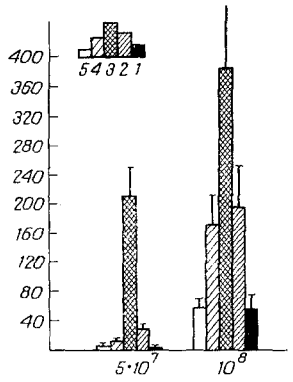

Fig. 3. Height of antibody response in CBA, C57BL/6, and BALB/c mice and in (CBA × C57BL/6)F_1 and (CBA × BALB/c)F_1 hybrids to intramuscular injection of SRBC. 1) C57BL/6; 2) (CBA × C57BL/6)F_1; 3) CBA; 4) (CBA × BALB/c)F_1; 5) BALB/c.

The results suggest that the weakly responding strains of mice investigated in these experiments possess a certain factor which is manifested within the zone of low immunizing doses of SRBC and is dominant in F_1 hybrids; the most likely cellular substrate of this factor, in the writers' view, is the macrophages. Direct proof of the role of macrophages is the manifestation of the immunologic phenomena described above may be obtained in experiments with transfer of macrophages of opposite parental lines to first generation hybrids.

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EFFECT OF THYMOSIN ON SPLENIC EXOCOLONY FORMATION IN ALLOGENEIC AND SYNGENEIC SYSTEMS

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Investigation of the role of the thymus in splenic colony formation probably ought not to be confined to an examination of the parts played by thymus-dependent cells only in this process, because the thymus not only produces T-lymphocytes but also secretes a humoral factor (or more than one). This factor, which is most frequently called thymosin, as the results of recent investigations have shown, plays an important role in the proliferation and, in particular, the differentiation of thymus cells [1]. In previous investigations the writers used two models with which to study the role of thymus cells in splenic colony growth. It was shown previously that injection of the synthetic polyribonucleotide polyI:polyC into recipient mice (F_1 hybrids) simultaneously with donor's bone marrow (C57BL mice) significant-

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TABLE 1. Abolition of Phenomenon of Allogeneic Inhibition of Colony Growth by Thymosin (M ± m)

Treatment of bone narrow	Additional treatment	Number of colonies	P
Incubation with thymosin	—	2,2±0,5 (45)	
0,6 μg/ml	—	1,8±0,4 (8)	
6 μg/ml	—	1,8±0,5 (13)	
60 μg/ml	—	6,3±0,9 (29)	<0,001
100 μg/ml	—	5,5±0,7 (18)	<0,001
200 μg/ml	—	4,2±0,7 (37)	<0,005
Without treatment	Thymocytes $3 \cdot 10^7$	4,8±0,3 (8)	<0,01
	Thymosin (intravenously):		
	30 μg	3,6±0,6 (23)	
	50 μg	2,3±0,5 (18)	
	100 μg	1,5±1,0 (15)	

Legend. Here and in Table 2, mean number of colonies given per 10^5 injected bone marrow cells; number of animals given in parentheses.

TABLE 2. Effect of Thymosin on Exogenous Splenic Colony Formation from Bone Marrow Treated with Anti-Q-Serum (M ± m)

Additional treatment	Number of colonies	P
—	11,1±1,1 (16)	
—	1,8±0,4 (17)	
Thymocytes $2 \cdot 10^7$	8,5±0,9 (11)	<0,001
Thymosin:		
0,6 μg/ml	2,9±1,0 (10)	
6 μg/ml	3,5±0,5 (12)	<0,05
60 μg/ml	4,1±0,3 (14)	<0,01
100 μg/ml	4,0±0,75 (10)	<0,05
200 μg/ml	2,6±1,0 (8)	
30 μg intravenously	1,5±1,0 (10)	
100 μg intravenously	1,2±0,3 (11)	

ly increases the number of exogenous splenic colonies from the parental bone marrow, i.e., administration of this preparation gave an effect similar to that of thymocytes [3]. Second, a syngeneic system in which cells of the bone marrow to be injected into the recipients were treated *in vitro* with anti-Q-serum, which led to sharp inhibition of the yield of splenic exocolonies. Colony formation was restored almost to the control level by additional injection of intact thymus cells together with the donor's bone marrow [2]. In other words, in both allogeneic and syngeneic systems the thymus had definite ability to restore processes of normal colony formation.

On the basis of these data, in the investigation described below an attempt was made to study the role of the thymus factor (thymosin) in splenic colony formation, by using the two types of system mentioned above.

