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), testostreone 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 lymphocutes accumulate in the depopulated hematopoietic tissue, where they selectively stimulate postradiation 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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7.0 Gy on the RUM-17 x-ray apparatus (dose rate 0.65 Gy/min). Removal of the thymus or mock thymectomy was performed 1 month before irradiation. Viable thymocytes, thymus cells treated *in vitro* with antibrain serum (ABS) [6] and complement (C') or with control serum (CS) from nonimmunized rabbits, thymocytes labeled *in vivo* with [^{3}H]thymidine, and thymus cells treated *in vitro* with cytosar (from Upjohn, Belgium) or actinomycin D (From Serva, West Germany), in a dose of $4 \cdot 10^7$ cells, were transplanted intravenously into the thymectomized mice 4.5 days after local irradiation. ABS also was used as marker of T cells [6]. The titer of the ABS was 1:64. When ABS was used to treat thymus cells, 1.5 ml of whole ABS and 3 ml of complement (1:8) were added to 8 ml of medium 199 containing $4 \cdot 10^8$ thymocytes. The resulting suspension was incubated (37°C) for 30 min. Cytosar (100 µg/ml) was used to block DNA synthesis in the cells, and actinomycin D (10 µg/ml) to block RNA and protein synthesis. Suspensions of mouse thymocytes ($6 \cdot 10^6$ cells/ml) were cultured in medium 199 (Tomsk Research Institute of Vaccines and Sera), with the addition of 10% embryonic calf serum (USA) at 37°C for 4 h. To test the effectiveness of action of the blockers, washed thymocytes were incubated for 2 h in the presence of labeled precursors of DNA, RNA, and protein synthesis (from the "Izotop" Combine, USSR). The cells were then sedimented by centrifugation and the nucleoprotein complex (NPC) isolated from them [7]. The NPC was transferred into a dioxane scintillator. Radioactivity of the samples was measured on a Mark 3 (USA) liquid scintillation counter with a counting efficiency of about 40%. All the tests were duplicated. To obtain labeled thymocytes, [^{3}H]-thymidine was injected intraperitoneally into intact mice every 3-5 h for 2 days in a dose of 18.5 MBq/kg body weight. The number of labeled thymocytes on autoradiographs of the thymus in this case was 51.6 ± 6.7%. During preparation of the autoradiographs of the thymus and bone marrow, type M emulsion was used as the recording medium (from Moscow Technical Photographic Plate Factory). In all cases the thymocytes were washed twice before transplantation with medium 199, by centrifugation at 1000 rpm for 10 min. On the 5th or 6th day after irradiation the recipients were killed and the total number of myelokaryocytes counted in the bone narrow from the irradiated femora. The myelogram or percentage of labeled lymphoid cells was counted on films of bone marrow. The diameters of the lymphocytes were measured with the AM-9-2 ocular micrometer.

EXPERIMENTAL RESULTS

ABS had a marked cytotoxic action on the bone marrow cells in locally irradiated hematopoietic tissue of mice not undergoing operation, on the 5th day of the experiment (29 ± 3.1), evidence that bone marrow tissue contained many T lymphocytes. Accumulation of T cells (during the same period) in the depopulated bone marrow (27.6 ± 3.9) took place also in thymectomized mice, after receiving an injection of viable thymocytes. The cytotoxic index for bone marrow cells of intact animals was 1.2 ± 0.3%. Accumulation of T lymphocytes in irradiated hematopoietic tissue of thymectomized animals after transplantation of thymocytes into them was evidently not sufficient to make it possible to consider migration of lymphocytes of donor origin into the depopulated recipient's bone marrow. Direct proof that the injected cells accumulate actually in the locally irradiated bone marrow was obtained in experiments with labeled lymphocytes. For instance, on injection of a suspension of labeled thymocytes (the number of labeled cells was 34%) into locally irradiated thymectomized recipients, 6.4 ± 1.1% of labeled lymphoid cells was found on autoradiographs of the irradiated bone marrow 10 h after transplantation. The population of labeled cells in the bone marrow consisted of small lymphocytes with a diameter of 5.8 to 7.9 µ. The mean cell diameter of the whole lymphoid population of irradiated bone marrow on the 5th day after irradiation, in thymectomized mice injected with viable thymocytes, was 7.74 ± 0.04 µ and did not differ significantly from that in locally irradiated mice not undergoing operation (7.75 ± 0.05 µ) and also from the mean diameter of thymocytes of intact donors (7.72 ± 0.04 µ). It must be pointed out that single labeled lymphocytes also were found on autoradiographs of the screened bone marrow (0.72 ± 0.2%).

