HAEMATOLOGICAL STUDIES IN EXPERIMENTAL TUBERCULOSIS[1, 2]

Variations in the Blood Cells of Rabbits Inoculated with Cultures Differing in Virulence

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As long ago as 1896, it was observed that there were changes in the cells of the circulating blood during tuberculous infection. Holmes (1) stated that the characteristic changes were: a decrease in small lymphocytes, an increase in phagocytes (neutrophiles), usually an increase in large lymphocytes (probably monocytes, as these cells were not then differentiated from lymphocytes), a decrease or disappearance of the eosinophiles, and certain qualitative changes of disintegrative nature involving in particular the phagocytes. Subsequent work has elucidated and elaborated upon Holmes's work, but the basic observations still stand. Arneth (2) and Lewis (3) observed qualitative and quantitative changes in the neutrophilic leucocytes, which were believed by Arneth (2) to be specific and diagnostic for tuberculosis. The latter was shown not to be the case by Lewis (3). In 1902 Lacapère (4) noticed changes in the blood cells very soon after inoculation of tubercle bacilli. The most constant change was an increase in the number of large mononuclear cells. Webb and his associates (5 to 8) later found that lymphocytosis was associated with increased resistance and that lymphopenia was evidence of advancing disease. Webb (8) believed that lymphocytes were able to engulf tubercle bacilli. But the identification of the monocyte by Schilling (9) and by Sabin (10), and subsequent studies of their phagocytic properties by Sabin and Doan (11), Lurie (12), Gottlieb (13) and others have shown that it is this cell and not the lymphocyte which has the capacity to engulf tubercle bacilli. However, the work of Webb on the relation of lymphocytes to resistance in tuberculosis was confirmed and extended by Murphy and his coworkers (14 to 18). It has also been indicated (39, 43) that the state of maturity of the lymphocyte

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may be important; induced lymphocytosis does not invariably result in enhanced resistance (39).

The identification of the monocyte (9, 10) made possible studies of the rôle of this cell in tuberculosis. Cunningham, Sabin, Sugiyama and Kindwall (19) observed that with advancing disease the monocytes increase in number, while the lymphocytes decrease, so that there is an elevation of the monocyte-lymphocyte ratio. Studies by Sabin, Doan and Cunningham (20) further emphasized the significance of the monocyte-lymphocyte ratio. These experimental studies have received ample confirmation in both the laboratory and clinic (21 to 26, 35).

Recently Medlar (27, 28) has studied the blood cells in clinical tuberculosis; he has stressed the rôle of the neutrophilic granulocytes and discussed the relation between the blood picture and the evolution of lesions in the tissues. The part played by each of the blood cells was discussed and three types of leucocytic patterns were observed in his patients. These were described as the septic, hyperplastic and nonseptic types (28). Crawford (29) devised a simple calculator for determining the leucocytic index, which takes into account the neutrophile-lymphocyte ratio and abnormal values for monocytes and total white cell count. This index was used by Medlar in extensive clinical studies (30 to 32). Sweany (26) has employed both the lymphocyte-monocyte and the neutrophile-lymphocyte ratios, together with erythrocyte sedimentation and certain chemical findings, and has devised charts which express graphically the course of events.

As result of these earlier studies, it is generally agreed that carefully performed total and differential leucocyte counts are of considerable prognostic value in experimental and clinical tuberculosis. It is believed that the results to be described in the following paragraphs throw new light on the rôle of the various blood cells in reflecting the course of tuberculous infection.

MATERIALS AND METHODS

The animals used in this study and the experimental procedures, except blood counts, were described in the first paper (36) and will be repeated here only briefly.

Forty normal, young adult, New Zealand red rabbits were used. Studies of the blood were made in the period from September to December, 1935. The animals were then divided into four groups of ten each with litter mates equally distributed. Animals of different age, sex

and weight were so distributed as to make the four groups as nearly alike as possible.

Four lines of cultures, each derived from the bovine strain 39, and grown for five successive generations at pH values of 6.0, 6.4, 6.8 and 7.2, were used. Differences in pathogenic properties of these bacteria were known to exist, as determined from results of a prior experiment. Each of the cultures was inoculated intravenously into one group of ten rabbits. A standard dose of 0.01 mgm. (moist weight, about 500,000 bacteria) was used throughout. Before and after inoculation, all animals were kept under similar conditions. None was subjected to any therapeutic procedure.