EXPERIMENTAL METHOD

Male (CBA × C57BL)F_1 and C57BL mice aged 2.5-3 months were used. The recipients were irradiated in a dose of 850 rads on the Luch-1 radiotherapeutic apparatus with a dose rate of about 60 rads/min. The colony-forming ability of the bone marrow was studied by the method of splenic exocolonies [7].

Rabbit immune serum (antibrain) against mouse θ-antigen was obtained by the method described in [5] and used in a working dilution of 1:3.

Thymosin — the purified 5th fraction — was obtained from the All-Union Research Institute of Technology of Blood Substitutes and Hormonal Preparations, by Goldstein's method [4].

A suspension of bone marrow cells containing 2×10^7 cells/ml was incubated for 1 h at 37°C with serum against mouse θ-antigens in the following proportions by volume: 0.1 ml suspension, 0.1 ml serum in the corresponding dilution, 0.1 ml medium No. 199 (in the control — 0.1 ml suspension and 0.2 ml medium No. 199). Intact thymus cells were injected intravenously into recipient mice in a dose of 2×10^7 cells per mouse (in 0.5 ml).

To prevent embolism the mice were given 15 units of heparin intraperitoneally 15-20 min before injection of the thymus cells.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of thymosin on the possibility of abolition of the phenomenon of allogeneic inhibition of colony growth was investigated. First, and attempt was made to increase the number of splenic colonies from allogeneic bone marrow by an intravenous injection of thymosin immediately after the bone marrow suspension (by analogy with polyI:polyC). The writers suggested previously [3] that the effect of the polyribonucleotide on abolition of the phenomenon of allogeneic inhibition of colony growth was associated either with replacement of the thymus cells by the polyribonucleotide in cooperative processes or with an effect analogous to the action of thymus hormones. However, it was discovered that intravenous injection of thymosin does not abolish the allogeneic inhibition phenomenon. Accordingly, the scheme of the experiment was modified. A suspension of parental bone marrow cells was made up in medium No. 199 or in a solution of thymosin of definite concentration. The suspensions were then incubated for 30 min at 37°C. It will be clear from Table 1 that incubation of allogeneic bone marrow with thymosin led to an increase in the number of exogenous splenic colonies compared with the control. A solution containing 60 μg/ml was particularly effective. Incubation of bone marrow in such a solution led to an almost threefold increase in the yield of colonies compared with the control. In the syngeneic combination, on the other hand, incubation of bone marrow cells in thymosin solution (60 μg/ml) did not change the number of splenic exocolonies (12.6 ± 1.6 in the control, 13.8 ± 0.5 after incubation with thymosin). Thymosin is thus a highly effective preparation for abolishing the phenomenon of allogeneic inhibition of colony growth, but only after preliminary incubation of cells of the donor's bone marrow in thymosin solution.

How can the effect of thymosin be explained in this case? We know that thymosin can restore the immunocompetence of spleen cells in neonatally thymectomized mice, i.e., that it can to some extent take over the function of the thymus [8]; approximately the same action, according to data in the literature, is given by polyI:polyC [6]. These substances also have similar effects on abolition of the allogeneic inhibition phenomenon. Very probably the action of both thymosin and polyI:polyC in an allogeneic system can be attributed to their effect on cyclic AMP formation, for both polyribonucleotides and thymosin are known to be very effective stimulators of adenylate cyclase activity [8, 9].

In the experiments of series II bone marrow suspension was incubated in a syngeneic system with anti-θ-serum, then washed by centrifugation in medium No. 199 in the cold at 1500g for 10 min. After washing, the bone marrow cells were resuspended in 10 ml thymosin solution (in medium No. 199) in concentrations of 0.6, 6, 60, 100, and 200 μg/ml; incubation was then repeated for 30 min at 37°C. After incubation, this bone marrow suspension was injected intravenously into irradiated recipients without preliminary washing in a dose of 0.5 ml. Table 2, which gives the results of these experiments, shows that incubation of bone marrow cells, previously treated with anti-Q-serum, with thymosin in concentrations of 6, 60, and 100 μg/ml led to an increase in the yield of exogenous splenic colonies compared with the control. However, the action of thymosin was only half as strong as the action of a suspension of thymus cells. It is an interesting fact that, just as in the case of allogeneic inhibition, only incubation of the bone marrow cells with thymosin was effective as regards increasing colony formation. Intravenous injection of thymosin had no positive action.

The results suggest that not only thymus cells, but also the humoral thymus factor play a definite role in processes of colony formation.

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