THE RHYTHMIC RANGE OF THE WHITE BLOOD CELLS IN HUMAN, PATHOLOGICAL LEUCOPENIC AND LEUCOCYTIC STATES, WITH A STUDY OF THIRTY-TWO HUMAN BONE MARROWS.

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In an attempted interpretation of the occasional unexplained inconsistencies in clinical blood counting it has been suggested (1) that there is normally a more or less rhythmic variation in the numbers of white blood cells from moment to moment in each individual. In general the total number of white blood cells has been found to vary, within physiological limits and without abnormal external or internal stimuli, 100 per cent during the course of a day in a single, apparently normal, adult individual. In other words, rather than a relatively fixed individual "norm" or concentration of circulating cells, our concept of the normal for each person now is a wide zonal range, with a fluctuation for example of from 4000 to 8000 or from 6000 to 12,000 white blood cells, any chance single count being the resultant of at least three variables, the person, the physiological conditions of the moment, and the time of day of the observation. A. F. Bernard Shaw (2) has recently confirmed this concept and has carried his observations further by describing diurnal tides of the leucocytes in man, finding consistently two low points during the 24 hours, one between 9 and 11 in the morning, the other between midnight and 2 a.m., entirely independent of starvation, food, rest, exercise, or sleep. Bushnell and Bangs (3) in surveying the large individual variations in both white and red cells occurring in normal rabbits, conclude from their mean of 10,675 that ± 4800.75 must be taken as the limits of normal for determinations of the white cells, and from a mean of 5,989,500, $\pm 1,682,100$ for the range of normal for the red cells. In one normal

animal daily and hourly counts gave as extreme limits for the white cells 10,200 and 13,600, for the red cells 4,322,000 and 6,976,000. For a review of the literature and an analysis of the normal rhythmic variations of the white cells the reader is referred to the three articles above mentioned.

The question which arises immediately in the mind of the clinician, habituated to depend more or less upon the leucocyte count in disease, is as to the dependence to be placed in single counts in the light of the demonstrated 100 per cent, normal individual fluctuation. To the end that we might possibly determine something concerning the mechanism of, and range for, the white blood cells in disease the series of studies here presented were undertaken. We have been guided in our experimental observations by an attempt to answer the following questions: Are the observed variations in total numbers of white blood cells in the same individual from time to time fancied or real? How large a factor is the error inherent in the technic of counting? Can rapid changes in blood volume account for the variable counts? How often and how regularly do the changes come? In short, is there an analyzable inherent rhythm to the fluctuation in the total white blood cells in disease, and do the different types of white cells maintain independent rhythms? If so, do they parallel the established normal variants, *i.e.* is a leucopenia simply the manifestation of a depression, a leucocytosis the result of an accentuation, of the normal mechanism? Is it important to do more than one white count for an accurate working knowledge of the available cells at any one period in the course of a disease, and, if so, how many and at what intervals should the repeated observations be made? Can we arrive at any idea of the rate and manner of delivery of cells from the bone marrow by an analysis of consecutive observations on the peripheral blood at 15 minute intervals? In summary, are the apparent fluctuations in total numbers of white cells actual changes in the absolute numbers of potentially available circulating units, or do such changes represent technical, observational inaccuracies plus a vasomotor, redistribution phenomenon with percentage importance only? Entirely irrespective, however, of the desire to understand and to explain the mechanism in these observations, is the fact that controlled consecutive counts do register large variations which must

be taken into consideration when the leucocyte count may be an important factor in diagnosis or prognosis and treatment in disease.

We have studied a wide series of clinical conditions in which both leucopenia and leucocytosis of varying degree have existed. The twenty selected cases presented in this report are but representative of similar findings in a much larger survey of cases, but in which the data are less complete. As a routine each patient has been followed for a period of 3 hours, at intervals of 15 minutes, certain cases having been observed on successive occasions for similar unit periods. This permits of the construction of a graphic record with a more accurate interpretation of the limits and meaning of the variations. An automatic lancet was used, thus theoretically insuring consecutive samples of freely flowing blood from the same depth each time. A different finger was used for each observation, or a different portion when it was necessary to use the same finger again toward the end of a 3 hour experiment. The order of procedure was as follows: first drop discarded, supravital preparation, cover-slip spreads for Wright's stain, total white blood cell count, total red blood cell count, hemoglobin, hematocrit, serum protein. In the complete studies two of us were making the preparations, each taking the same type of sample each time, so that usually within 2 minutes all tests had been secured and the individual personal error in each particular series should have been fairly constant. In several of the cases a double check of the total white blood cell count was made by two persons independently to determine the error inherent in this technic. The same Bureau of Standards equipment was used throughout in each series of observations. The hemoglobins were read in a Duboscq colorimeter with the Newcomer standard. The hematocrit determinations were made with the Van Allen pipette (4). Refractometric readings were made for serum protein determinations. Our problem has not been concerned so directly with the establishment of exact absolute values for hemoglobin, serum protein, and hematocrit readings as with the relationship existing between consecutive *ad seriatum* determinations made under identical, standardized, closely controlled conditions and bearing to each other a definite time relationship. This might be expected to give at least the relative variations in a given determination over the period of observation. In some instances a 100 cell supravital differential count was made, with vital neutral red (5, 6); in others cover-slip preparations were made with Wright's stain and 200 cells counted by two observers from both cover-slips and the average percentages taken; in some cases both supravital and fixed film observations were made and compared. We have found that while it is essential to count larger numbers of cells and average the counts from both cover-slips to obtain accurate data from fixed films, with the supravital preparations, in which the whole drop of blood is evenly and uniformly spread out, 100 white cells counted will give in the majority of instances the same percentage incidence of cell types as a larger number of cells counted or as is given by a count of two consecutive preparations by two different individuals. In certain of the cases we have made an

