

TABLE 1. Parameters of EPR Spectrum of Spin Probe in Erythrocyte Membranes

Group of subjects tested	Distance between extrema, (2A <sub>pp</sub> ), G	Constant of hyperfine interaction (a'), G	Parameter of orderliness (S), relative units
Healthy individuals	51,16±0,32	14,52±0,15	0,611±0,007
Patient with IMD: without vascular involvement	51,00±0,22	14,60±0,02	0,603±0,007
with vascular involvement	53,00±0,47	14,87±0,10	0,642±0,009

TABLE 2. Molar Cholesterol/Phospholipid (CH/PL) Ratio and Na,K-ATPase Activity of Erythrocyte Membranes

Group of subjects tested	CH/PL, mole/mole	Na,K-ATPase activity, $\mu$ moles P <sub>i</sub> /mg protein/h $\times 10^2$
Healthy individuals	0,87±0,01	148,93±4,35
Patients with IMD: without vascular involvement	0,92±0,05	116,00±4,60
with vascular involvement	1,15±0,02	45,07±2,39

The results are evidence of a profound change in structure of the erythrocyte membranes in IHD, leading to inhibition of the membrane-bound enzyme Na,K-ATPase. The presence of such changes indicates a possible connection between damage to the arteries, beginning probably with injury to smooth-muscle cell membranes [6], and the state of the erythrocyte membranes.

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EFFECT OF TESTOSTERONE ON RATE OF TOTAL PROTEIN SYNTHESIS IN THE FETAL RABBIT REPRODUCTIVE TRACT *IN VITRO*

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KEY WORDS: testosterone, protein synthesis, reproductive tract, rabbit fetuses.

In the modern view androgens determine the development of the male reproductive system in the critical period of embryonic development [4]. Whereas the molecular basis for the direct effect of androgens on tissues of target organs has been studied quite well [3], the mechanism of their action on growth and differentiation of the reproductive tract of mammalian fetuses has not hitherto been investigated.

The most active androgen is testosterone, secreted by Leydig's cells in the testis. Its stimulating effect on the synthesis of various proteins, including receptor proteins, in target organs has been demonstrated in adult mammals [3, 7]. Nevertheless, the study of the action of testosterone on biosynthesis in fetuses is of great interest for elucidation of the role of hormones in early ontogeny.

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TABLE 1. Rate of Synthesis of Total Protein in Rabbit Fetal Tissue ( $M \pm m$ )

Day of development of fetus	Tissue	Intensity of incorporation of $^3\text{H}$ leucine, cpm/mg protein/30 min	
		testosterone	control
18	I	178 $\pm$ 261	360 $\pm$ 91
	II	969 $\pm$ 30	343 $\pm$ 17
19	I	246 $\pm$ 33	153 $\pm$ 15
	II	149 $\pm$ 18	146 $\pm$ 6
20	I	593 $\pm$ 70	158 $\pm$ 39
	II	161 $\pm$ 53	98 $\pm$ 22
21	I	224 $\pm$ 36	94 $\pm$ 10
	II	170 $\pm$ 40	42 $\pm$ 11
22	I	679 $\pm$ 35	68 $\pm$ 17
	II	116 $\pm$ 9	73 $\pm$ 16

Legend. I) Reproductive tract; II) muscle.

Experiments *in vitro* have shown that the character of specific and selective uptake of [ $^3\text{H}$ ]testosterone by anlagen of the genital tract of rabbit fetuses of both sexes is similar and corresponds to the male type of morphogenesis [1]. However, it is not clear whether the changes observed are the result purely of the acceptor capacity of the target tissue, or whether the hormone not only is actively bound in this case, but also exerts its physiological effect *in vitro*.

The aim of this investigation was to determine the rate of protein synthesis in tissues of the reproductive system of rabbit fetuses under the influence of testosterone *in vitro* in the critical period of development.

#### EXPERIMENTAL METHOD

The reproductive tract and a piece of thigh muscle from rabbit fetuses at the 18-22-day stage of development (counting from the time of copulation) were incubated for 30 min at 38°C in Hanks' solution containing 5  $\mu\text{Ci/ml}$  of [ $^3\text{H}$ ]leucine (specific radioactivity 27 Ci/mmol, Hungary) and 20  $\mu\text{g/ml}$  testosterone (from Searle, England).

