et al. [4], who stimulated ovulation in mice by serum gonadotrophin and also by chorionic gonadotrophin and found, besides an increase in the number of ovulating eggs a decrease in their percentage fertility and an increase in the number of stillbirths.

The results of culture of follicular oocytes from ovaries of animals stimulated by LHRF indicate that in their cytogenetic characteristics these oocytes correspond in general to the oocyte population from ovaries of control animals in the estrus–metestrus stage [1]. However, the increase in the number of cells resuming meiosis, the number of spontaneously cleaving oocytes, the increase in the number of degenerating cells, and also some increase in the number of chromosomal anomalies must be taken into consideration. These particular features are probably associated primarily with the predominantly luteotrophic action of LHRF, which raises the blood LH level and can intensify the processes of follicular atresia in the ovaries [5].

The study of the effect of single and repeated injections of LHRF on the state of the follicular oocytes and on the population of ovulating oocytes in intact rats thus shows that LHRF, which simulates follicle formation, leads to some increase in the intensity of follicular atresia, with a consequent increase in the degree of heterogeneity of the resulting gamete population, without, however, having any significant effect on the frequency of chromosomal aberrations among the ovulating oocytes.

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EFFECT OF THE THYMUS ON ENDOCRINE FUNCTIONS OF THE GONADS AND ADRENALS IN MICE

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KEY WORDS: nude mice; transplantation of the thymus; 11-hydroxycorticosteroids; sex hormones.

The thymus is closely interconnected with the glands of internal secretion [4, 7, 10]. This fact is demonstrated particularly vividly by experiments on mutant nude mice with genetically determined absence of the thymus. In such animals the onset of sexual maturity is delayed, fertility is reduced [10], and the morphology [5] and function [2, 7] of many endocrine organs are disturbed.

The aim of this investigation was to study whether these changes in the endocrine sphere of genetically athymic mice are directly connected with absence of the thymus, or whether it is an independent pleiotropic effect of the nude mutation. It was interesting to study mutant animals into which the thymus was transplanted from normal mice during the first days of life.

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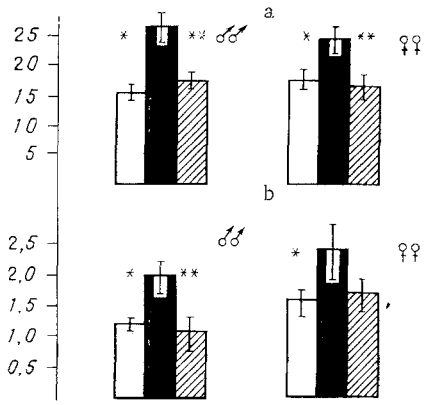

Fig. 1. Adrenal function in athymic nude mice (intact and after transplantation of the thymus): a) plasma 11-HCS concentration (in μg %); b) specific production of 11-HCS by adrenals *in vitro* (in μg/100 mg weight of gland/h). Unshaded columns — control normal animals; black columns — intact nude mice; obliquely shaded columns — nude mice after transplantation of thymus. *Differences between control animals and intact nude mice significant; **differences between intact mice and nude mice undergoing operation significant.

EXPERIMENTAL METHOD

In the experiments of series I mutant athymic female and male nude mice, obtained at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR by introducing the nu gene into line BALB/c, were used at the age of 2 months. Normal BALB/c mice of the same age served as the control. In the experiments of series II one thymus gland from a BALB/c mouse aged 7 days was transplanted into the left subscapular region of mutant athymic mice aged 5 days. These animals were investigated 2 months after the operation. Adrenal function was judged from the 11-hydroxycorticosteroid (11-HCS) concentration in the peripheral blood plasma and the level of production of these hormones by the adrenals *in vitro*. The corticosteroid concentration in plasma and incubation material was determined fluorometrically [1]. Activity of the gonads was judged from the testosterone concentration in peripheral blood plasma and sex hormone production by the gonads *in vitro*. The concentration of sex hormones in the plasma and incubation material was determined by radioimmune assay with standard kits from CEA-Ire-Sorin (France). The results were subjected to statistical analysis by Student's and Fisher's tests.

EXPERIMENTAL RESULTS

The results of experiments on genetically athymic mice showed that the level of adrenal function is significantly raised in these animals. In both male and female nude mice the peripheral blood plasma 11-HCS concentration was much higher than in normal control mice (Fig. 1a); the effect observed was the result of increased production of these hormones by the adrenals (Fig. 1b). Pierpaoli and Besedovsky [7] also found a raised corticosteroid level in the blood of 14-day-old genetically athymic mice but the reaction of the adrenals to exogenous ACTH was indistinguishable from normal. It can be tentatively suggested that in this case the inhibitory influence of the thymus on the adrenals is abolished, although it is impossible to judge whether the thymus exerts its effect directly on the adrenal cortex or indirectly through other endocrine glands.

Transplantation of the thymus into nude mice in the early period of postnatal development restored normal adrenal function. For instance, the plasma corticosteroid concentration and the production of these hormones *in vitro*, which were much higher than normal in male nude mice, fell practically to the control values if these animals were grafted with a thymus from normal mice. The same effect also was obtained in females (Fig. 1a, b). The experiments showed that transplantation of the thymus into genetically athymic mice during the first days of life prevents disturbances of adrenal function which otherwise would develop in them and, consequently, absence of the thymus during early ontogeny is the direct cause of changes observed in that function in mutant animals.