Following inoculation, counts of the blood cells were done on each rabbit once weekly until death from tuberculosis occurred, or until the last remaining animals were sacrificed 6 months following inoculation. Haemoglobin determinations were done by the Newcomer method; all were read by one disinterested person to whom the identity of the individual animals was unknown. Standardized blood diluting pipettes and standardized double-ruled Levy-Hauser counting chambers were employed for the blood counts. Differential leucocyte counts were done by the authors, using the supravital technique; identity of the rabbits was unknown to the individuals making the counts. As routine, 100 cells were counted on each of two coverslip preparations; when there were appreciable differences between the two counts, as many as 1,000 cells were counted. Total erythrocyte and leucocyte counts were done by competent technicians experienced in this work.

Statistical analyses of the blood studies were made following the termination of the experiment. Data for each group of ten animals were treated separately. The 75 counts done on each group prior to inoculation served as specific controls. Counts following inoculation were analyzed by months, that is, all the counts on one group during the first month after inoculation were treated together, etc. Values for the absolute number of each of the blood cells were used. Employing the methods and formulae outlined by Davenport and Ekas (33), the mean, standard deviation and probable error of the mean were determined for each type of blood cell in each group of animals for each time interval, namely, control period, first month after inoculation, second month, etc. The significance of changes in the various blood cells was determined by calculating the deviation from the control mean, determining the probable error of this deviation, and dividing the former by the latter.

This quotient expresses the deviation as a multiple of its probable error. The statistical significance (probability) of various values of such quotients was taken from Pearl's (34) table 40. From this table (or Davenport and Ekas's (33) table 11, p. 178), it may be seen that deviations having the value of PE $\times$ 3.8 occur by chance only once in 96 times, and those having the value PE $\times$ 3.9 occur by chance only once in 117 times. Therefore the value PE $\times$ 3.85 was taken as the limit for normal variation; and deviations of order greater than this have a probability of 100 to 1, or more, of being significant. Deviations of lesser order were considered not to be of certain significance. The shaded areas on charts 1 to 5 show the magnitude only beyond which deviations of the mean counts were considered to be certainly significant. Comparisons of the deviations in any one type of cell in one group of rabbits with those in a group inoculated with a different culture were thus made, not on the basis of a common normal value, but on the basis of the normal value obtained in each particular group. It should be mentioned, however, that differences in the mean normal values among the four groups were not of high order.

The monocyte-lymphocyte ratios, proposed by Cunningham, Sabin, Sugiyama and Kindwall (19), and the neutrophile-lymphocyte ratios, proposed by Crawford (29), were calculated for each individual count; these data were thereafter treated in precisely the same manner as those for the blood cells.

The data included 300 counts done before inoculation and 609 counts done after inoculation. The grouping of data during the disease period was such that the mean values represented not less than seven and not more than forty-five counts.

RESULTS

The marked differences in virulence of the four cultures used for inoculation of the four groups of animals (36) made possible a study of the blood cells in various grades of tuberculous infection from mild to severe. It was shown (36) that the culture grown at pH 6.0 was markedly attenuated (virulence +), that grown at pH 6.4 was moderately attenuated (virulence ++), that grown at pH 7.2 was slightly attenuated (virulence +++), and that grown at pH 6.8 was fully virulent (++++). Hereafter, in order to facilitate discussion, the four groups of animals will be referred to by numbers 1 to 4, corresponding to the virulence of

the culture, group 1 being that inoculated with the least virulent culture, etc. Among the four groups of animals, each type of blood cell at some time in the disease showed significant change from the normal. In presenting the data, each type of cell and the two cellular ratios will be discussed separately. Finally, the efficiency of each cell in showing significant change will be discussed.

Haemoglobin: Each of the four groups of animals showed a significant fall in haemoglobin during the first month after inoculation, as shown on chart 1. The magnitude of this fall was greatest in those inoculated with the most virulent culture and least in those receiving the most attenuated culture. During the second month of the disease, only the animals of groups 3 and 4 showed significantly low values for haemoglobin. Paradoxically, however, the surviving animals of group 4 did not show abnormal values for haemoglobin during the third month. This may have been due to the fact that only seven counts were obtained and five of these were on the one animal of the group which survived more than 72 days. During the third and fourth months, only the animals of group 3 showed abnormally low haemoglobin. But in the fifth and sixth months, those of groups 1 and 2 also showed low values. Every significant deviation of haemoglobin from the normal was in the direction of a decrease. Of the nineteen means determined according to group per month, twelve were significantly low, giving an efficiency of 63.15 per cent.

