

THE EFFECT OF LIVER EXTRACT AND METHYL ACETAMIDE WITH PARA-CHLORO-XYLENOL ON ARTIFICIALLY INDUCED LEUKOPENIA IN RATS*

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Alteration in the vitamin requirements of the white rat results from the feeding of highly insoluble sulfonamides—sulfaguanidine or succinylsulfathiazole (sulfasuxidine)—incorporated into specific purified diets.^{1, 7} The animals develop a leukopenia and granulocytopenia, with marrow studies revealing decreased numbers of cells of the myeloid series, particularly the adult polymorphonuclear cells. Welch and Wright⁹ attributed these hematopoietic changes to an alteration in the bacterial flora of the lower intestinal tract whereby the coliform bacteria are greatly reduced in number. According to Hogan and Parrott⁵ this creates in the rat an exogenous requirement for some component of a preparation containing “folic acid.” Associated with the reduction in the number of coliform bacteria, a quantitative diminution in “folic acid” has been demonstrated by Topley, Krebl, and Elvehjem⁸ in the cecal contents of rats on highly purified diets containing 4 per cent succinylsulfathiazole. Crystalline “folic acid” will overcome in rats the experimental leukopenia and granulocytopenia resulting from treatment with insoluble sulfonamides.² Recently, Zondek and Bromberg¹⁰ described another leukopoietic agent, viz., methyl acetamide with para-chloro-xyleneol which evidently acts by direct stimulation of the granulopoietic function of the bone marrow.

With this indirect method of producing neutropenia through the use of purified diets containing insoluble sulfonamides, and the well-known effect of the subcutaneous injection of benzol in olive-oil solution,⁴ two contrasting ways of attaining a similar result are available. Two methods of stimulating granulocytosis also are possible: indirectly, through the oral administration of a preparation of liver extract[†] containing 20 to 40 micrograms of “folic acid” per gram, and directly, through the intramuscular injection of 25

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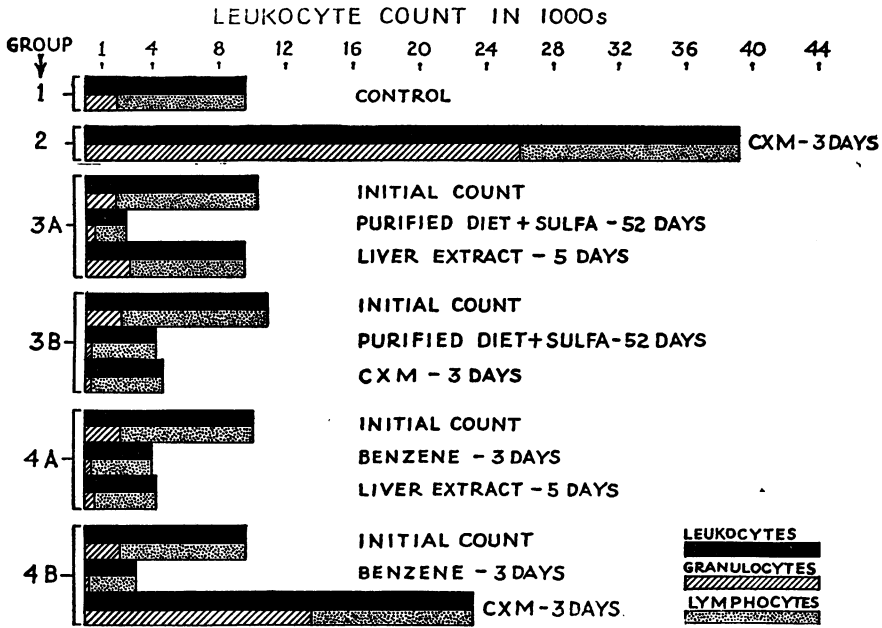
† Furnished through the courtesy of Dr. E. L. R. Stokstad of Lederle Laboratories, Incorporated.

per cent CXM* solution. The report that follows deals with the effects of these two leukopoietic agents on the two varieties of experimentally induced neutropenic states.

Experimental

Sixty young albino rats were subdivided into four series as shown on the Chart.

Group one consisted of four control animals. They were placed on a normal diet of Purina fox-chow pellets. Five peripheral total white blood cell counts were made on the tail blood of each animal, using the Levy chamber with improved Neubauer ruling. Differential leukocyte counts were obtained by counting at least one hundred cells in blood smears stained by the Wright polychrome technic. The average peripheral blood values



gave a total leukocyte count of 8,550 with 6,650 lymphocytes and 1,900 granulocytes per cu. mm. Qualitative marrow studies made at intervals from the diaphyseal ends of the femur showed normal myeloid-erythroid proportions.

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Group two contained twelve animals studied over a three-day period as above. Then each rat was injected intramuscularly with 0.5 cc. of methyl acetamide in 25 per cent para-chloro-xyleneol (25 per cent CXM). The total white blood cell count rose to an average of 39,200 per cu. mm. with 26,000 granulocytes 24 hours after the last injection. Femoral myelograms showed an overwhelming number of myelocytes and granulocytes with an occasional myeloblast.

