The rapid effect of dexamethasone on the circulating CFUs pool can be linked with a redistributive mechanism by analogy with that for lymphocytes [3].

The results are further evidence of the regulatory role of glucocorticoids in the migration and recirculation of CFUs.

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EFFECT OF *Mycoplasma arthritidis* ON RECOVERY OF ERYTHROPOIESIS IN MICE AFTER ADMINISTRATION OF 5-FLUOROURACIL

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Experimental infection of mice with *Mycoplasma arthritidis* is accompanied by stimulation of endogenous colony formation in mice irradiated in a sublethal dose [1, 4] and contributes to the more rapid regeneration of erythropoiesis in plethoric mice [5] and also in mice which have previously been given repeated injections of small doses of actinomycin D [2]. The problem of the nature of the target cells for *M. arthritidis* and the mechanism of its action on hematopoietic cells remains unsolved.

It was therefore decided to study the action of *M. arthritidis* on recovery of erythropoiesis in mice in which hematopoiesis was disturbed as a result of a single injection of a sublethal dose of the cycle-specific cytostatic 5-fluorouracil (5-FU). It was postulated that if *M. arthritidis* affects the proliferative state of hematopoietic cells, its injection at different times relative to 5-FU ought to lead to significant changes in the time course of recovery of erythropoiesis after the action of the cytostatic.

EXPERIMENTAL METHOD

Experiments were carried out on (C57BL/6 × A/Sn)F_1 mice weighing 20-22 g, obtained from the Rappolovo Nursery, Academy of Medical Sciences of the USSR. *M. arthritidis* was obtained as described previously [3]. Mice were infected intraperitoneally with *M. arthritidis* in a dose of 10^8 CFU in 0.5 ml physiological saline. The 5-FU (from Sigma, USA) was injected intraperitoneally in a dose of 150 mg/kg.

To estimate erythropoiesis quantitatively, ^{59}Fe citrate (specific activity 0.2 mCi/ml) was injected into mice in a dose of 0.5 μCi in 0.5 ml physiological saline. The mice were

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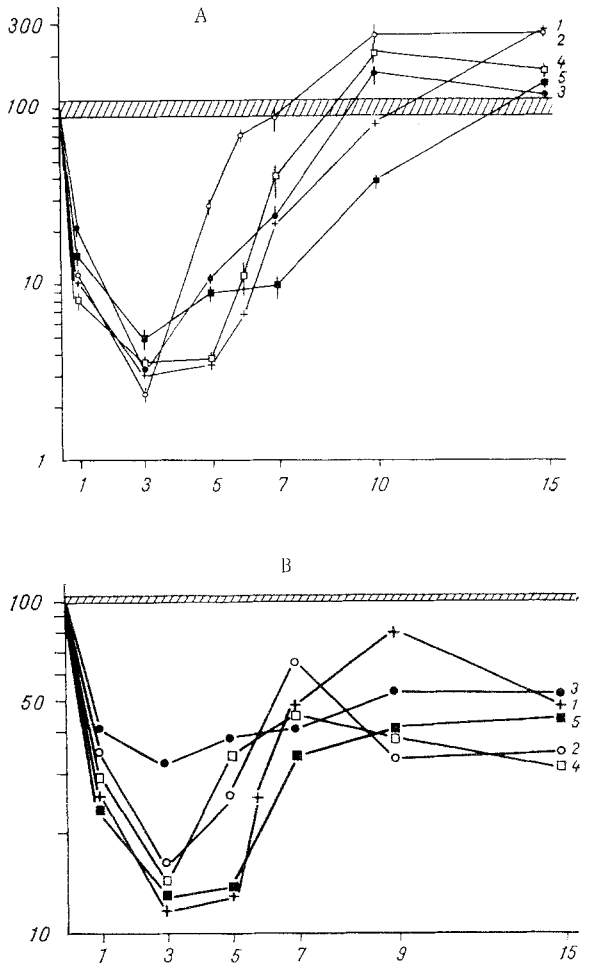

Fig. 1. Dynamics of incorporation of ^{59}Fe into erythroid cells after injection of 5-FU. A) Spleen; B) bone marrow. Abscissa, time (in days) after injection of 5-FU; ordinate, incorporation of ^{59}Fe (in % of control). 1) 5-FU; 2) *M. arthritidis* 24 h after 5-FU; 3) *M. arthritidis* 4 h after 5-FU; 4) *M. arthritidis* 4 h before 5-FU; 5) *M. arthritidis* 24 h before 5-FU. Short vertical lines in Fig. 1a indicate confidence interval (P = 0.05).

killed 6 h after injection of the isotope and incorporation of the label into bone marrow and spleen was measured on a gamma counter (Nuclear Chicago, USA).