Injection of viable thymus cells into locally irradiated thymectomized mice stimulated regeneration of erythropoiesis (Table 1). Meanwhile thymus cells, treated *in vitro* with ABS and complement, has no such action. Treatment of thymocytes with cytosar did not affect their ability to stimulate postradiation regeneration of the erythron. Thymus cells treated with actinomycin D has a much weaker stimulating effect than intact thymocytes.

Data showing the effectiveness of blocking of DNA, RNA, and protein synthesis in the thymocytes by the preparations studied are given in Table 2. Cytosar and actinomycin D inhibited synthesis of DNA and RNA respectively virtually completely in the cells. Although

TABLE 1. Effect of ABS, Cytosar, and Actinomycin D on Ability of Thymocytes to Stimulate Erythropoiesis in Locally Irradiated Thymectomized Mice (M ± m)

Group of irradiated animals	Number of animals irradiated	Transplanted platelets	P	Absolute number of erythroid cells on 6th day after irradiation in irradiated bone marrow, $\times 10^6$
Not undergoing operation	10	—	<0,001	9,89±1,23
Undergoing mock operation	9	—	<0,001	9,18±1,14
Thymectomized	11	—	—	1,98±0,31
	9	Untreated	<0,001	9,62±1,12
	8	ABS+C′	>0,02	2,56±0,40
	9		<0,001	9,70±1,41
	9	CS+C′	<0,001	8,91±1,20
	10	Cytosar	<0,001	9,43±1,15
	10	Actinomycin D	<0,01	4,11±0,71

Legend. P) For comparison of group of thymectomized mice not receiving thymocytes with other groups of animals.

TABLE 2. Effectiveness of Blocking of DNA Synthesis in Thymocytes by Cytosar and RNA and Protein Synthesis by Actinomycin D

Test	Mean relative radioactivity of sample, cpm $\times 10^6$ cells	Percentage inhibition of incorporation
Thymidine — control	2622	—
Thymidine — cytosar	140	95
Uridine — control	3007	—
Uridine — actinomycin D	68	98
Amino acids — control	112	—
Amino acids — actinomycin D	99	12

Legend. In all cases the precusors were added in a quantity of 0.08 MBq/ml; specific activity (in GBq/mmole): thymidine-5(methyl-$^3H_1$) 925; D,L-alanine-2-$^3H_1$ 222; D,L-lysine-4,5-$^3H_2$ 407.

actinomycin D, on incubation with thymocytes for 4 h, depressed protein synthesis in them only very slightly, it can be postulated that normal functioning of T regulator cells requires preservation of synthesis not only of RNA, but also of protein. Actinomycin D, which inhibits transcription processes, can inhibit protein synthesis in the cell [1].

Hence, during local irradiation, many T lymphocytes accumulate in the depopulated bone marrow in animals not subjected to thymectomy, and accelerated postradiation regeneration of the erythron, but they did not accumulate in thymectomized mice. A similar effect (stimulation of erythropoiesis) also was observed after transplantation of viable thymocytes into thymectomized animals. It was shown that small thymocytes migrate directly into locally irradiated bone marrow. It can also be postulated on the basis of these findings that the regulatory function of T cells is not directly linked with DNA synthesis in them. To exhibit a stimulating effect, processes of RNA and, evidently, of protein synthesis must be preserved in the cells.

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