Red blood cells: During the first month of the disease, only the rabbits of groups 4 and 2 showed marked decrease in the red blood cells, as shown on chart 1. During the second and third months, none of the groups showed abnormal values for red blood cells. In the fourth month, those of group 3 showed a decrease. In the fifth and sixth months, the rabbits of group 2 had red blood cell counts which were significantly low. The rabbits receiving the most attenuated culture (group 1) showed significantly low erythrocytes only in the sixth month. Roughly then, the anaemia was proportional to the severity of disease; but of nineteen mean values after inoculation only six were significantly low, giving an efficiency of only 31.57 per cent. All significant deviations were in the direction of a decrease, however.

Total leucocytes: In the first and second months following inoculation, only the animals of group 4, those inoculated with the most virulent bacteria, showed a significant change in total leucocyte count; this was a decrease. In the third month, none of the groups showed significant

deviation from the normal mean values. In the fourth month, groups 1 and 2 showed values which were abnormally high. Only group 2 exhibited an abnormal level for leucocytes in the fifth month, namely, a rise. In the sixth month, groups 1 and 2 showed significant elevation.

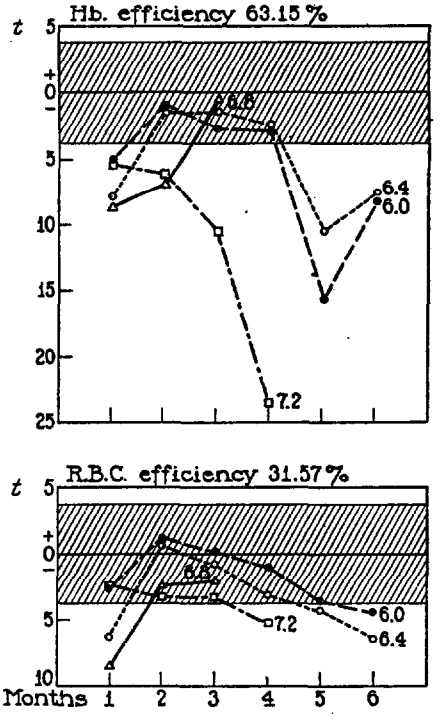

CHART 1.[3] Monthly mean deviations of haemoglobin and erythrocytes expressed as multiples of their probable error.

The decreases, recorded on chart 2, occurring in the animals inoculated with the more virulent organisms, exemplify the leucopenic phase described by Sabin, Doan and Cunningham (20). Of the nineteen mean

[3] Deviations in charts 1 to 5 inclusive are shown as multiples of the probable error of the deviations. The odds against occurrence by chance are small in the case of all points falling in the shaded zone and their significance is not certain. But the odds against occurrence by chance are high in the case of deviations falling outside the shaded zone; such deviations are considered significant. The horizontal line in the centre of the shaded zone represents the normal value; the direction of each deviation from the normal is shown. The whole number and decimal at the end of each curve indicate the pH of the medium on which the culture was grown for inoculation of the animals represented by that curve. The curves differ in length according to the survival time of the rabbits represented.

values in the four groups, seven deviated significantly from the normal, so that the efficiency was 36.84 per cent. Of these seven significant deviations, two were below the normal level and five were above. It was interesting that group 3, receiving the culture grown at pH 7.2 (virulence +++), at no time showed deviations of high order (chart 2).

Neutrophiles: Only the animals of group 4 showed abnormal values for neutrophiles in the first two months following inoculation. This change, a sharp decrease (chart 2), corresponded to the anaemia-leucopenia phase of the disease as demonstrated in table 1. In the third month

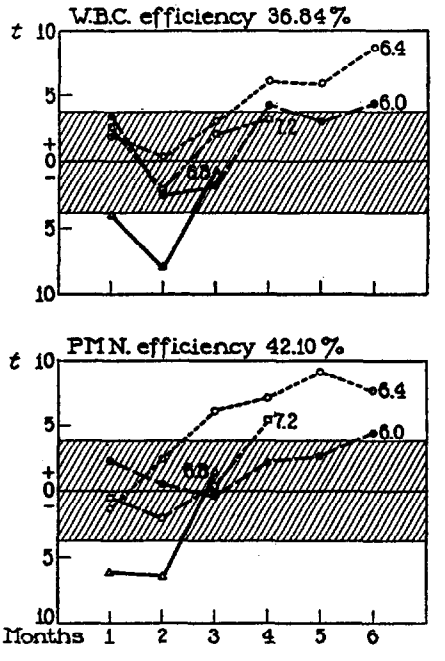

CHART 2. Monthly mean deviations of white blood cells and neutrophiles expressed as multiples of their probable error.

