THE BONE MARROW OF RATS MADE ANEMIC BY ADMINISTRATION OF SULFANILAMIDE

GEORGE M. HIGGINS
Division of Experimental Medicine
AND
THOMAS E. MACHELLA[1]
Fellow in Experimental Physiology, The Mayo Foundation, Rochester, Minnesota

Recently we reported the details of an anemia induced in rats by the daily oral administration of sulfanilamide (para-aminobenzenesulfonamide). The daily administration of 1 gm. of the drug per kilogram of body weight produced in 10 days a macrocytic type of an anemia characterized by marked reductions in the erythrocyte count, a corresponding reduction in the hematocrit determinations, an increase in the mean corpuscular volume and an increase in the percentage of reticulocytes in the peripheral blood. Toxic symptoms, such as cyanosis, gastric distention, irritability and occasional convulsions, were seen. This report is concerned with the changes observed in the bone marrow of rats in which changes in the peripheral blood, as indicated, were induced by administration of sulfanilamide.

Although there is a rapidly growing literature on both the clinical and experimental use of sulfanilamide, detailed studies on the effects of the drug on the cells of the bone marrow have not been reported. Much of the data contained in the reports of experimental studies have concerned the toxicity of the drug and the effects exerted upon the formed elements of the peripheral blood; there are only occasional references to the changes in the bone marrow.

[1] Commonwealth Fund Fellow from the University of Pennsylvania.

In studying lesions in white mice Hageman ('37) observed an increased incidence of eosinophils in the bone marrow preparations of animals which had been given sulfanilamide. Apparently no actual counts were made for no data regarding such counts were included in the report. Finklestone-Sayliss, Paine and Patrick ('37) deduced from studies made on the peripheral blood of animals which had been given sulfanilamide that there was marrow stimulation. The number of polymorphonuclear leukocytes was increased but there was no increase in the percentage of reticulocytes. No report was made of direct observations upon the bone marrow. Rimington and Hemmings ('38) gave daily doses of sulfanilamide to rats and observed a marked increase in the urinary and fecal excretion of porphyrin and a polyuria, but observations on the bone marrow were not reported. Kreutzmann and Carr ('38) gave prontosil to rabbits for 21 days. They observed an increase in the percentage of eosinophilic polymorphonuclear leukocytes in the peripheral blood and regarded this increase as indicative of depression of bone marrow. No statistical data were assembled. Wood ('38) made a numerical study of the cells in the bone marrow in a case in which acute hemolytic anemia developed during a course of sulfanilamide therapy. Marrow preparations of the sternum, vertebra and distal end of the femur were made. Cells were counted and a differential count was made. Wood deduced that there was a definite hyperplasia of the erythropoietic elements of the bone marrow.

PROCEDURE

Fifteen apparently healthy male rats of the Wistar strain were carefully selected. They ranged in weight from 200 to 250 gm., the average weight being 217 gm. The number of erythrocytes ranged from 8,400,000 to 11,100,000 (average 9,825,000) per cubic millimeter of blood, and the number of leukocytes ranged from 10,300 to 24,800 (average 17,850) per cubic millimeter of blood. Although there was a considerable variation in both the erythrocyte and leukocyte counts, for the purpose of this study we concluded that we had selected

a group of animals in which the bone marrow could well be accepted as normal.

Three of the fifteen animals were lightly anesthetized and killed by exsanguination. The femurs were dissected free and disarticulated, and the proximal portion was gently crushed in order to expose the marrow. Serial imprint preparations were made and then stained with the May-Grünwald Giemsa stain (Pappenheim's modification). A minimum of 500 cells were counted from each preparation and the percentage distribution of the erythroid and myeloid elements was computed. The data assembled from preparations made of the marrow of these three animals constituted the body of control data with which all the experimental data were contrasted.

The remaining twelve animals were given by mouth 1 gm. of sulfanilamide (para-aminobenzenesulfonamide) in 6 cc. of tap water daily in the morning. Two were lightly anesthetized and killed by exsanguination on the fourth day. Imprint preparations of the bone marrow were made in a manner identical to that described for the three control animals. Likewise, two animals were killed on the sixth day, two on the eighth, and one on each of the tenth, twelfth and fourteenth days. One animal died of toxic reactions during the experiment and two others were allowed to recover. Imprint preparations were made of the femoral marrow of all ten animals. One thousand cells were counted of each preparation and the percentage distributions of the cells were established.