Group three consisted of twenty animals placed on a purified diet containing succinylsulfathiazole (sulfasuxidine*), 1 per cent; "Smaco" (vitamin-free casein), 18 per cent; cod-liver oil, 2 per cent; cottonseed oil, 3 per cent; salt mixture No. 550 (prepared according to Osborne and Mendel⁶ except that sodium fluoride is reduced to 1 per cent and 0.313 gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ is added), 4 per cent; glucose, 72 per cent. The daily oral vitamin supplement[†] consisted of: thiamin chloride, 100 micrograms; pyridoxine hydrochloride, 200 micrograms; riboflavin, 200 micrograms; calcium pantothenate (dextro-rotatory), 200 micrograms; nicotinic acid, 1 milligram; choline dihydrogen citrate, 10 milligrams; crystalline biotin, 2 micrograms. The white blood cells of the peripheral blood fell to 1200 with 400 granulocytes and 760 lymphocytes within a period of 9 weeks (average time 52 days). The femoral marrow patterns showed maturation arrest at the premyelocyte and myelocyte stages.

Group 3A—For five days eight of these rats were then fed, one gram per day, a liver extract containing 20 to 40 micrograms of "folic acid." Subsequent peripheral blood studies revealed an average rise in the total number of leukocytes from 1,200 to 8,350 with a normal ratio of granulocytes (1,600) and lymphocytes (6,650). This was associated with a definite increase in metamyelocytes and granulocytes and a concomitant disappearance of lymphocytes from the femoral marrow pattern.

Group 3B—The other twelve animals of group three were injected for three successive days with 0.5 cc. of 25 per cent CXM. Peripheral blood and marrow studies made before and after the CXM injections revealed the following trend. Before therapy, the total peripheral white blood cell count averaged 4,150 with only 270 granulocytes, and the femoral myelogram showed characteristic concomitant changes as previously described. The administration of the 25 per cent CXM did not materially affect the total number of leukocytes (4,500) and granulocytes (320).

Group four consisted of twenty-four rats on a normal fox-chow diet and after standardization each rat received 0.6 cc. of benzene in 2 cc. of olive-oil

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† Furnished through the courtesy of Dr. William T. Strauss of Hoffman La-Roche, Incorporated.

daily until the total leukocyte count reached 4,000 and the polymorphonuclear cell count was 150 per cu. mm. Studies of the peripheral blood confirmed this marked neutropenia and relative lymphocytosis, and the bone marrow demonstrated marked lymphocytic infiltration and an occasional myelocyte.

Group 4A—One gram of the liver extract preparation was then fed for five consecutive days to twelve animals, and the blood and marrow studies were repeated. The peripheral blood and diaphyseal marrow patterns were not significantly altered.

Group 4B—The remaining twelve albinos received 0.5 cc. of 25 per cent CXM intramuscularly daily over a three-day period and were followed by peripheral blood and femoral marrow studies. These revealed an extreme reversal in the leukocytic picture, the granulocytic level rising from 180 polymorphonuclears per 3,000 white blood cells to 12,500 polymorphonuclears per 22,000 leukocytes. The bone-marrow pattern showed extensive myelocytic, metamyelocytic, and polymorphonuclear granulocytic hyperplasia.

Discussion

A purified diet containing succinylsulfathiazole will produce neutropenia and leukopenia in albino rats. This dietary neutropenia⁶ is associated with a quantitative reduction in the coliform bacteria and with a marked diminution in the "folic acid" content of the lower intestinal tract. It may be assumed that the insoluble sulfonamides interfere with the production of this essential factor by reducing the number of "folic acid" synthesizers. To substantiate this viewpoint, a study was made of the efficacy of a liver extract preparation and the inefficacy of a proven non-protein leukotactic agent (25 per cent CXM) in the reversal of the agranulocytosis produced indirectly by this "folic acid" deficiency. The results seem to indicate, as Fitz-Hugh and Krumbhaar³ have previously predicated, that some forms of agranulocytosis result from a deficiency of a maturation factor which seems to be supplied or partially formed by coliform bacteria.

Agranulocytosis produced by a toxic chemical agent (benzol), which apparently acts directly on the bone marrow, is reversed by a chemoleukopoietic agent (CXM) provided the bone marrow has not been injured too severely. A preparation containing "folic acid" has no effect on the leukopenia produced by direct bone marrow depressants (benzol).

Conclusions

(1) Oral administration of a liver extract preparation containing "folic acid" reverses the neutropenia and leukopenia produced by a purified diet containing an insoluble sulfonamide. This same extract does not correct a similar hematopoietic state produced by the injection of benzol.

(2) The liver extract preparation containing "folic acid" apparently acts indirectly on the bone marrow by supplying this deficiency factor depleted by the activity of an insoluble sulfonamide by the reduction of the number of its synthesizers, the coliform bacteria.

(3) Methyl acetamide with para-chloro-xyleneol reverses the agranulocytosis and leukopenia induced by benzol, but fails to correct the neutropenia produced by a purified diet containing succinyl-sulfathiazole.

(4) Methyl acetamide with para-chloro-xyleneol apparently acts directly on the bone marrow by virtue of its peripheral leukotactic effect.

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