values show a tendency to return to normal. Shaded region denotes period of PD; continuous line — values for rats after IFP; broken line — values for untreated rats. Abscissa, time (in days).

age of 2 months. Hence it follows that the critical age for development lies between the neonatal period and the beginning of sexual maturity (in rats, sexual dimorphism is reflected in a difference between body of females and males), second, during this period of development it is particularly important to provide the growing animal with a balanced protein diet [1, 5].

So far as the pathogenesis of the disturbances is concerned, there is evidently a certain threshold of PD above which the body cannot maintain normal development of such vitally important organs as the pancreas. The results confirm the effect of PD on the endocrine function of the pancreas. As regards the mechanism of these disturbances, it can be tentatively suggested that an essential role is played by disturbance of the exocrine function of the pancreas, which could explain known changes in the structure and function of the intestine [4]. These changes may determine the malabsorption syndrome and preservation of the protein deficiency, leading to inadequate insulin production in the rehabilitation period, observed in rats after prolonged starvation. IFP, it can be assumed, provides both specific replacement therapy (hormone production by the implant) and also, perhaps, nonspecific stimulation of regeneration of the pancreas, and even of other organs, so that the body is able to escape from the vicious circle of protein deficiency.

LITERATURE CITED

1. I. A. Drzhevetskaya, Endocrine System of the Growing Organism [in Russian], Moscow (1987).
2. V. P. Kulik, V. K. Novikov, T. P. Pisareva, et al., Sov. Med., No. 7, 16 (1987).
3. Diabetes Mellitus. WHO Expert Report [Russian translation], Moscow (1987), pp. 22-26.
4. E. P. Shuvalova (ed.), Tropical Diseases [in Russian], Moscow (1989), p. 460.
5. N. A. Tyurin et al., Diseases of the Newborn and Infants [in Russian], Moscow (1986), p. 79.

EFFECT OF CORTICOSTEROID IMBALANCE ON THE CATECHOLAMINE SYSTEM OF THE FETAL RAT BRAIN

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KEY WORDS: tyrosine hydroxylase; corticosterone; metyrapone; prenatal development

Administration of glucocorticoids which pass through the placenta is used in medical practice during pregnancy to prevent the syndrome of respiratory disturbances in the newborn [4]. Meanwhile, in children exposed during early development to a high glucocorticoid level as a result of hormone therapy of the mother or to the action of stressors, disturbances of neuropsychological and behavioral development are observed [7]. In model experiments on animals, psychoemotional disturbances also are found in the offspring, due evidently to changes in function of the catecholamine mediator system of the brain [2]. However, it is not clear what changes take place in the mediator system of the fetal brain when the hormone balance is disturbed. Solving this problem would help to establish the role of glucocorticoids in prenatal development of the catecholamine mediator system and explain the mechanism of realization of the remote aftereffects of damaging influences of the environment and of hormone therapy in early ontogeny on the functions of the adult.

The aim of this investigation was to study levels of catecholamines and activity of the key enzyme of their biosynthesis, namely tyrosine hydroxylase (TH) in the fetal rat brain after disturbance of the glucocorticoid balance in the blood of the pregnant mothers.

EXPERIMENTAL METHOD

Female Wistar rats received a subcutaneous injection of corticosterone ("Calbiochem," 2.5 mg/0.2 ml/100 g), the corticosteroid synthesis blocker metyrapone (Metypirone, CIBA, 12 mg/0.2 ml/100 g), or distilled water (0.2 ml/100 g), subcutaneously on the 16th and 18th days of pregnancy, and the 21-day fetuses of these groups of females were studied 3 days later. In other experiments, on the 20th day of pregnancy the mothers were given corticosterone in the same dose or were left intact, and their fetuses were studied 6 h later. After sacrifice of the mothers, the fetuses were removed by cesarean section and quickly weighed. The brain was isolated in the cold and cut along a plane passing from the pineal gland to the optic chiasma into brain stem and anterior parts. Some of the brain tissue samples were used for fluorometric measurement of the DNA concentration [12] and of protein, by Lowry's method [13]. The dopamine and noradrenalin concentrations or TH activity [1] were determined fluorometrically in other samples, in the presence of saturating concentrations of coenzyme ($DMPH_4$) and tyrosine.

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