were sick in the early neonatal period (groups 10 and 12-19). It is impossible to characterize the embryotoxic effect of sera of newborns depending on the mother's disease because of the relatively small number of observations and the difficulties encountered in differentiating the diagnostics of a vast array of pathologies. However, it should be noted that the embryotoxic effect is considerably potentiated by hypoxia. The sera of 4 newborns after prenatal hypoxia (groups 14-17, as evidenced by the presence of meconium in the amniotic fluid) induced death of mouse embryos in 100, 100, 73, and 23% of cases, respectively. A 100% embryolethal effect was seen in the serum of a newborn after hemolytic (ABO) disease (group 18). This effect may be due to the similarity of the ABO system of humans and mice.

Thus, our results indicate that *in vitro* culture of preimplantation mouse embryos can be used for the evaluation of embryotoxicity of sera from newborns.

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Effect of Stress on the Reticular Zone of the Adrenal Cortex

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The reticular zone (RZ) of the adrenal cortex is shown to be involved in the formation of the organism's response to stress. A new scheme of physiological regulation of RZ is presented.

Key Words: *stress exposure; adrenal reticular zone; cholesterol esterification*

The adrenocortical reticular zone (RZ) secretes androgens (mainly dehydroepiandrosterone and dehydroepiandrosterone sulfate [2,12]) in amounts surpassing the total sum of all the other adrenal hormones, including glucocorticoids. However, the physiological function of the RZ in humans and animals is still unknown [1,8,12], as is its contribution to the development of the organism's response to stress. The system of physiological regulation of RZ is not clear either [14].

The aim of our research was to study changes in the activity and physiological regulation of the adrenocortical RZ under the effect of stress.

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MATERIALS AND METHODS

Experiments were carried out with male Wistar rats weighing about 200 g. The animals were castrated

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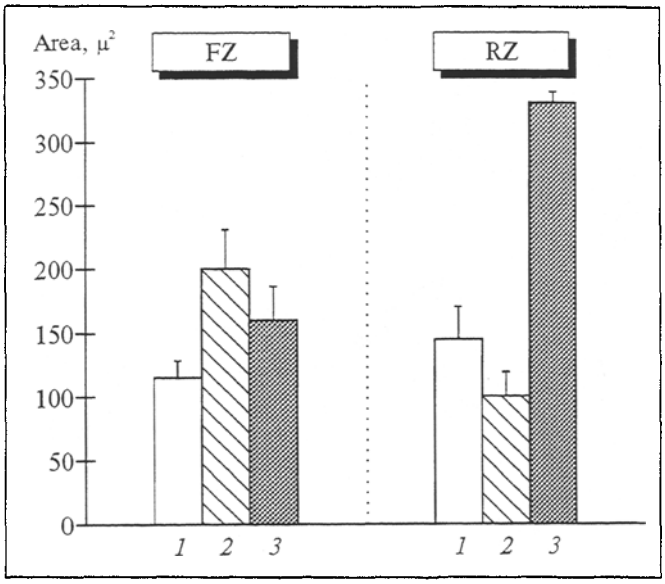

Fig. 1. Area of cell nuclei of FZ and RZ in male rats. *1*) control; *2*) single stress exposure; *3*) multiply (9 days) repeated stress exposure.

4 days before the experiment. Stress was induced by shaking the animals using an AVB-4p laboratory shaker at a frequency of 180 vibrations per min for an hour once or repeatedly (for 9 and 18-19 days once daily). The animals were sacrificed by decapitation. Retabolil was intramuscularly injected to rats in a dose of 5 mg/100 g two times, 22 and 11 days before decapitation.

For morphometry transverse slices of the adrenals were stained with hematoxylin-eosin and measurements were carried out using an ocular micrometer, the size of cell nuclei being measured in 30-50 cells of the zone in 5-12 animals of every group. The esterifying capacity of the blood plasma was assessed by the radioisotope method [13] by estimating the percent share of cholesterol acylated in an hour in 1 liter of plasma. The data were statistically processed after Student (the mean values and mean error are presented).