The number of cells in the bone marrow and spleen was counted in a hemocytometer.

EXPERIMENTAL RESULTS

Incorporation of ^{59}Fe into the spleen 24 h after injection of 5-FU was reduced by 90%, and reached a minimum (3%) on the 3rd day after injection of the cytostatic (Fig. 1A). Incorporation of the isotope remained stable during the next 2 days, and began to rise after the 6th day. On the 10th day incorporation of ^{59}Fe was almost back to the control level, and this was followed by an overshoot.

Injection of *M. arthritidis* 24 h after 5-FU led to more rapid regeneration of erythropoiesis in the spleen. Starting from the 5th day the level of incorporation of the label was significantly higher than that in the spleen of mice receiving 5-FU alone (Fig. 1A). On the 7th day incorporation of the isotope had regained its normal level, after which an overshoot also was observed. If *M. arthritidis* was injected 4 h after 5-FU the stimulating effect was much weaker.

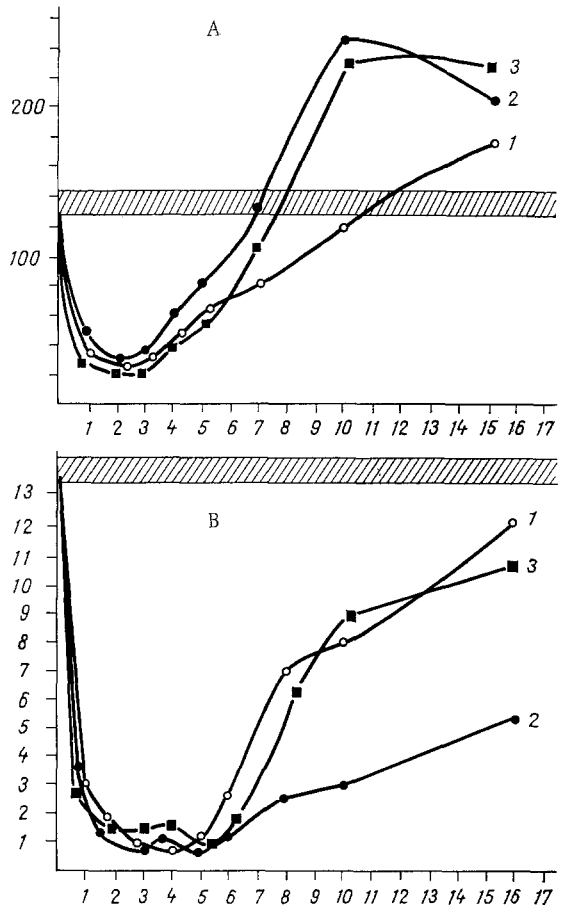

Fig. 2. Changes in number of nucleated cells in spleen (A) and bone marrow (B) of mice at different times after injection of 5-FU. Ordinate, number of cells (millions). 3) *M. arthritidis* given 4 h before 5-FU. Remainder of legend as to Fig. 1.

If *M. arthritidis* was injected 24 h before 5-FU the level of incorporation of ^{59}Fe into the spleen was restored more slowly than in mice receiving 5-FU alone. If the mycoplasma was injected 4 h before the cytostatic, regeneration of erythropoiesis was delayed compared with animals receiving 5-FU alone.

The time course of recovery of erythropoiesis after 5-FU alone and together with *M. arthritidis* was similar in the bone marrow (Fig. 1B).

