EFFECT OF POLY-4-VINYLPYRIDINE ON THE NUMBER OF COLONY-FORMING CELLS IN MOUSE BONE MARROW AND SPLEEN

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Injection of poly-4-vinylpyridine in the maximal tolerated dose into mice doubles the number of colony-forming units (CFU) in the bone marrow and reduces the number of nucleated cells by 22%. The increase in the CFU pool takes place from the second through the seventh day. The number of bone marrow cells starts to decrease 2 h after injection of the polymer and remains below the initial level for 5 days; after the second day, however, it starts to return gradually to normal, which it reaches by the seventh day. The number of CFU in the spleen increases ninefold, and this is accompanied by the development of marked splenomegaly on account of the increase in the number of cells.

KEY WORDS: *synthetic polymer; colony-forming units; bone marrow; spleen.*

A number of common manifestations can be distinguished in the reaction of synthetic ionogenic polymers on mammals, namely the effect on hematopoiesis and immunogenesis, as well as some specific reactions. The participation of poly-4-vinylpyridine (P4VP) in the immune response of the organism has been demonstrated [3]: P4VP enhances cooperation between T and B lymphocytes in the immune response and replaces the function of the T cells in B mice [4]. It facilitates migration of colony-forming units (CFU) from the region of bone marrow screened during irradiation [1]. Many other aspects of the action of P4VP on hematopoiesis still await investigation, including the response of hematopoietic stem cells, which are ascribed the leading role in this process.

The investigation described below was undertaken to assess the action of P4VP on the number of CFU (stem cells) in the bone marrow and spleen of mice.

EXPERIMENTAL METHOD

Experiments were carried out on 950 (CBA × C57BL)F_1 hybrid mice. The weight of the donor mice was 20-22 g and of the recipient mice about 25 g. The donor mice (130 animals) received an intravenous injection of P4VP with a mean coefficient of polymerization of 10^3 (mol. wt. 100,000) in a dose of 100 mg/kg body weight. An isotonic solution in 0.5% acetic acid with the addition of 0.5% sodium chloride was used for injection. At different times after injection of the polymer solution the mice were decapitated and the number of nucleated cells counted in their spleen and femoral marrow. To determine the CFU, suspensions of bone marrow and spleen cells prepared under sterile conditions in medium No. 199 and diluted to a definite concentration were injected intravenously into recipient mice irradiated in a lethal dose (^{60}Co, 875 R, dose rate 220-290 R/min) shortly before transplantation.

On the ninth day after transplantation the number of colonies growing in the recipients' spleens was counted and, allowing for the injected dose of cells, the number of CFU was calculated in the donor's spleen and in the femur, using the method described earlier [14]. The calculated number of CFU is that fraction of the cells which settles in the spleen and it amounts to 17% of the total number of cells of this type [13]. Three repetitions of

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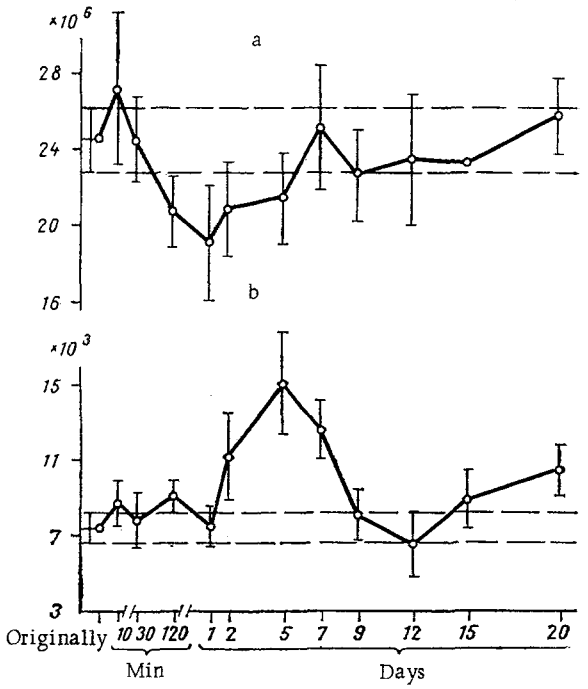