Arneth differential count uniting for charting the non-lobulated and two lobed nuclei as significant of the younger mature polymorphonuclear neutrophils, the three and four lobed nuclei as fully mature, and, those having five or more lobes as indicating old cells. In the supravital preparation we have differentiated the actively motile leucocyte from the round inactive form and from the specific "non-motile" neutrophil of Sabin (5), the last being interpreted as the morphological state denoting the physiological death of the cell.

In addition to the series of twenty cases, which form the basis of the clinical observations herein analyzed, differential cell counts of the bone marrow have been

TABLE I.

Case	Age	Sex	Diagnosis	W.B.C.	Range 3 hr. period with 15 min. counts
	yrs.				
1 (T71)	35	M.	Chr. osteomyelitis	Leucopenia	1,400– 2,800
2 (T44)	40	"	Primary anemia	"	2,900– 5,300
3 (T5)	65	"	" "	Low normal	4,000– 6,500
4 (T86)	25	"	Ac. rheumatic fever	Normal	4,200– 7,000
5 (T87)	22	"	" " "	"	4,200– 8,000
6 (T89)	25	F.	" " "	"	4,400– 10,200
7 (T111)	30	"	Erythrodermia	Leucocytosis	7,400– 15,100
8 (T81)	75	M.	Chr. cystitis	"	9,500– 15,500
9 (T88)	24	F.	Ac. rheumatic fever	"	8,800– 16,500
10 (T90)	18	"	Chr. otitis media	"	10,800– 16,500
11 (T41)	40	M.	Lobar pneumonia	"	10,000– 20,000
12 (T4)	55	"	Polycythemia vera	"	10,500– 23,000
13 (T92)	40	"	Amebic dysentery	"	14,700– 24,600
14 (T78)	16	F.	Pertussis	"	17,000– 25,000
15 (T82)	15	M.	Rheumatic endocarditis	"	16,000– 32,000
16 (T109)	30	"	Lung abscess	"	23,000– 34,600
17 (T110)	40	"	Septicemia (strep.)	"	23,900– 34,700
18 (T63)	30	"	Lobar pneumonia	"	50,000– 75,000
19 (T70)	60	"	Chr. lymph. leucemia cutis	"	35,000– 52,000
20 (T112)	45	"	" myeloid leucemia	"	208,000–284,000

made from thirty-two selected cases coming to autopsy in the Pathological Department of the Boston City Hospital. This has given additional independent evidence in confirmation of the probable part played by the bone marrow in white blood cell fluctuations, more spectacularly in the leucocytoses. These latter preparations were made originally (L.G.Z.) with an entirely different end in view and only on subsequent analysis was it seen how strikingly an interpretation of these data corroborates the rhythmic concept developed from a study of the peripheral white cell fluctuations in health and disease. In the postmortems performed within 5 hours after death, supravital differential studies were made to

check with the fixed films (7). However, in the majority of cases the counts were taken from fixed preparations. In all instances, except as specifically indicated, the femoral bone marrow was used. Occasionally it was convenient to examine