RESULTS

For a description of the cells of both the myeloid and erythroid series occurring in the bone marrow of rats the reader is referred to the paper by Stasney and one of us (Higgins). It will be observed that a considerable shift in the percentages of the immature myeloid forms, from the data recorded, in 1935, has been tabulated in the control preparations of the current report. Twenty-four animals supplied the data from which the earlier report was prepared, whereas but three were killed to serve as a control for this study. Since the transition

from myeloblast to granulocyte is more or less a continuous one, it is conceivable that constancy in the percentage of any one stage of myeloid development would hardly maintain in different groups of animals. Thus differences in the actual percentages of the various stages are likely to occur. More significant is the fact that the total number of myeloid cells in this control group of three animals constituted 59.56% of all cells counted, while Stasney and one of us (Higgins) in a study of the marrow of twenty-four animals, found that the

TABLE 1

Percentage distribution of cells in the bone marrow

	DAYS OF ADMINISTRATION OF SULFANILAMIDE						
	Control	4	6	8	10	12	14
Myeloblast	0.42	0.59	0.75	0.47	0.42	0.74	0.46
Leukoblast	2.83	1.89	1.29	1.28	0.84	0.74	0.57
Promyelocyte	2.31	6.29	3.22	2.04	3.64	2.97	5.72
Myelocyte	2.78	9.11	8.97	9.23	8.27	6.78	9.15
Metamyelocyte	21.08	17.88	15.68	14.12	19.07	13.28	11.89
Granulocyte	16.93	26.27	7.50	7.43	10.37	6.13	7.67
Eosinophils	11.75	3.64	4.53	2.97	2.66	5.94	6.06
Basophils	0.95	0.13	0.08	0.51	0.28	0.46	0.23
Normoblasts	36.51	30.07	56.30	60.49	52.31	61.61	57.55
Megakaryocyte	0.51	0.39	0.72	0.56	0.31	0.33	0.46
Plasma cells	0.97	1.05	0.46	0.27	0.42	0.27	0
Lymphocytes	2.96	2.69	0.50	0.63	1.40	0.74	0.23

myeloid cells constituted 62.75% of all cells counted. Furthermore, the percentage of erythroid cells, including both erythroblasts and normoblasts, constituted 36.51% of all cells tabulated from smears of marrow of these three control animals, while the data of Stasney and Higgins showed a mean erythroid percentage of 35.89.

The essential changes which have been observed in the smears of marrow of animals given sulfanilamide and killed at intervals during the administration of the drug have been condensed into tables 1 and 2. It seems evident from the data that even as early as 4 days changes occurred in the marrow which indicated an initial myeloid stimulation. Counts on the

formed elements of the peripheral blood of the two animals killed after 4 days of treatment showed a slight reduction in the erythrocyte count and a considerable decrease in the total number of leukocytes. This decrease in the number of leukocytes in the peripheral blood was reflected in the myeloid stimulation in the marrow, for an average relative increase of 6.63% in total myeloid cells was recorded for the two animals killed on the fourth day. The myeloid-erythroid ratio which had been established at 1.63 : 1.00 for our control group increased to 2.20 : 1.00 on the fourth day.

The myeloid stimulation which occurred in the bone marrow on the fourth day was clearly shown in the peripheral blood of the animals killed on the sixth day. The average leukocyte

TABLE 2

Myeloid-erythroid ratios in bone marrow

	DAYS OF ADMINISTRATION OF SULFANILAMIDE						
	Control	4	6	8	10	12	14
Total myeloid cells (per cent)	59.56	66.19	42.74	38.61	45.86	37.37	42.21
Total erythroid cells (per cent)	36.51	30.07	56.30	60.49	52.31	61.61	57.55
Myeloid-erythroid ratio	1.63 : 1	2.20 : 1	0.76 : 1	0.64 : 1	0.87 : 1	0.61 : 1	0.75 : 1

count made of the peripheral blood on two animals killed on the sixth day was 29,000 cells per cubic millimeter, while 6 days before, at the onset of the experiment, the mean leukocyte count of these two animals was 19,000. The mean erythrocyte count of the two animals killed on the sixth day was 7,400,000 cells per cubic millimeter which was a marked decline from the count of 10,200,000 recorded for these same animals before the administration of any drug. These changes in the peripheral blood were also reflected in the percentage distribution of cells in the marrow. As a result of the anemia there was a marked erythroid stimulation in the marrow resulting in a relative increase from 30.07% of all cells tabulated, to 56.30%. Whereas there was a decline in the relative

myeloid percentage from 66.19 to 42.74, on the sixth day, the conclusion is not indicated that there was any myeloid suppression. The marrow was exceedingly cellular and abundant and we are of the opinion that an absolute increase of both myeloid and erythroid elements occurred. Total counts of marrow cells have, of course, not been attempted. This rapid increase in erythroid components over and above the myeloid percentage had resulted in an inversion of the myeloid-erythroid ratio, which on the sixth day was 0.76 : 1.00.