Fig. 1

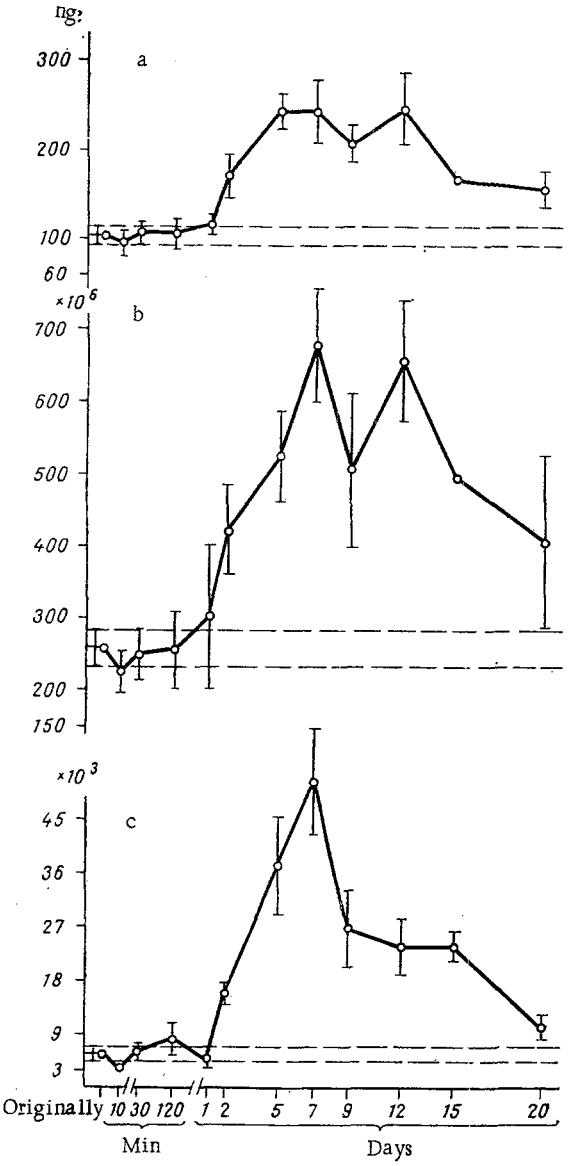

Fig. 2

Fig. 1. Changes in number of nucleated cells and CFU in bone marrow of mice receiving P4VP. Abscissa: time after injection of preparation; ordinate: a) number of nucleated cells in femoral marrow; b) number of CFU in femoral marrow. Vertical lines show confidence limits (P = 0.05); broken lines indicate range of variations under normal conditions.

Fig. 2. Changes in weight of spleen and number of nucleated and colony-forming cells in mice receiving P4VP. Abscissa: time after injection of preparations; ordinate: a) weight of spleen (in ng); b) number of nucleated cells in organ; c) number of CFU. Vertical lines indicate confidence limits (P = 0.05); broken lines show range of variations under normal conditions.

experiment were carried out and the results were analyzed together. The significance of differences between the indices was assessed by the t test.

EXPERIMENTAL RESULTS

Injection of P4VP led to changes in the number of bone marrow cells: After 2 h it was 16% below its initial level, and 22% below after 24 h. It was back to normal again on the seventh day (Fig. 1a). Among bone marrow cells which responded to injection of P4VP, the CFU are of special importance. Whereas the total number of bone marrow cells decreased, the number of CFU increased, and three successive waves of increase were observed: after 2 h,

on the second to seventh day, and on the 20th day (Fig. 1b). After 2 h the number of CFU rose from 7500 to 9000. The longest and most marked (by 1.5-2 times) increase in the number of CFU took place from the second through the seventh day. The dynamics of the CFU was not studied after 20 days.

Injection of P4VP induced splenomegaly. An increase in weight of the spleen was observed after 2 days. The maximal weight of the spleen was 2.3 times greater than its initial weight (Fig. 2a). The number of splenocytes exceeded its initial level for 3 weeks, by the greatest degree (2.5 times) in the period from the 7th to the 12th days (Fig. 2b). Determination of the number of nucleated cells showed that 1 mg spleen tissue of intact mice contained 2.6 ± 0.1 million cells. In mice receiving P4VP this figure was not substantially changed, i.e., the weight of the organs was increased on account of an increase in number of cells.