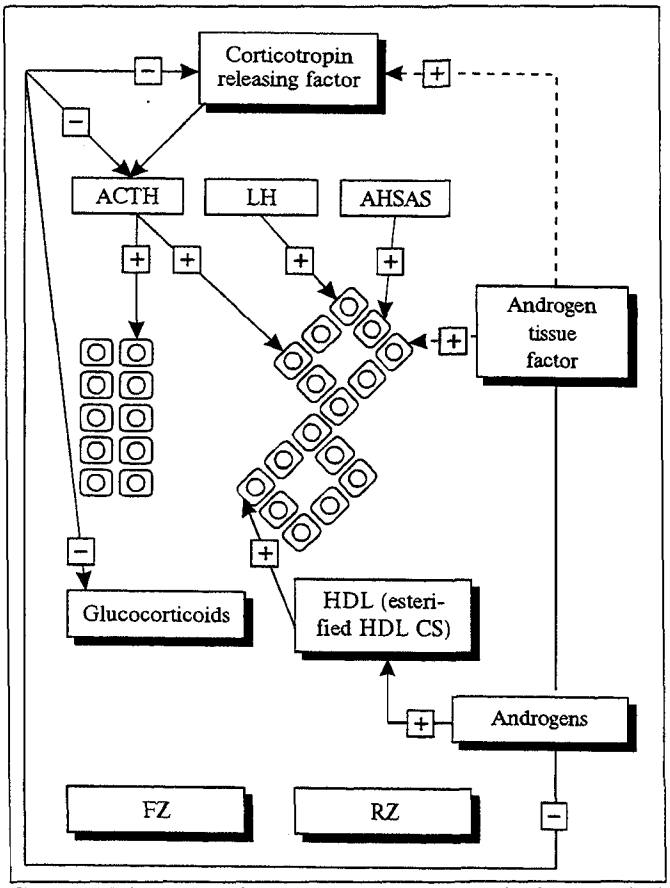

Fig. 2. Scheme of physiological regulation of adrenocortical RZ and FZ activity under stress. AHSAS: androgenic hormone stimulating adrenal secretion; LH: luteinizing hormone. Solid line: relationships whose existence is proved; dashed line: hypothetical relationships.

RESULTS

Morphometric examination of cell nuclei of the fasciculate zone (FZ) and RZ of the adrenal cortex of rats revealed a reliable increase in the size of the nuclear area in both zones under the effect of repeated stress exposures (19 days) (Table 1), this increase being indicative of their activation.

Experiments performed to compare the effects on rats of a single and multiple (9 days) exposures showed (Fig. 1) that, first, multiple exposures induced activation of both FZ ($p<0.02$) and RZ ($p=0.001$); second, that FZ was activated by both a single ($p<0.04$) and multiple ($p<0.02$) exposures; and, third, that RZ was activated solely by multiply repeated exposures ($p=0.001$).

The activation of the adrenocortical glucocorticoid-secreting FZ in stress conforms to Selye's notion of stress. It is noteworthy that we revealed activation of the adrenocortical RZ after multiple exposures, this indicating the involvement of this adrenal zone in the response of the body to stress.

The hypophyseal adrenocorticotropic hormone (ACTH) is considered to be the principal regulator-activator of the RZ and FZ. In this connection it is not clear why in our experiments an acute exposure led to activation of the FZ but failed to affect the RZ. It is possible that for the RZ ACTH is not the only and, perhaps, not even the principal regulator. Some scientists think that the adrenocortical RZ is regulated, along with ACTH, by a hormone "stimulating the secretion of adrenal androgens" [6], but it is not clear to which specific structural compound it should be referred. Gonadotropins may contribute to the regulation of the RZ [10]. Blocking of the peripheral tissue receptors with antiandrogens has been shown to disturb the mechanism of

compensatory hypertrophy of the RZ which develops in men after castration [4]. Hence, for RZ hypertrophy, there has to be a "hormone requirement" on the part of the tissues, with the participation of the tissue androgen-binding receptors. Such a regulatory impact appears to be formed during chronic stress exposures, particularly so in exercise: the RZ is activated because of the signals coming from a tremendous number of "androgenic" receptors, primarily from the muscles. Muscular exercise in athletes has been shown to have a strong activating effect on the RZ [14].

Based on these data, a scheme is presented, offering a new viewpoint on the complex regulation of the function of the adrenocortical RZ (Fig. 2). This scheme shows that high-density lipoproteins (HDL) help regulate RZ function, this deserves special note.

Esterified blood lipoprotein cholesterol (CS) is mainly utilized in the biosynthesis of steroid hormones, androgens included; in rats it is esterified HDL CS.

Our experiments carried out to study the effects of androgens on the rate of CS esterification demonstrated that castration of male rats caused a reliable decrease of the esterification rate in the blood plasma of animals (Fig. 3, *a*). Exposure of normal male rats to stress using a laboratory shaker did not induce changes in the rate of esterification, whereas in castrated males multiple stress exposures led to a reduction of the rate of CS esterification which was normalized by the injection of retabolil, a synthetic analog of adrenocortical RZ androgens (Fig. 3, *b*).