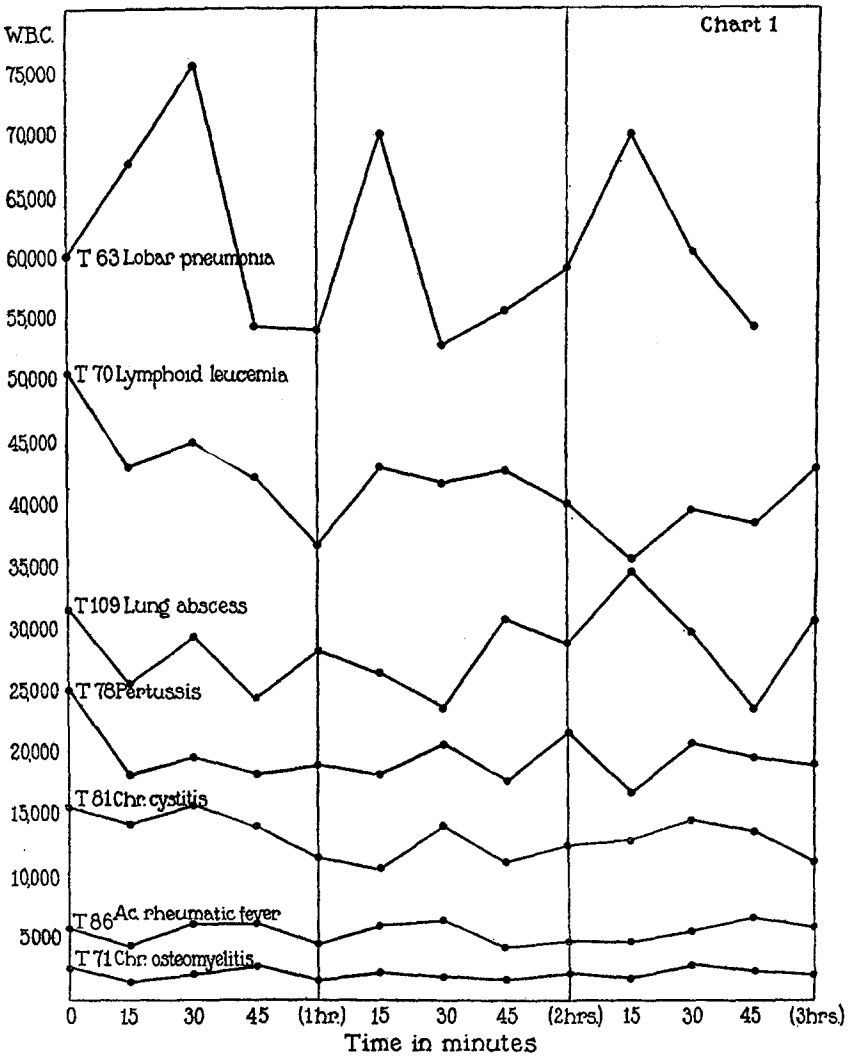


CHART 1. Fluctuations in total white cell counts.

vertebral marrow. In the empyema cases, the marrow from ribs removed at operation formed the basis for the counts. All fixed preparations were made as follows: A small piece of bone marrow was emulsified in 2½ per cent sodium citrate, and centrifuged at high speed (2600 R.P.M.) for 10 minutes. The supernatant

fluid with the fat was pipetted off and the cells were rewashed three times with sodium citrate, the suspension being centrifugalized each time. The washed cells were then suspended in a few drops of autogenous serum, from which coverslip preparations were made. After drying in the air, they were stained with Wright's stain and mounted in balsam. No fewer than 1000 cells were counted in any case and as many as 2000 cells were counted in some instances, by two observers, and the average percentage recorded.

In Table I will be found the clinical cases on which at least one 3 hour period of observation was made. The patients' age, sex, diagnosis, and range of total white blood cells during the 3 hour period are given. In Chart 1 are to be seen graphic representations of the white blood cells in selected cases showing the variations in the total white cells at different levels—from a leucopenia of from 1400 to 2800 cells to a leucocytosis of from 50,000 to 75,000.

Controls in the Establishment of the White Cell Variations.

In the attempt to establish in so far as might be possible the nature of the apparent fluctuations in the total white cell count, coincident and simultaneous observations of the total red blood cells, hemoglobin, hematocrit, and serum protein were made, all or in part, in five cases, as recorded in Charts 3A, 8A, 10A, 11A, and 12A. The curves of the red cells, hemoglobin, and hematocrit tend to follow each the other but with no relationship to the total white cell curve. The serum protein produces the most constant finding, the very slight changes noted in Chart 12A bearing no relationship to the fluctuations in the other curves, and in Chart 8A there was a constant reading throughout the whole period except for one slight rise. It has been generally accepted that the serum protein (8), under normal basic, fasting conditions is one of the most constant constituents of the blood, relatively, and by pediatricians, in particular, has been used extensively as an index of dehydration. This fact, together with the ease of refractometrically determining its per cent on small samples of blood caused us to decide to make these consecutive determinations.