this group of animals exhibited normal values for neutrophiles, as did those of groups 3 and 1. However, the rabbits of group 2 showed an increase in neutrophiles at this time and thereafter the values were always significantly high in this group. In the fourth month the animals of group 3 showed an increase in neutrophiles. The group 1 which received the most attenuated culture exhibited only one abnormal monthly mean value, a rise in the sixth month. Of the nineteen mean values after inoculation in the four groups, eight showed significant deviation from the normal, giving an efficiency of 42.10 per cent. Two of these

eight significant deviations were in the direction of a decrease (in animals inoculated with highly virulent organisms) and six were in the opposite direction. It was interesting and probably important that the increases in neutrophiles which did occur, did not take place until the third month in any of the groups, despite the fact that the disease was sufficiently established in three of the four groups that deaths were occurring. Terminal neutrophilic leucocytoses were noted in certain individual animals but were not of sufficient magnitude to cause significant elevation of the mean for the groups. In the group receiving the most virulent microörganisms, in which most of the deaths occurred in 60 days or less, even individual counts taken just prior to death exhibited neutropoenia, although their lesions exhibited the most extensive caseation.

Basophiles: The counts of basophiles preceding inoculation in these groups of animals were lower than those reported by Pearce and Casey (37) and by Thomas (40). It was shown by Sabin, Miller, Smithburn, Thomas and Hummel (38) that the basophiles do not reach maximum levels until the time of maturity. Although all animals in the present experiment were of mature age when inoculated, at the midpoint of the period before inoculation twenty-four, or 60 per cent, were between 4.2 and 5.9 months old. Higher values might, therefore, have been obtained had the base-line counts been made later. However, the mean of the final preinoculation counts of basophiles in the forty animals was 631, a figure still below that obtained by the investigators mentioned; at this time each animal was at least 6 months old.

No significant change in basophiles occurred in the animals receiving the most virulent culture, at any time. But in the first month, the other three groups each exhibited an increase. In the rabbits of group 2 the rise extended through the second and third months. No other groups exhibited significant deviation in the second month. In the third month, those of group 3 again showed a significant rise, but in the fourth month there was a fall of equal magnitude. In the fourth, fifth and sixth months, the rabbits of group 1 again showed a rise in basophiles, while those of group 2 exhibited no significant deviation from the normal. Thus there were deviations of apparent significance in three of the four groups, some above and some below the normal. These changes were neither related to the virulence of the infecting agent, nor to the resistance of the host. Ten of the nineteen values following in-

oculation showed significant deviations from the normal. Of these, nine were in the direction of an increase, while one was in the opposite direction, as shown on chart 3. It is interesting to note that, during the leucopenic phase, the animals receiving the most virulent microörganisms did not exhibit a decrease in basophiles, although there was a decrease in all other cellular elements originating in the bone marrow. It is also notable that none of the apparently significant elevations of the basophile counts (see table 1) exceeded the normal values obtained by Pearce and Casey (37).

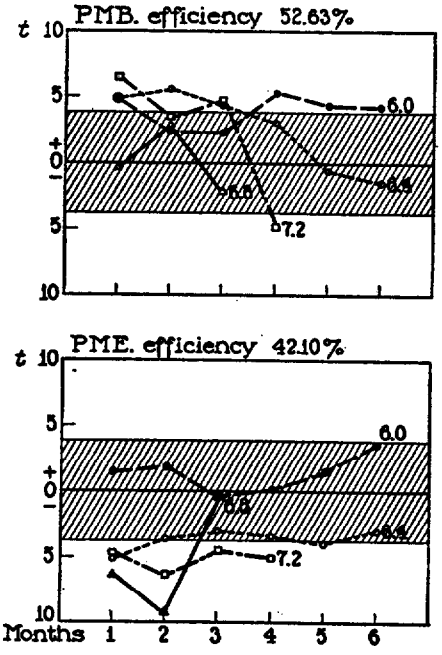

CHART 3. Monthly mean deviations of basophiles and eosinophiles expressed as multiples of the probable error.

Eosinophiles: Chart 3 shows that the ten animals of group 1 at no time showed significant deviations from the normal count of eosinophiles. Group 2 showed a significant decline in the first and fifth months only. Those of group 4 showed a significant decline in the first and second months, but not in the third. Group 3 showed significantly low eosinophiles in each of 4 monthly periods following inoculation. Thus, of eight significant deviations, all were downward and all were in the three

groups receiving the more virulent inoculum. The decline was not dependent on the leucopenic phase, as may be seen in group 3; these animals had no leucopenia (see charts 2 and 3). (The eosinophiles do not disappear entirely, as previously reported by some workers, but are present in such small numbers during severe tuberculosis that often many cells must be counted to find a single eosinophilic leucocyte.)