The time course of the change in cell composition of the spleen was comparable with that of ^{59}Fe uptake (Fig. 2A). The number of cells in the spleen 1-3 days after injection of 5-FU reached a minimum, about 30% of the control level. The cell composition of the spleen was almost restored on the 10th day, after which an overshoot occurred. When *M. arthritidis* was injected 4 h before the cytostatic and, in particular, when injected 24 h after 5-FU, the overshoot was observed considerably earlier and the splenomegaly lasted 1 week.

An increase in the cell population in the bone marrow of mice receiving 5-FU alone was observed starting from the 6th day, but regeneration was not yet complete by the 15th day (Fig. 2B). A similar time course was observed for bone marrow cells of mice receiving *M. arthritidis* 4 h before 5-FU. If the mycoplasma was injected 24 h after the cytostatic the cell population in the bone marrow was restored after a long delay.

The writers showed previously that *M. arthritidis* stimulates erythropoiesis in mice irradiated in a sublethal dose [1, 4]. The maximal stimulating effect was observed when the mycoplasma was injected 24 h before irradiation, but a stimulating effect also was observed when *M. arthritidis* was injected 4 h or even 24 h after irradiation. This last feature distinguishes *M. arthritidis* from most other biological factors stimulating endogenous colony

formation. Such a strong stimulating agent as bacterial endotoxin is ineffective when injected into mice 4-24 h after irradiation [8]. If, however, the endotoxin was injected 4 h after 5-FU, regeneration of hematopoiesis and, in particular, restoration of the population of colony-forming cells (CFUs) was sharply accelerated; conversely, injection of endotoxin 24 h before 5-FU was followed by marked inhibition of regeneration of hematopoiesis [12]. 5-FU is known to damage chiefly proliferating cells, but hematopoietic cells outside the cycle are evidently not affected by the cytostatic [7, 10]. The effect of endotoxin has been explained by its ability to trigger the entry of resting CFUs into the cell cycle [12]. In the present experiments stimulation of ^{59}Fe incorporation into the spleen was observed not only when the mycoplasma was injected 24 h after 5-FU, but also when it was injected 4 h before the cytostatic. It is not yet clear how this effect can be explained. *M. arthritidis* contributes to the more rapid recovery of hematopoiesis in mice irradiated in a lethal dose [1], evidently by increasing the proliferative pool of hematopoietic cells; possibly only a small proportion of cells commences the cycle 4 h after injection of mycoplasma, and they thereupon die under the influence of 5-FU. The remaining cells evidently start to proliferate after the action of the cytostatic has ceased, and this could account for stimulation of incorporation of ^{59}Fe into the spleen. When the mycoplasma was injected 24 h before 5-FU, most of the cells had already started the cycle and died after injection of the cytostatic, thus leading to delayed regeneration of erythropoiesis. No answer can yet be given on the basis of these data to the question whether true stimulation of erythropoiesis takes place in the spleen under the influence of *M. arthritidis* injected 24 h after 5-FU, or whether the increased incorporation of ^{59}Fe into heme of the erythroid cells of the spleen reflects increased liberation of cells from the bone marrow. Evidence that the last mechanism is possible is given by the marked decrease in incorporation of the isotope into bone marrow erythroid cells of mice infected with *M. arthritidis* at times when incorporation of ^{59}Fe is maximal.

Exogenous erythropoietin, injected into mice at different times after 5-FU, also stimulates restoration of erythropoiesis, as shown by the increased concentration of radioactive iron in the peripheral blood [10, 11]. However, the mechanism of action of *M. arthritidis* evidently differs from that of the stimulating action of erythropoietin, for *M. arthritidis* can stimulate erythropoiesis in plethoric mice, in which erythropoietin production is temporarily interrupted [5], and also in mice in which erythropoiesis is blocked as a result of repeated injections of actinomycin D, i.e., under experimental conditions when exogenous erythropoietin is ineffective [2]. This last fact, and also its ability to stimulate endogenous colony formation when injected 4-24 h after irradiation of mice, distinguish the action of *M. arthritidis* from that endotoxin [1, 4]. Considering the ubiquitousness of the distribution of mycoplasmas and the diversity of the pathology produced by them, it must be concluded that elucidation of the mechanism of action of mycoplasmas on hematopoietic cells will provide a better understanding of the nature and the distinguishing features of their complex relationships with the carrier organism.

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