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## Hematopoietic Changes in Mice Following *Bordetella pertussis* Vaccine.* (31837)

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Injection of *B. pertussis* vaccine into mice causes marked hematologic changes which include hyperleukocytosis, depletion of small lymphocytes from lymphatic tissues, increased numbers of polymorphonuclear leukocytes in the splenic red pulp, and splenomegaly(1). Although both the spleen and the bone marrow were examined in the aforementioned study, no erythropoietic changes were noted in these organs. In light of the well-known ability of certain bacteria to stimulate splenic erythropoiesis in laboratory animals(2,3), this problem was reinvestigated with the use of morphologic and isotopic technics.

*Methods.* Adult, male CF#1 mice (Carworth, Inc., New City, N.Y.) weighing 25-30 g were housed individually and were fed a standard laboratory ration and water. Mice were injected intraperitoneally with 0.3 ml *B. pertussis* vaccine (Parke-Davis and Co., Detroit, Mich.) which contained approximately $18 \times 10^9$ killed bacteria in 0.9% NaCl solution with 0.01% thimerosal as a preservative. Control mice were injected intraperitoneally with 0.3 ml sterile, nonpyrogenic saline (McGaw Lab., Inc., Milledgeville, Ga.).

Touch preparations of fresh spleen and bone marrow were stained with a Wright-Giemsa mixture; histologic sections of spleen were stained with hematoxylin and eosin. Mice were injected subcutaneously with 0.1 $\mu$c $Fe^{59}$ (E.R. Squibb and Sons, New Brunswick, N.J.) and its uptake measured in bone marrow and spleen(4). Quantitative studies of the local peritoneal fluid cellular response were made with the aid of an electronic cell counter(5). Circulating erythrocyte concentrations were determined with an electronic cell counter. Reticulocytes were stained with new-methylene blue.

*Results.* Seven days following injection of the vaccine, splenic weight was 320 $\pm$ 6 mg (M $\pm$ S.E.) as compared to 104 $\pm$ 3 mg in saline-injected control mice. Spleen sections revealed a marked decrease in white pulp and hyperemia in the red pulp. Cytologic examination of spleen prints disclosed the presence of numerous foci of developing nucleated erythroid and myeloid cells, their numbers far exceeding those seen in control animals (Fig. 1-3). In contrast to the increased splenic erythropoiesis, the fresh tibial and femoral bone marrow of vaccine-injected mice was grossly yellow as compared to the normal pink appearance seen in all the control animals, and the percentage of nucleated erythroid cells in bone marrow of vaccine-treated mice was only 6 $\pm$ 0.7% as compared to 19 $\pm$ 2.0% in control mice. Nine mice were examined in each group. Granulopoiesis appeared to be stimulated in the vaccine-injected mice as evidenced by the preponderance of myeloblasts and myelocytes, and the frequent appearance of mitosis in these cells.

Peritoneal fluid studies gave further indication of an intense leukocytic stimulation caused by the vaccine (Table I). Although 7 days had elapsed, neutrophils were still abundant. There were many enlarged macrophages containing phagocytic vacuoles; some were in mitosis. Cells resembling plasmocytes were seen and this cell type was never observed in control mice.