Lymphocytes: None of the four groups of animals exhibited abnormal values for lymphocytes in the first month after inoculation, as can be

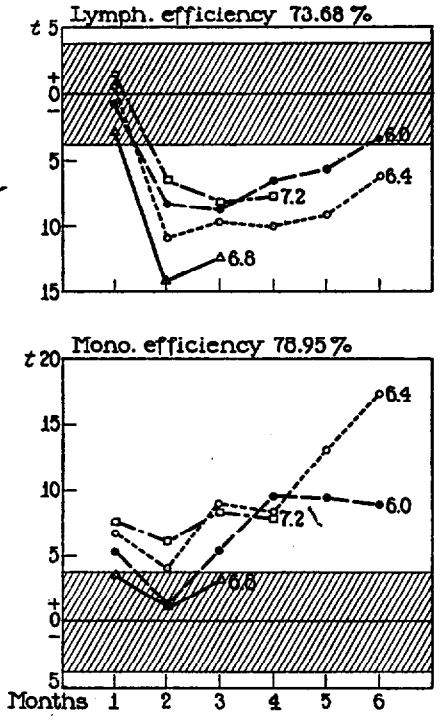

CHART 4. Monthly mean deviations of lymphocytes and monocytes expressed as multiples of their probable error.

seen from chart 4. A significant decline in circulating lymphocytes occurred in each group during the second month. The lymphopenia was marked in each case, but most profound in group 4; it persisted in every group throughout the disease, except in group 1. The latter animals exhibited a fall in lymphocytes, and then a rise, so that by the sixth month the mean value was not significantly low. From chart 4 it may be seen that the curves for lymphocytes more faithfully reflected the

course of the disease than any of the other blood cells, that is, the magnitude of change most closely paralleled the severity of the infection. However, as will be shown, they were less sensitive than the monocytes in that the latter showed abnormal values earlier. Fourteen of the nineteen mean monthly values for lymphocytes following inoculation were significantly low, giving an efficiency of 73.68 per cent for these cells.

Monocytes: The rabbits of group 4 at no time showed significant deviations from the normal counts of monocytes, as shown on chart 4. The absolute values were at all times above the base-line mean (as shown in table 1) but the deviation was not statistically significant. The animals of group 1 exhibited a mean value for monocytes in the second month which was within the normal range. All other monthly mean values in all the groups showed significant deviations in the direction of an increase. Thus, in three of the four groups there was a significant rise in the first month after inoculation, and in two groups this rise persisted throughout the disease. Fifteen of the nineteen monthly mean values for monocytes showed significant elevation, giving an efficiency of 78.95 per cent. Thus these cells were more sensitive than any others in reflecting the course of events; but the fact remains that, in the animals exhibiting the most severe infection, the circulating monocytes did not show an increase of high order.

Neutrophile-lymphocyte ratio: The animals of group 4 at no time showed significant deviation from the mean base-line value for the neutrophile-lymphocyte ratio. In this group there was a rise in the second and third months (table 1) but it proved not to be statistically significant. Group 3 showed significant elevation of the neutrophile-lymphocyte ratio only in the fourth and final month of disease. Again the mean values were elevated in the second and third months, but the rise was not significant as the probable error of the deviation was very high. The two groups receiving the more attenuated cultures showed significant elevation of the neutrophile-lymphocyte ratio during the second to sixth months following inoculation. This elevation was in large part due to the decrease in lymphocytes, since nine of the ten corresponding values for lymphocytes were significantly low, whereas only five of the ten corresponding values for neutrophiles were significantly elevated. Eleven of nineteen mean values showed significant elevation, so that the efficiency of the neutrophile-lymphocyte ratio was 57.89 per cent. But it is important to note that only one of seven mean values following in-

oculation showed significant elevation from the normal in the two groups of animals inoculated with the two more virulent cultures. Deviations of the neutrophile-lymphocyte ratio are shown on chart 5.