Medium not containing the hormone was used as the control. After incubation the tissue was homogenized in the cold in physiological saline. The precipitation of protein and hydrolysis, followed by centrifugation, were carried out in 5% TCA. Protein was dissolved in 0.1 M NaOH. Radioactivity was determined on the SL-30 scintillation counter. The counting efficiency was 12%. The protein concentration was determined by a micromethod in tetraborate buffer, pH 9.0 [6], on the SF-4A spectrophotometer. The rate of protein synthesis was judged by the intensity of incorporation of labeled leucine per milligram protein in 30 min.

#### EXPERIMENTAL RESULTS

During incubation of the reproductive tract and muscles of rabbit fetuses at 18-22 days of intrauterine life in medium not containing testosterone no differences were observed in the rate of protein synthesis, judging by incorporation of [ $^3\text{H}$ ]leucine (Table 1). Only a small decrease in biosynthesis was observed with the age of the fetus. Testosterone increased the rate of protein synthesis. The intensity of leucine incorporation in tissues of the reproductive tract was always higher than in muscle tissue ( $P \leq 0.05$ ). The highest rate of protein synthesis was found on the 18th day of intrauterine development.

Incorporation of [ $^3\text{H}$ ]testosterone by anlagen of the reproductive tract of rabbit fetuses from the 18th through the 25th day of development was studied previously under similar conditions and with the same hormone concentrations in the medium [1]. It was found that although specific incorporation took place in 18-day-old fetuses, no selectivity was present relative to testosterone incorporation in the muscles discovered at subsequent stages of development. An increase in uptake of labeled testosterone on the 19th and 21st days of development preceded the beginning of morphogenesis of the different anlagen of the fetal reproductive system.

Comparison of these data shows that under the influence of the hormone an increase in the rate of protein synthesis (18th, 20th, and 22nd days) were accompanied by intensive incorporation of [ $^3\text{H}$ ]testosterone (19th, 21st, and 24th days) [1]. It can be tentatively sug-

gested that during this period of development synthesis of various proteins is accompanied by synthesis of receptor proteins which, in turn, enable subsequent binding of the androgen to a high degree.

Much evidence has now been obtained to show that steroid hormones stimulate synthesis of various proteins at not only the genomal, but also the extragenomal level [5]. The possibility cannot be ruled out that on the 18th day of development of rabbit fetuses testosterone exerts its action not only at the transcription level, bearing in mind the absence of selectivity in uptake of the hormone by anlagen, the high rate of protein synthesis in the muscles, and also the low level of entry of the hormone into the nucleus. Judging by the distribution of radioactivity in subcellular fractions after administration of labeled testosterone, most label is found in the cytoplasm [2].

Meanwhile on the 21st day of fetal development, when morphogenesis of anlagen of the reproductive tract begins and the hormone is concentrated mainly in the nucleus, and maximal uptake of testosterone also is observed, preceding an increase in the rate of protein synthesis (20th day), testosterone evidently exerts its action at the nuclear level.

The results thus indicate that testosterone increases the rate of synthesis of total protein in tissues of the reproductive tract in rabbit fetuses. Under these circumstances synthesis of the various cell proteins is probably accompanied by synthesis of receptor proteins, necessary for realization of the action of the hormone on morphogenesis of anlagen of the target organs, also.

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#### SOME MECHANISMS OF STIMULATION OF ERYTHROPOIESIS BY T LYMPHOCYTES DURING LOCAL IRRADIATION *IN VIVO*

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It was shown previously that during local irradiation of bone marrow many T lymphocytes accumulate in the depopulated hematopoietic tissue, where they selectively stimulate post-radiation regeneration of the erythron [2, 3]. In thymectomized mice, when the phenomenon of T-cell accumulation in the irradiated bone marrow is abolished, recovery of erythropoiesis is delayed [3, 5]. It is an interesting fact that postradiation regeneration of the erythron in athymic mice can be accelerated by transplantation of viable thymocytes into them [4].

The aim of the present investigation was to continue the study of mechanisms of stimulation of erythropoiesis by T cells after local irradiation *in vivo*.

#### EXPERIMENTAL METHOD

Experiments were carried out on 200 male BALB/c mice weighing 18-20 g (obtained from the "Rassvet" nursery, Tomsk). The mice were irradiated locally (right hind limb) in a dose of

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