Ferrokinetic studies confirmed and extended the morphologic data dealing with

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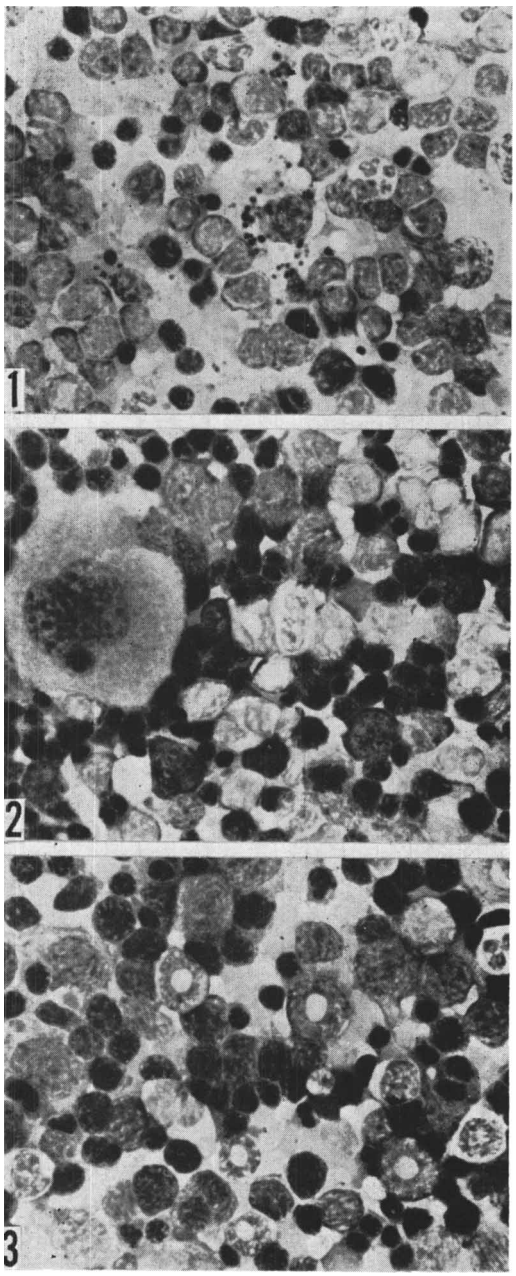

FIG. 1-3. Spleen prints. Red pulp. Stained with Wright-Giemsa; pH 6.6. Enlarged 560 times.

FIG. 1. Saline control. Moderate numbers of darkly stained nucleated erythrocytes, primarily normoblasts, are scattered throughout the red pulp of the spleen.

FIG. 2. *B. pertussis* vaccine. Clusters of darkly stained nucleated erythrocytes in all stagees of development predominate. Myelocytes with ring shaped nuclei are also present and a megakaryocyte appears at the left.

FIG. 3. *B. pertussis* vaccine. Nucleated erythroid cells predominate. Increased numbers of myelocytes are also seen.

erythropoiesis (Fig. 4). Splenic $Fe^{59}$ uptake increased about 4-fold following vaccine injection whereas bone marrow $Fe^{59}$ uptake was severely depressed. In addition, $Fe^{59}$ uptake into circulating erythrocytes was significantly increased in the vaccine-injected mice.

There was no significant change in the concentrations of circulating erythrocytes after 7 days whereas there was a doubling in reticulocyte percentages (Table II).

TABLE I. Effects of a Single 0.3 ml Injection of *B. pertussis* Vaccine on Peritoneal Fluid Cellularity After 7 Days (Means ± standard error of means).

| | Saline | *B. pertussis* vaccine |
|---|---|---|
| Number of mice | 9 | 9 |
| Peritoneal fluid cells: | | |
| Total leukocytes, millions | 6.4 ± .7 | 9.1 ± .6* |
| Neutrophils, " | .2 ± .01 | 4.0 ± .8* |
| Mononuclears, " | 5.9 ± .1 | 4.9 ± .3 |
| Eosinophils, " | <.2 | <.2 |

* P <.01.

TABLE II. Effects of a Single 0.3 ml Injection of *B. Pertussis* Vaccine on Circulating Erythrocytes After 7 Days (Means ± standard error of means).

| | Saline | *B. pertussis* vaccine |
|---|---|---|
| Number of mice | 9 | 9 |
| RBC/mm³ (millions) | 8.6 ± .2 | 8.2 ± .1 |
| Reticulocytes (%) | 1.6 ± .1 | 3.2 ± .2* |

* P <.01.