Monocyte-lymphocyte ratio: Statistically significant deviations from the normal monocyte-lymphocyte ratio occurred in all the counts following inoculation except in that for the first month in group 4 and that for the second month in group 1. Thus, of nineteen mean values at all stages of the disease, seventeen were significantly elevated. The effi-

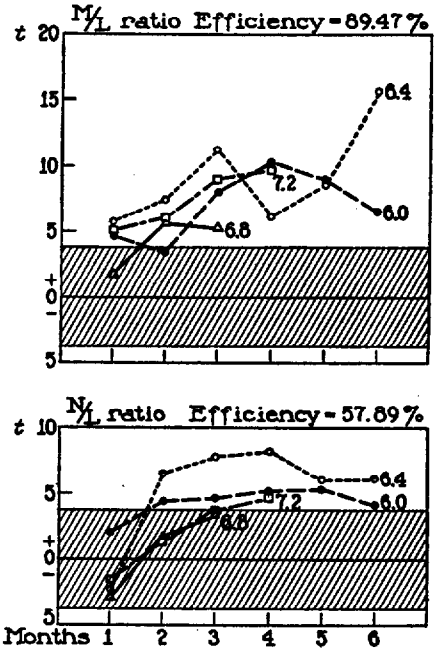

CHART 5. Monthly mean deviations of the monocyte-lymphocyte and neutrophile-lymphocyte ratios expressed as multiples of their probable error.

ciency of this ratio was therefore 89.47 per cent, and it was more sensitive in detecting the presence of infection than the neutrophile-lymphocyte ratio, the haemoglobin, or any of the blood cells considered separately. Furthermore, although the values given on chart 5 show no parallelism with the severity of infection, the actual mean values for the index given in table 1 do show greater change in the groups receiving the more virulent microörganisms, and progressively higher mean values in each group. Table 1 also shows that the mean values for the monocyte-lymphocyte

TABLE 1

Mean normal and mean monthly postinoculation values for each type of blood cell, for the monocyte-lymphocyte and neutrophile-lymphocyte ratios, and for haemoglobin in each of the four groups of animals

	INOCULATED WITH CULTURE GROWN AT pH	BASE-LINE	FIRST MONTH	SECOND MONTH	THIRD MONTH	FOURTH MONTH	FIFTH MONTH	SIXTH MONTH
Haemoglobin	6.0	82.2	*77.2*	81.3	79.6	78.6	*67.5*	*71.1*
	6.4	79.9	*71.7*	78.4	78.2	76.5	*61.1*	*60.8*
	6.8	78.9	*69.7*	*72.1*	77.3			
	7.2	80.5	*74.9*	*75.0*	*72.0*	*61.0*		
Red blood cells	6.0	5,814	5,611	5,887	5,818	5,742	5,567	*5,493*
	6.4	5,597	*5,152*	5,641	5,530	5,329	*5,054*	*4,365*
	6.8	5,683	*5,123*	5,431	5,211			
	7.2	5,691	5,526	5,478	5,307	*4,990*		
White blood cells	6.0	10,030	11,064	9,139	9,433	*11,997*	11,137	*11,988*
	6.4	9,470	10,132	9,592	10,380	*12,380*	*11,888*	*13,705*
	6.8	10,268	*8,654*	*7,083*	9,778			
	7.2	9,969	11,155	9,166	10,851	12,185		
Neutrophiles	6.0	4,345	4,850	4,461	4,287	4,941	4,895	*5,484*
	6.4	4,003	3,727	4,661	*5,181*	*5,942*	*6,591*	*7,068*
	6.8	4,417	*3,094*	*2,931*	5,324			
	7.2	4,687	4,540	4,071	4,776	*6,224*		
Basophiles	6.0	537	*785*	643	642	*870*	*845*	*925*
	6.4	440	*641*	*885*	*709*	757	406	318
	6.8	590	578	772	485			
	7.2	586	*880*	935	*889*	*351*		
Eosinophiles	6.0	154	175	180	150	155	192	210
	6.4	122	*65*	81	84	78	*68*	77
	6.8	147	*64*	*45*	142			
	7.2	156	*102*	*81*	*100*	*67*		
Lymphocytes	6.0	4,108	3,996	*2,864*	*2,945*	*3,165*	*3,066*	3,538
	6.4	3,975	4,062	*2,460*	*2,654*	*2,616*	*2,222*	*3,006*
	6.8	3,971	3,434	*2,030*	*1,948*			
	7.2	3,469	3,732	*2,472*	*2,246*	*1,927*		
Monocytes	6.0	872	*1,230*	963	*1,393*	*2,067*	*2,114*	*2,401*
	6.4	914	*1,619*	*1,479*	*1,753*	*2,954*	*2,575*	*3,221*
	6.8	1,123	1,463	1,251	1,879			
	7.2	1,058	*1,890*	*1,584*	*2,815*	*3,552*		

