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Studies on the blood of the albino rat.

Its normal cellular constituents. Their reaction to sarcoma growth and to benzol treatment.

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While working with a transmissible sarcoma of the white rat it was found desirable to note the changes in the blood picture during the growth of the tumor. With the idea of establishing the normal, a careful search of the available literature on the blood of the albino rat (and also the common wild rat) was made. No reports on the blood plates and only a few on the leucocytes of this animal could be found. For this reason, and because of the growing importance of the white rat as a laboratory animal and its availability for tumor work, it has seemed advisable to contribute this small series of studies on the blood of albino rats, with special reference to the blood plates in the normal animal. The changes in the blood picture due to sarcoma growth and benzol treatment have been observed.

Hans Hirschfeld¹ in a paper on the differential morphology of the white cells of the blood reported the usual types of cells to be present in the blood of rats of mixed and albino breeds. He noted especially a cell with annular nucleus and fine eosinophilic granules. He did not report the usual number of white cells or their differential count.

Pappenheim² was the first to report in brief the changes in the blood of the albino rat coincident with the progress of fatal transmissible sarcoma. His conclusions were that, except for a very slight secondary anemia and a great degree of polychromatophilia and granular degeneration of the red cells, there was little reaction on the part of the blood until the tumor ulcerated, when a marked leucocytosis appeared.

¹ *Virchow Archiv*, 1897, CXLIX, p. 22.

² *Folia Hematol.*, Vol. X, 2, p. 393.

Hirschfeld¹ reached much the same conclusions, but reported finding normoblasts in the blood. He recorded a leucocyte count of 180,000 in one rat with an ulcerating sarcoma. No normal standard of number or of differential count of leucocytes was reported in either of these papers. The blood plates were not enumerated or described.

In the work presented here the leucocytes and blood plates were counted after the method of Wright and Kinnicutt² designed for plate counting. It was modified by the procedure of first drawing the fixing fluid to the mark .5 in the stem of the white cell pipette in order to prevent the blood from coming in contact with a dry surface. A 1-20 dilution was invariably used. Two pipettes were used for each count and five drops counted from each to determine the number of leucocytes. One hundred small squares (Turk's stage) from the dilutions in each pipette were counted to determine the number of blood plates. The following table shows the results of eighteen studies on the blood of eight apparently healthy albino rats. For the blood plates the figures given represent the nearest 50,000; for the leucocytes the nearest 500.

Average number blood plates per ccm.....	1,000,000
(Variation 850,000 to 1,200,000)	
Average number of leucocytes per ccm.....	19,000
(Variation 12,000 to 30,000)	
Average number of red blood cells per ccm.....	10,000,000
(Variation 9,000,000 to 10,500,000)	

Differential leucocytes (300 cells counted):

Average polymorphonuclear cells.....	50 per cent.
(Variation 30 per cent.-60 per cent.)	
Average mononuclear cells (small 30 per cent., large 14 per cent.)..	44 per cent.
(Variation in proportion great)	
Average transitional cells.....	5 per cent.
(Variation 2 per cent.-14 per cent.)	
Average eosinophil cells.....	1 per cent.
(Variation 0-3 per cent.)	

It will be seen from the table for normal rats that the blood plate count shows an unusually small degree of variations. The

¹ *Fol. Hemat.*, Vol. X, 2, p. 393.

² *Jr. A.M.A.*, 1911, LVI, p. 1457.

plates themselves, however, differ greatly in size, measuring from two to five micra in long diameter. In the diluting fluid they usually appear slightly oval and cup-shaped. They are granular. Varied forms, however, may be seen in the dry and stained preparations. The red blood cells, even in healthy rats, always show considerable granular degeneration and polychromatophilia. The total number of leucocytes is high as compared with the human count and, as shown in the table, has a wide variation in apparently normal animals. The reaction to an inflammatory process commonly results in a high leucocytosis. Several counts of over 45,000 per c.c. were made. Two were in cases of large subcutaneous abscesses, two in cases of ulcerating sarcoma.

The differential count of leucocytes in normal animals shows a fairly constant percentage of polymorphonuclear cells. The total number of mononuclear cells is also fairly definite, but there is great variation in the proportion of large and small cells in different animals. While the small mononuclear cells usually greatly outnumber the large, two apparently normal rats showed more large than small cells.

In five albino rats inoculated with sarcoma the blood was observed. The results agree in general with those of Pappenheim and of Hirschfeld. No marked change in the blood picture is noticeable during the growth of the tumor except an increase in the amount of polychromatophilia of the red cells and a slight increase of small mononuclear leucocytes at the expense of the large mononuclear and transitional cells. When the tumor was of an ulcerating character, however, leucocytosis was marked: 45,000 in one, 50,000 in another rat. Blood plates remained stationary.

Benzol leucopenia was readily produced in two rats, the leucocyte count falling from 15,000 to 1,200 in one, and from 25,000 to 2,200 in the other; while the plates showed a corresponding drop from 850,000 to 500,000, and from 1,000,000 to 500,000 respectively inside of ten days. The fall in leucocytes was proportionately greater in the large mononuclear and transitional cells than in the polymorphonuclear and small mononuclear cells.

SUMMARY.

1. In the blood of the normal albino rat the blood plate count approximates 1,000,000 per c.c. and is fairly constant.

2. The total leucocyte count is variable and reacts violently to ulcerative processes.

3. The reaction of the cellular elements of the blood to the growth of transmissible sarcoma is slight and probably in no way characteristic.

4. Benzol injections produce a more rapid and proportionately greater reduction in the leucocytes of the blood than in the plates.

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Gravimetric determination of beta-oxybutyric acid.

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If beta-oxybutyric acid is oxidized with dichromate in the presence of sulfuric acid and mercuric sulfate, a precipitate of the acetone compound of mercury sulfate can be obtained in an amount proportional to the beta-oxybutyric present. Thus, if 175 c.c. of a beta-oxybutyric solution containing 9 per cent. of sulfuric acid, 2 per cent. of mercuric sulfate, and 0.25 gram of potassium dichromate are boiled under a reflex for an hour, 7.7 milligrams of mercury-acetone compound are precipitated for each milligram of beta-oxybutyric acid present. The beta-oxybutyric acid may vary from 1 to 9 mg. without affecting the ratio, if the concentrations of the other reagents are kept constant.

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Complement fixation in tuberculosis.

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