Simultaneously with the increase in the total number of cells in the spleen, the number of CFU increased. By the seventh day it had risen from 6000 to 51,000. On the ninth day the number of CFU had fallen to half its maximal value. Subsequent restoration of the normal number of CFU took place gradually, over a period of 2 weeks (Fig. 2c). It should also be noted that the reaction of CFU in the spleen to injection of the polymer had a transient primary phase: The number of CFU in the organ 10 min after injection of P4VP was reduced by 2500.

These experiments thus showed that P4VP affects the number of CFU in the bone marrow and spleen. When the results were analyzed, an attempt was made to answer two questions which are of greatest interest: 1) Is the action of the polymer on the stem cells direct, and 2) do the bone marrow cells participate in the development of splenomegaly?

To judge from the indirect evidence, intermediate mechanisms can be demonstrated in the action of the polymer on the stem cell pool. The increase in the pool was preceded (by about 46 h) by a decrease in the number of cells in the bone marrow. The response of the stem cell pool to injection of the polymer could evidently be induced by natural regulators of hematopoiesis, activated in response to the decrease in the cell population in the bone marrow. A definite role in the regulation of the stem cell pool [9, 10] and the blood leukocytes [6, 7] is ascribed to the plasma proteins and, in particular, to complement and interferon, the concentrations of which change after administration of certain natural and synthetic polymers [8, 12].

When the second question is answered it must be remembered that the reaction of the stem cells on the bone marrow compared with the change in CFU in the spleen was less marked and shorter in duration. Furthermore, the appearance of a surplus number of CFU in the bone marrow was not accompanied by any overproduction of hematopoietic tissue cells such as occurred in the spleen. The CFU of the bone marrow can evidently choose other pathways of development, such as migration or maturation by a shortened cycle. In this connection it should be pointed out that mainly semistem cells [11], i.e.. committed CFU, participate in the process of migration. Intensification of the process of CFU migration into the spleen during the first few hours after injection of P4VP or of endotoxin has been established for an endogenous model [1] and a system *in vitro* [11], respectively. In investigations by other workers [2, 5] an increase in the growth of endogenous colonies after injection of polysaccharides or synthetic polymers was explained differently, allowing for changes in the fixing properties of the spleen with respect to circulating cells. In the present investigation, when an exogenous model was used, migration of CFU into the spleen was not found. In fact, CFU accumulate simultaneously in the organs. However, it must be borne in mind that the endogenous model is more suitable for the assessment of migration than the exogenous model, for it enables the migration even of single cells to be recorded. Consequently, after injection of P4VP, migration of CFU into the spleen could take place, but within narrow limits.

As regards the other mechanism responsible for the increase in the number of CFU, namely proliferation of cells in the spleen, it must evidently be accepted as the dominant mechanism in the later stages, when the rate of accumulation of CFU corresponded clearly to the standard model of development of the splenic colony.

The stem cell pool of the body can thus be significantly modified (increased) with the aid of P4VP.

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STATE OF THE PITUITARY–THYROID SYSTEM IN PREGNANT RABBITS, FETUSES, AND NEWBORN RABBITS

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The functional state of the pituitary–thyroid system was studied in pregnant rabbits and in their fetuses during the last third of pregnancy. Between the 23rd and 27th day of pregnancy the uptake of ^{131}I and the intensity of hormone synthesis in the thyroid glands of mother and fetus were increased; this, together with changes in the histological structure of the gland, was evidently connected with an increase in the thyroxine requirement at this period in the mother + fetus system. The above-mentioned changes in the mother were preceded by increased thyrotropic function of the pituitary, but no such correlation was found in the fetus. The results are evidence that the functional activity of the maternal pituitary–thyroid system changes rapidly in accordance with the body's requirements of thyroid hormone.

KEY WORDS: *pregnancy; fetus; thyroid gland; pituitary gland.*

This investigation consists of an analysis of the function of the pituitary–thyroid gland system of the mother and fetus in rabbits.

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