The relations between the numbers of cells of the peripheral blood and those of the bone marrow of animals killed on the sixth day were all the more apparent in those animals killed on the eighth day of administration of the drug. At that time the periperal blood contained an average of 31,000 leukocytes per cubic millimeter, while the average number of erythrocytes for the two animals was 5,900,000 per cubic millimeter. This further decline in the total number of erythrocytes, together with the increase in the total number of leukocytes was again reflected in the bone marrow by an even higher rise in the relative erythroid percentage coupled with a corresponding decline in the relative myeloid percentage. The data indicated that the anemia induced in the peripheral blood by the drug produced a marked stimulation of cells in the bone marrow.

The conditions which were observed on the eighth day were not essentially different in those animals killed on the tenth, twelfth and fourteenth days respectively. In all animals there was a greater relative increase in the erythroid elements, so that a myeloid-erythroid ratio of less than 1 : 00 was always found.

Although in the tabulations the eosinophilic leukocytes were not grouped into the eosinophilic myelocytes, metamyelocytes and mature eosinophils, there was no indication of any significant increase in the number of eosinophils in the marrow. Most of the cells were of the mature type with ring-shaped nuclei, but the relative percentages established for this cell

type did not indicate any eosinophilic stimulation, such as has been described hitherto.

One animal showed marked toxic reactions. These were first seen on the fourth day shortly after giving the drug, when the animal had definite convulsive symptoms. These reactions were more marked on the fifth day, and on the sixth day, when it appeared that the animal apparently would not recover, the rat was killed and the bone marrow was examined. Unfortunately red and white cell determinations on the peripheral blood were not made, but the bone marrow showed a marked relative erythroid suppression and a relative myeloid stimulation. The myeloid-erythroid ratio was nearly 4.00:1.00. Most of the erythroid cells were erythroblasts; very few normoblasts were seen. There were a large number of hypersegmented granulocytes in the smears, indicating perhaps some inhibition in the release of these cells into the blood stream.

In our early study on the peripheral blood of rats given sulfanilamide we observed the frequent appearance of granulocytes of the neutrophilic order, with a large number of lobes. Nuclei with as many as ten or twelve lobes were encountered. Smears of the marrow seem to indicate that these multilobular granulocytes begin to appear as early as the promyelocyte stage. Unusual and bizarre types of nuclear patterns were observed in certain promyelocytes. These continue to differentiate through the succeeding stages of myeloid development and give rise, we believe, to the hypersegmented granulocytes in the peripheral blood.

SUMMARY AND CONCLUSIONS

Bone marrow preparations of white rats which were given daily doses of sulfanilamide (1 gm. per kilogram of body weight) have been studied. Imprint preparations were made of femoral marrow removed from animals killed on the fourth, sixth, eighth, tenth, twelfth and fourteenth days respectively. The differential percentage distribution of the cells compris-

ing the marrow was established and these data were compared with those assembled from the marrow preparations of three control animals which had not received sulfanilamide. The following observations and deductions have been made.

1. On the basis of the relative distribution there is, after 4 days of administration of the drug, a greater myeloid stimulation than erythroid stimulation.

2. At 6 days, when anemia in the peripheral blood was seriously indicated, we observed that the erythroid stimulation was relatively greater than the myeloid stimulation.

3. The myeloid-erythroid ratio, whereas normally greater than one, was always less than one from the sixth day of the experiment to the end.

4. Eosinophilic stimulation in the bone marrow was not apparent.

5. The frequent appearance of multilobular granulocytes in smears of the peripheral blood of animals receiving sulfanilamide seems to be explained on the basis of the early modifications in the nuclear patterns of the promyelocytes and myelocytes.

LITERATURE CITED

FINKLESTONE-SAYLISS, H., C. G. PAINE AND L. B. PATRICK 1937 The bacteriostatic action of *p*-aminobenzenesulphonamide upon haemolytic streptococci. Lancet, vol. 2, pp. 792–795.

HAGEMAN, P. O. 1937 Toxicity of sulfanilamide; a study of the pathological lesions in white mice. Proc. Soc. Exper. Biol. & Med., vol. 37, pp. 119–122.

KREUTZMANN, W. B., AND J. L. CARR 1938 Effect of prontosil on blood cells. Proc. Soc. Exper. Biol. & Med., vol. 38, pp. 19–21.

MACHELLA, T. E., AND G. M. HIGGINS 1939 Anemia induced in rats by the administration of sulfanilamide. Proc. Staff Meet., Mayo Clin., vol. 14, pp. 183–185.

RIMINGTON, CLAUDE, AND A. W. HEMMINGS 1938 Porphyrinuria following sulphanilamide; sulphanilamide dermatitis. Lancet, vol. 1, pp. 770–776.

STASNEY, JOSEPH, AND G. M. HIGGINS 1935 A quantitative cytologic study of the bone marrow of the adult albino rat. Anat. Rec., vol. 63, pp. 77–89.

WOOD, H. 1938 Fatality from acute hemolytic anemia which developed during administration of sulfanilamide. South M. J., vol. 31, pp. 646–648.