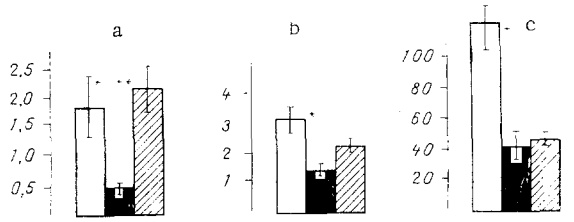

Fig. 2. Endocrine function of gonads of nude mice (intact and after transplantation of thymus): a) plasma testosterone concentration (in ng/ml); b) specific testosterone production by testes *in vitro* (in ng/100 mg weight of gland/h; c) specific estradiol production by ovaries *in vitro* (in pg/100 mg weight of gland/h). Remainder of legend as to Fig. 1.

Investigation of the endocrine function of the gonads of mutant athymic mice showed that it was appreciably reduced in these animals: The plasma testosterone concentration of 2-month-old nude male mice was significantly lower than in normal animals (Fig. 2a). The reason for this is evidently the decrease in production of this hormone by the testes observed *in vitro* (Fig. 2b). Reba et al. [8] also observed a marked decrease in the concentrations of testosterone and Δ-androstenedione in the blood of nude mice. Transplantation of the thymus into mutant males during the first days after birth prevents dysfunction of the testes which these animals develop in the adult state. The blood level of testosterone and its production by the gonads *in vitro* were significantly increased in animals with a transplanted thymus (Fig. 2a, b). Other workers observed a decrease in the gonadotrophin concentration in the pituitary of genetically athymic mice and of neonatally thymectomized mice [8]. The same workers showed that early transplantation of the thymus completely prevented this disturbance [9]. On the basis of the experimental data given above and information from the literature it can be concluded that the depression of testicular function observed in genetically athymic animals is due to absence of the thymus in the early stages of postnatal development, and it is mediated through pituitary regulatory centers.

Estradiol production by the ovaries *in vitro* was significantly lower in nude females than normal animals (Fig. 2c). This was probably because of profound structural changes in the ovaries of these mice: the glands are reduced in size, interstitial cells predominate in them, and there are no large follicles or corpora lutea [5]. At the same time it has been shown that disturbances of a central character are present in female nude mice: The content of luteinizing and follicle-stimulating hormones is much lower in the pituitary of these animals than of normal mice [9]. The results of the present experiments showed that transplantation of the thymus into female athymic mice has no effect on the low sex hormone production (Fig. 2c). However, according to Rebar et al. [9], transplantation of the thymus in the neonatal period completely prevents the fall in the concentration of gonadotrophins in the pituitary gland of such animals. Inability of the implanted thymus to restore the endocrine function of the ovaries is evidence that the thymus participates in the morphogenesis of this organ, and that its absence in the embryonic period and during the first few days of life leads to irreversible structural and functional changes. Data obtained by other workers [6], showing that the early stages of follicle development in the ovary are under thymic control, confirmed this conclusion. Anlagen of the follicles, from which subsequently oocytes develop, appear during the embryonic period in females and the early stages of their development take place actually in the early postembryonic period, and absence of the thymus at that time has a dramatic effect on development of the ovary. Transplantation of the thymus into such animals during the first days of life can restore the normal pituitary gonadotrophin level but cannot restore the morphological structure or endocrine function of these glands.

Transplantation of the thymus into genetically athymic animals in the early stages of postnatal development thus prevents disturbances of adrenal and testicular function from arising. Consequently, absence of the thymus at a certain period is the immediate cause of disturbances of these functions observed in mutant animals. It can be postulated that the thymus plays an essential role also in the formation of a functionally active ovary by its participation in the morphogenesis of these glands, but this takes place in the embryonic period.

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NUCLEOLUS-ORGANIZING REGIONS OF CHROMOSOMES IN EARLY EMBRYOGENESIS OF LABORATORY MICE

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Both transcription and translation of several genetic loci take place in mammals starting from the 2-4 blastomere stage [1]. In particular, biochemical methods have reliably demonstrated the presence of rRNA synthesis starting from the 4 blastomere stage [8, 9]. However these methods cannot establish whether all chromosomes carrying nucleolus-organizing regions (NOR) participate in transcription in initial embryogenesis. In 1975 Goodpasture and Bloom [5] developed a method of selective demonstration of NOR of chromosomes which, as was subsequently shown [10, 12, 14], reveals precisely those NOR which functioned in the preceding interphase as organizers of the nucleolus, i.e., staining with silver reflects the function of ribosomal genes and not simply their presence. A method of silver staining was used in [4, 6] to study the time of onset of NOR activity in early mouse embyrogenesis but, as our own observations have shown, the methods used by these workers to prepare their specimens and the method of staining with silver are not optimal.

The object of this investigation was to determine the conditions for most complete demonstration of NOR in early mouse embryogenesis and to study changes in the number of NOR starting from the first cleavage division and until the 10th day of development.

EXPERIMENTAL METHOD

Experiments were carried out on CBA × C57B1 mice obtained from the "Rappolovo" nursery. The mice (females) were given an intraperitoneal injection of 0.2 ml of 0.02% colchicine 1-1.5 h before they were killed on the 1st, 2nd, 3rd, 4th, and 10th days of development, after which specimens were prepared by the method adopted in the writers' laboratory: The embryos were placed in 0.9% sodium citrate for 4-15 min, fixed in a mixture of methanol with glacial acetic acid (3:1), and then macerated on a slide in a mixture of 75% acetic acid and methyl alcohol (1:1). For selective staining with silver a modified method of Howell and Black [7] was used. There were two solutions: A 2% solution of gelatin to which formic acid was added (1 ml to 50 ml gelatin) and a 50% solution of silver nitrate, purified beforehand by Khachaturov's method [4]. Both solutions were made up in deionized water. One drop of gelatin and two drops of silver were applied to the freshly prepared specimens, a coverslip was placed over them, and they were incubated in a humid chamber for 5-15 min at 60°C until they were stained a golden brown color. The specimens were then vigorously rinsed in running water and dried

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