TABLE 1—*Concluded*

	INOCULATED WITH CULTURE GROWN AT pH	BASE-LINE	FIRST MONTH	SECOND MONTH	THIRD MONTH	FOURTH MONTH	FIFTH MONTH	SIXTH MONTH
Neutrophile-lymphocyte ratio	6.0	1.17	1.36	*1.87*	*1.57*	*1.63*	*1.87*	*1.90*
	6.4	1.15	1.00	*2.13*	*2.22*	*2.45*	*3.77*	*2.47*
	6.8	1.27	1.00	1.50	3.08			
	7.2	1.71	1.47	2.01	2.54	*3.63*		
Monocyte-lymphocyte ratio	6.0	0.236	*0.346*	0.434	*0.500*	*0.712*	*0.820*	*0.853*
	6.4	0.255	*0.438*	*0.653*	*0.723*	*1.307*	*1.387*	*1.113*
	6.8	0.313	0.446	*0.666*	*1.025*			
	7.2	0.372	*0.543*	*0.807*	*1.384*	*1.938*		

Note: Statistically significant deviations from the normal appear in italics. Values shown in ordinary type proved not to be statistically significant deviations from the normal.

TABLE 2

Efficiencies of blood cells and cellular ratios in showing abnormal values in tuberculosis

	PER CENT
Ratio monocytes-lymphocytes	89.47
Monocytes	78.95
Lymphocytes	73.68
Haemoglobin	63.15
Ratio neutrophiles-lymphocytes	57.89
Basophiles	52.63*
Eosinophiles	42.10
Neutrophiles	42.10**
Total white cells	36.84**
Erythrocytes	31.57

* Data open to question as base-line values were lower than those of Casey and Pearce, and of Thomas. Animals may have shown rise in basophiles due to influence of state of maturity.

** Less reliable than might be indicated, since some values were high and others low, indicating that both determinations are subject to variation from a variety of causes, such as tuberculosis of bone marrow, secondary infections, etc.

ratio were invariably elevated following inoculation, whereas only sixteen of the values for the neutrophile-lymphocyte ratio were elevated. The greater efficiency of the monocyte-lymphocyte ratio is therefore borne out by the mean values without resort to statistical formulae.

Table 2 lists in order the efficiency values for each of the blood cells, the haemoglobin, and the two cellular ratios in showing abnormal changes in tuberculosis. These values were obtained by dividing nineteen, the

whole number of mean counts of each type of cell following inoculation, by the number of these counts showing significant deviation from the normal mean, and expressing the result as per cent.

DISCUSSION

There are certain fundamental differences between experimental tuberculosis and pulmonary tuberculosis in man. Fortunately the human being is apparently more resistant than the usual experimental animal. Many humans recover, but inoculation of animals with virulent microorganisms invariably results in death. Moreover, the disease in experimental animals usually becomes generalized in contrast to that in the human, which most frequently involves the lungs and lymph nodes draining them. Furthermore, after intravenous inoculation of virulent microörganisms, experimental animals invariably exhibit lesions in the bone marrow. Such lesions in man occur with less regularity. In view of these facts, it is our opinion that the experimental disease in our two groups of animals receiving the more attenuated microörganisms represented more closely the usual picture in man than did the two groups inoculated with the more virulent microörganisms. The latter have a clinical parallel only in the most rapidly advancing cases of pulmonary tuberculosis, or in patients with acute miliary tuberculosis. It is therefore clear that our experiments include individuals in which any test showing, with some regularity, a significant deviation from the normal may be considered a sensitive test. By the same token, any test showing significant deviation from the normal in all four of these groups of animals may be considered highly efficient and reliable. And if the deviations be consistent in one direction, either above or below the normal, it may be considered that intercurrent factors are less likely to exert an influence than if deviations occur in both directions from the normal. Therefore, the monocyte-lymphocyte ratio may be seen to be more sensitive and reliable as a detector for tuberculous infection than the neutrophile-lymphocyte ratio, the haemoglobin, or any of the blood cells alone. It exhibits statistically significant deviations more frequently and consistently than any of the above, and always in the direction of an increase over the normal. Minimal tuberculosis is capable of influencing the ratio significantly, and the magnitude of the change is roughly proportional to the severity of disease.

The efficiency of the monocyte-lymphocyte ratio in tuberculosis is due

to the fact that the lymphocytes and monocytes are the most sensitive of all the blood cells to tuberculosis, and that the changes in these cells are in opposite direction. While the monocyte proves to be the more sensitive of the two in that it shows change earlier, the decline in lymphocytes more closely parallels the decline of the individual resistance (therefore the advance of the disease) than any of the other cells. The fact that the monocyte-lymphocyte ratio has greater efficiency than the neutrophile-lymphocyte ratio is to be expected, since the denominator in the two is the same and the numerator of the one ratio, the monocyte, has greater efficiency than that of the other, the neutrophile.