*Discussion.* In contrast to the findings reported by Morse in NCS mice(1), these studies indicate that a single injection of *B. pertussis* vaccine causes a marked increase in splenic erythropoiesis and granulopoiesis accompanied by a severe inhibition of erythropoiesis in the bone marrow of CF#1 mice. It is, therefore, possible that strain differences exist. These effects are similar to those seen following an injection of a bacterial lipopolysaccharide(4). The cause of these erythropoietic changes is obscure, but it may be related to the intense leukocytic stimulation generated by the vaccine. It is suggested that when large numbers of stem cells

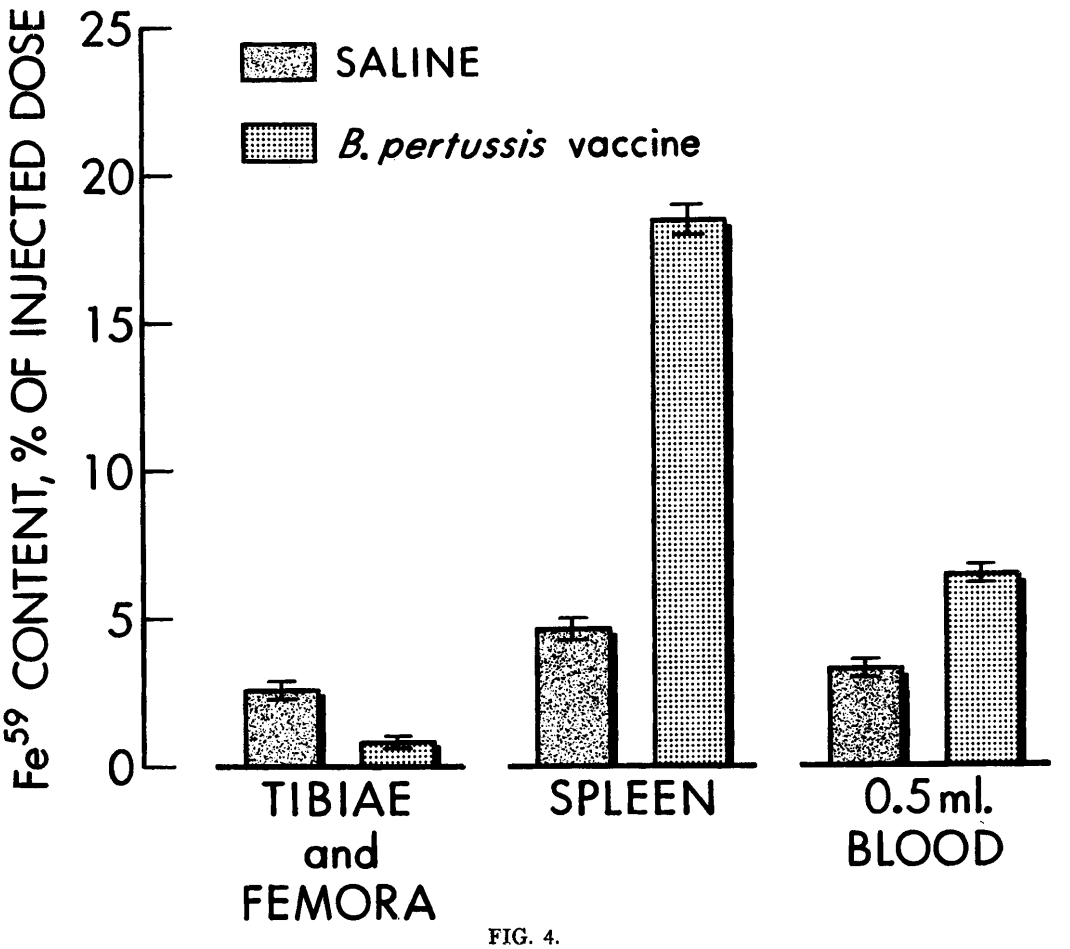


FIG. 4.

in the bone marrow are induced to develop into leukocytes, a temporary shunting of erythropoiesis from the bone marrow to the spleen occurs.

*Summary.* Cytologic and ferrokinetic studies in mice following a single injection of *B. pertussis* endotoxin reveal a marked increase in splenic erythropoiesis accompanied by a decrease in bone marrow erythropoiesis. It is suggested that the intense leukocyte stimulation noted at the site of injection as well as in the bone marrow and spleen, may be responsible for this shift in erythropoiesis.

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