

ENDOCRINE FUNCTION OF THE GONADS AND ADRENALS IN NUDE
AND THYMECTOMIZED MICE

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The immune status of an organism is reflected in function of its endocrine system, in which an important role is played by the thymus — the central organ of immunity. The thymus has been shown to have antagonistic relations with the adrenals [3, 5, 13] and to influence hormonal activity of the gonads [5, 14]. A further study of the interconnection between the immune and endocrine systems is interesting. An extremely convenient model for such investigations is provided by mutant athymic nude mice, in which sexual maturation is retarded, fertility considerably reduced, and serious changes are present in the adrenals and ovaries [3, 14].

The aim of the present investigation was to study the function of the adrenals and gonads in mice with genetically determined absence of the thymus and to examine how removal of the thymus in the early postnatal period affects the endocrine status of normal animals.

EXPERIMENTAL METHOD

Mutant athymic nude male and female mice, obtained at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, by introduction of the nu gene into the BALB/c line were used at the age of 2 months in the experiments of series I. Normal mice of the same line and the same age were used as the control. In the experiments of series II BALB/c mice were thymectomized on the 3rd day after birth. To rule out any possible action of operative stress at an early age, a mock thymectomy was performed on control animals at the same age. The animals were decapitated 2 months after the operation. Adrenal function was judged from the 11-hydroxycorticosteroid (11-HCS) concentration in the peripheral blood plasma and production of these hormones by the adrenals *in vitro*. The corticosteroid concentration in the plasma and incubation media was determined fluorometrically [1]. Activity of the gonads was judged from the plasma testosterone concentration and production of sex hormones by the gonads *in vitro*. The concentration of sex hormones in the plasma and incubation media was determined by a radioimmune method, using standard kits from Cea-Ire-Sorin (France). The results were subjected to statistical analysis by Student's t test and Fisher's test.

EXPERIMENTAL RESULTS

The results obtained in experiments on nude mice showed that function of the adrenals and gonads was significantly modified in animals with genetically determined absence of the thymus. For instance, the corticosteroid concentration in the peripheral blood plasma of male and female nude mice was significantly higher than in control mice (Fig. 1A). Since this effect may be linked with changes in the function of the adrenals themselves in the athymic animals, 11-HSC production by the adrenals was investigated *in vitro*. It was found that an increase in corticosteroid production did in fact occur in the mutant mice (Fig. 1A). In this case the inhibitory effect of the thymus on the adrenals, demonstrated by experiments in which thymus extract was injected into rats *in vivo* [5, 6], was probably abolished.

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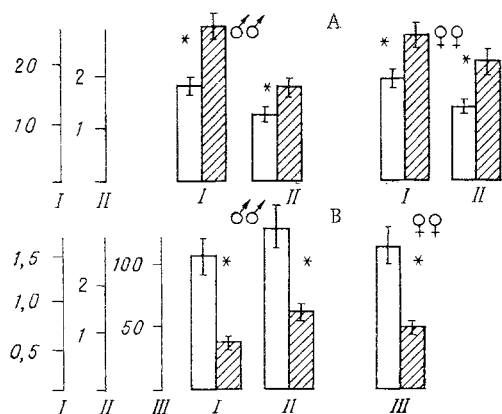


Fig. 1

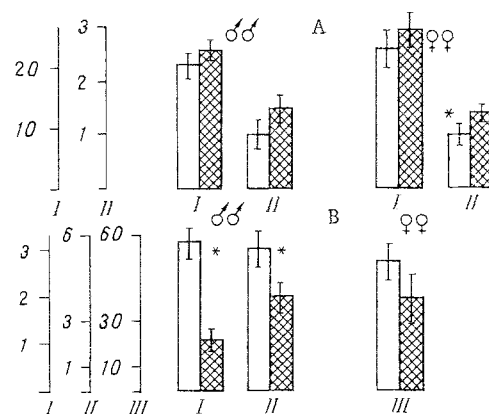


Fig. 2

Fig. 1. Endocrine function of adrenals and gonads in mice with genetically determined absence of the thymus. Shaded columns represent nude mice, unshaded columns control animals. *) Differences significant ($P < 0.05$). A: I) 11-HCS concentration in blood plasma (in $\mu\text{g}\%$), II) specific 11-HCS production by adrenals *in vitro* (in $\mu\text{g}/100$ mg weight of gland/h); B: I) testosterone concentration in blood plasma (in ng/ml); II) specific testosterone production by testes *in vitro* (in $\text{ng}/100$ mg weight of gland/h); III) specific estradiol production by ovaries *in vitro* (in $\text{pg}/100$ mg weight of gland/h).