In regard to the rôle of the neutrophile in tuberculosis, we are in agreement with Medlar, that the general trend, especially in advanced disease, is for the neutrophiles to increase. It is likewise true that, in the acute tuberculous lesions (41, 42) the neutrophile plays a more important part than in chronic or retrogressive lesions. However, in certain instances, namely, early, severe infection, the neutrophiles may diminish in number, which renders their efficiency less than if the change were in the opposite direction. This early decline in neutrophiles appears not to be caused wholly by lesions in the bone marrow, since all the cells having origin there are not equally affected. There may be neutropenia without anaemia, as shown by the mean values for neutrophiles and for erythrocytes in the group inoculated with the culture grown at pH 6.8 during the second month (table 1). Moreover, our results indicate that an increase in neutrophiles occurs only with well established or advanced disease, whereas the changes in monocytes occur earlier.

It seems probable, for reasons stated before, that the elevated values we have obtained for basophiles are less significant than is apparent. It appears likely that the basophiles are little changed in tuberculosis.

The decline in number of eosinophiles cannot be explained due to lack of knowledge of the function of these cells. While not a sensitive indicator, the decline is quite characteristic in advanced tuberculosis; it does not occur in very mild tuberculosis.

Our results indicate that the anaemia in tuberculosis is of the microcytic type, since the decline in haemoglobin is proportionally greater than the decline in erythrocytes. Also the haemoglobin is more sensitive than the erythrocytes, abnormal values being obtained when the number of erythrocytes was in the normal range. The lesions to be found in the bone marrow seem wholly inadequate to account for the anaemia and leucopenia which occur in severe infection. It seems more likely

that the anaemia and leucopenia are due to toxic effects of products of the microörganisms, as indicated by Sabin, Doan and Cunningham (20).

The report of Boissevain, Forster and Good (44) records results at variance with most recent studies of the blood in tuberculosis. It seems likely that their failure to confirm earlier reports is due to three facts, namely, their series of cases was very heavily weighted in favor of those with far advanced disease (1,171 of 1,569 counts); the questionnaire method of ascertaining the ultimate condition might be open to serious question; and the number of counts per individual was so small that little information could reasonably have been expected. (Their report includes statistical analyses of 1,569 counts of the blood from 431 patients, an average of less than four per patient, taken over a period of 10 years.) We believe, as do others (28), that if blood studies in tuberculosis are to be of value they must be repeated at relatively frequent intervals.

The results which we have obtained indicate that studies of the blood cells are of great value in tuberculosis. While the blood picture can hardly be said to be pathognomonic, it should be of considerable aid in diagnosis. But perhaps its greatest value is in prognosis, since the quantitative changes in the cells are progressive with advancing disease. We have laid no stress on qualitative changes which we believe to be of equal importance. Profound morphological and physiological changes are known to occur among the monocytes (19, 20) and neutrophiles (1, 2, 3). Wiseman and Doan (43) have also demonstrated qualitative changes in the lymphocytes during tuberculous infection. Since each change in the blood cells is an expression of some particular phase of the pathological picture, it seems that careful consideration of each quantitative and qualitative change is more likely to be informative than any blood index in which the identity of such changes is hidden by an arithmetical formula.

SUMMARY

Carefully controlled studies of the blood cells were made in groups of rabbits subjected to tuberculous infection of four grades of severity. The most significant changes noted were as follows.

1. The monocyte-lymphocyte ratio is a more sensitive indicator of tuberculous infection than any of the blood cells alone or the neutrophile-lymphocyte ratio.

2. Of the individual cells, the monocyte is most sensitive in that it is first to show significant change, but the lymphocyte is the more reliable

for prognosis, as the degree of decline is roughly proportional to the severity of the infection.

3. When anaemia occurs in tuberculosis, it is of the microcytic type. The haemoglobin is more sensitive to tuberculous infection than are the erythrocytes.

4. There is a tendency, especially with advanced disease, toward a rise in neutrophiles and a decline in eosinophiles.

5. The basophilic granulocytes are probably little affected in tuberculosis.

6. In very acute tuberculous infection the blood cells may fail to show the changes which are so characteristic of more slowly progressive disease. In such instances the decline in lymphocytes may be the only significant quantitative change.

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