It is noteworthy that at first castration of males seemingly does not cause a decrease in CS esterification (Fig. 3, *b*), in contrast to the picture observed 22 days after the operation (Fig. 3, *a*). The cause of this difference consists in the following: Fig. 3, *a* reflects the status of males castrated 4 days before the experiment, when compensatory hypertrophy of the adrenocortical RZ has not yet developed [3]. Such animals are characterized by the absence of gonadal androgens and a low level of adrenal androgens [3,5].

A pronounced hypertrophy and activation of the adrenocortical RZ (Fig. 3, *b*) with a corresponding compensation of androgen insufficiency is attained in males 22 days after castration (castration 4 days before stress exposure + 18 days of shakings) [5,10]. That is why the rate of CS esterification in them is normalized at rest, but during stress exposure (shaking) androgen insufficiency is observed, presenting as insufficiency of the CS-esterifying mechanism. All this points to a close

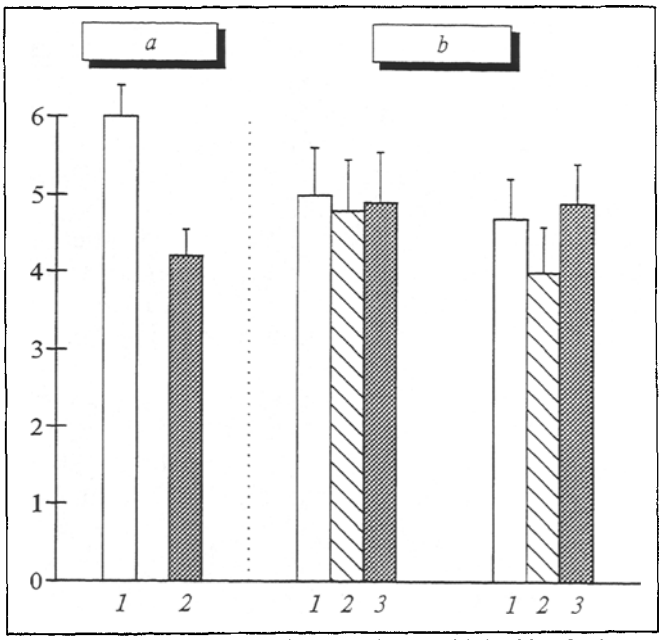

Fig. 3. Rate of CS esterification (% per h) in blood plasma of normal and castrated male rats. *a*) at rest (*1*), 4 days after castration (*2*); *b*) effect of multiply (18–19 days) repeated stress exposures and intramuscular retabolil, 22 days after castration. *1*) control; *2*) multiple stress exposures; *3*) multiple stress exposures in parallel with retabolil injections.

relationship between the rate of CS esterification and androgen levels, both gonadal and adrenal, and to a possible regulatory role of androgens in the process of CS esterification.

The revealed capacity of androgens to activate CS esterification may determine their participation in autoregulation of a biosynthetic process in the RZ with the involvement of esterified HDL CS, and, hence, of HDL. HDL in such a case interact with adrenal cells by binding with specific HDL receptors via apoA-1 [11].

Moreover, ACTH, by influencing steroid biosynthesis via activation of the enzymatic processes of steroidogenesis, may regulate CS absorption from HDL [7] and, together with these lipoproteins, participate in activating the biosynthesis of steroid hormones.

TABLE 1. Effect of Multiple (19 Days) Stress Exposures on the Area of Cell Nuclei in the FZ and RZ of the Right Adrenal Cortex of Rats

Group	Area of cell nuclei, μ^2	
	FZ	RZ
Control	46.4±7.13 (5)	50.0±5.57 (5)
Stress	127.8±21.25 (6) $p<0.01$	78.3±7.71 (6) $p<0.02$

Note. p shows reliability of differences vs. control; in parentheses: number of rats in group.

Lipoproteins contribute to the biosynthesis of glucocorticoids only as modulators of the process; in the biosynthesis of androgens they are true regulators due to the presence of feedforward and feedback consisting in androgen activation of CS esterification and utilization of esterified CS, in its turn, for androgen biosynthesis. Such a scheme of regulation of the function of the adrenocortical RZ (Fig. 2) helps explain the observed regulatory peculiarities of the RZ and FZ, as well as the involvement of the RZ mainly in multiply repeated stress exposures.

These data indicate activation of the adrenocortical RZ in multiple, but not single stress exposures, with CS esterification in rat blood plasma playing a certain role in the regulation of RZ activation.

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