Fig. 2. Effect of thymectomy on endocrine function of adrenals and gonads. Shaded columns represent thymectomized mice, unshaded columns animals undergoing mock thymectomy. Remainder of legend as to Fig. 1.

The study of the blood testosterone concentration showed that the level of this hormone in nude mice was considerably lower than in normal animals (Fig. 1B). This was evidently due to deficient hormone production by the testes, for the gonads of the mutant mice secreted only half as much testosterone as in the control. According to Pierpaoli and Besedovsky [11], the blood testosterone level in athymic mice on the 14th day of life is appreciably lower than in normal animals. Our own results are in good agreement with these findings, despite differences in the age of the experimental animals. There is little information in the literature on interaction between thymus and testes. Hypertrophy of the thymus has been observed after removal of the testes [8], whereas involution of the thymus follows administration of gonadotrophins or directly of sex steroids [15]. The mechanism of the influence of the thymus on the gonads has not yet been explained. Japanese workers found no direct effect of the thymus on the testes [10], and it has been suggested that the thymus exerts its action through the pituitary [5].

According to our own observations, estrogen production by females with genetically determined absence of the thymus was significantly lower than in normal animals (Fig. 1B). It was shown previously that the most marked morphological disturbances in nude mice are observed in the ovaries: The glands are reduced in size, they consist mainly of interstitial cells and follicles and corpora lutea are absent [3, 4]. The observed fall in hormone production is evidently directly connected with the profound structural changes observed by other workers in the gland [3, 4].

To determine whether the changes observed in the hormonal status are directly connected with absence of the thymus or whether they are the result of the pleiotropic effect of the nude gene, a series of experiments was carried out on normal BALB/c mice subjected to thymectomy on the 3rd day after birth. No significant difference was found between the 11-HCS levels in the blood plasma of the thymectomized animals and animals undergoing the mock operation, although there was a tendency for the concentration of these hormones to be higher in the experimental animals. These differences were more marked when the ability of the gland itself to produce hormones was studied, especially in the group of females, where the differences were significant (Fig. 2B). There is evidence that the 11-HCS level in the blood plasma is increased only during the first weeks after neonatal thymectomy [2, 4, 7], after which it falls to normal [7]. In the light of these data, the absence of significant differences in the present experiments between the glucocorticoid concentrations in the experimental animals

killed 2 months after the operation conforms to the rule. Removal of the thymus at the age of 3 days, unlike the situation observed in genetically athymic mice, evidently affects adrenal function only during the first weeks after the operation; females, moreover, are more sensitive to removal of the thymus than males.

The results of the present experiments showed that the ovaries of females were less sensitive to removal of the thymus than male gonads. No significant differences were obtained between females undergoing thymectomy and the mock operation as regards estradiol production by the ovaries *in vitro* (Fig. 2B), whereas the genetically athymic mice differed highly significantly from the control animals with respect to this feature. We know from the literature [10, 11] that thymectomy at an early age causes changes very similar to those observed in the ovaries of mutant athymic mice. However, it was also shown that although thymectomy was carried out at the same age, the response to the operation varied: 20% of the female mice were sterile, and of the fertile females only 50% had a normal progeny, and in the rest the number was sharply reduced [9]. An important role is evidently played by individual sensitivity of the animals to absence of the thymus. This heterogeneous response of the mice to thymectomy may perhaps also be the reason for the absence of significant differences between the animals undergoing operation and the control animals.

As regards function of the gonads in thymectomized males, just as in mutant athymic mice both the blood testosterone concentration and production of this hormone by the testes *in vitro* were significantly higher than the corresponding levels in control animals (Fig. 2B). Just as in the case of glucocorticoids, it is possible that the difference found may disappear with time.

Serious disturbances of function of the endocrine glands are thus found in mutant mice, whereas changes in the endocrine system after thymectomy performed at the age of 3 days are not so marked as in genetically athymic mice. The thymus perhaps exerts its influence on formation of the functions of the adrenals and gonads chiefly in the embryonic period.

Since changes in the endocrine system observed in thymectomized mice coincide only partially with changes observed in genetically athymic animals, it can be tentatively suggested that the disturbances present are not the result of the pleiotropic action of the *nu* gene, but are more probably due to absence of the thymus. However, further investigations are needed to confirm this hypothesis.

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