Table II summarizes the range of total red blood cells and hemoglobin observed in selected cases in this series. Roughly, the hemoglobins run in percentage variation about half that of the cells, *i.e.*, 1 per cent variation in hemoglobin is the equivalent of a 2 per cent

variation in red cells. The tendency toward a relatively large variation between the minimum and the maximum count when consecutive observations on the same individual are compared, whether the time interval be in minutes or days, has been so constant in the experience of the last several years that, for the red cells too, we believe there is a normal, individual zonal fluctuation rather than an exact point in total count which it is necessary to consider. Thus when a single count, particularly in the female patient, reveals the total red blood cells near 4,000,000 it is necessary to know whether that figure represents a minimum, the average, or a maximum point in the physiological range for that individual. As is not infrequently the case,

TABLE II.

Red Blood Cells.

Range during 3 Hour Period with Counts at 15 Minute Intervals.

Case No.	Minimum count	Maximum count	Hgb. range	R.B.C. var.	Hgb. var.
			per cent	*per cent*	*per cent*
3 (T5)	3,616,000	4,808,000	74– 85	24	13
10 (T90)	4,112,000	5,232,000	90– 96	21	6
12 (T4)	5,760,000	6,470,000	125–130	10.9	4
13 (T92)	3,696,000	4,624,000	64– 72	20	11
14 (T78)	4,880,000	5,632,000	83– 89	13	7
Normal individual (R44)	5,230,000	6,180,000	79– 88	15	10

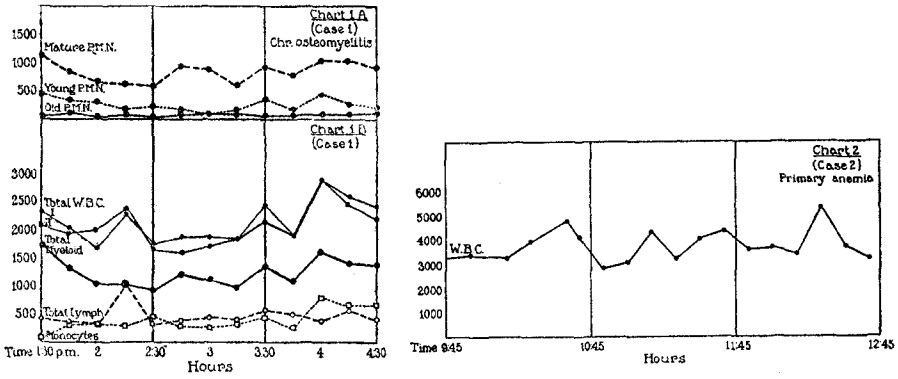
it may well be only the lower limit of a normal swing in the count for that patient if subsequent counts register 4,500,000 to 5,000,000. Only a consistent trend upward or downward in both hemoglobin and total cells as determined in repeated observations on the same individual over a period of days may provide a reliable basis for an estimate of the state of equilibrium, or the lack of it, in the supply of red cells from the hemopoietic organs. The lack of correlation between the curves of the serum protein, the white and the red cells in the present series of cases minimizes the factors of blood volume and circulatory disturbance as solely responsible for the observed variations. Rather the now well recognized reservoir for red cells in the spleen (9) and the known periodic delivery of new cells from the bone marrow (as re-

vealed, for example, in "normoblastic crises") must be considered as potential factors of importance.

Leucopenia.

The lowest maintained white blood cell count which was observed during the year was that of Case 1 (Charts 1A and 1B) in the series.

The diagnosis of chronic osteomyelitis of the right tibia was made. Repeated white blood cell counts made singly on successive days during the patient's course in the Hospital consistently registered below 4000. During the 3 hour period of the consecutive 15 minute observations the lowest number of white cells counted



was 1350, the highest 2850. In this instance two of us took white blood cell counts simultaneously and counted them independently in an endeavor to check the error inherent in the technic of counting (see also Charts 7B, 17A). Because of the marked leucopenia blood was drawn to the 1 mark in the pipettes instead of the usual 0.5. The greatest single variation from the two pipettes was 700 cells; the greatest variation in the 3 hours, 1500 cells. Thus the maximum error attributable to technic in this particular case is 47 per cent. However, the majority of the counts checked within a narrower range and the technical error in general where the total number of cells is larger may be depended upon not to exceed 10 per cent. The actual cell differences in the two counts each time are as follows:

400	250	500
690	650	0
700	250	25
160	100	225
		525

Obviously the smaller the number of cells the greater the percentage of error in the counting on the basis of the computations necessary in the present method of estimating total cells per c.mm.

At such points as the counts taken at 2.00 and at 2.45, where instead of the usual paralleling of the total cell change in the two pipettes the two counts diverge slightly, the average of the two, of course, gives a concept no nearer the true count than either of the extremes. Consequently such results admit of no analysis or interpretation. It is only in counts such as those at 3.45 and 4.00 where the changes are relatively marked and registered in both observations that one feels justified in trying to explain the variation on some basis other than technical. The practical importance of this point is, of course, only apparent in later cases with higher counts.

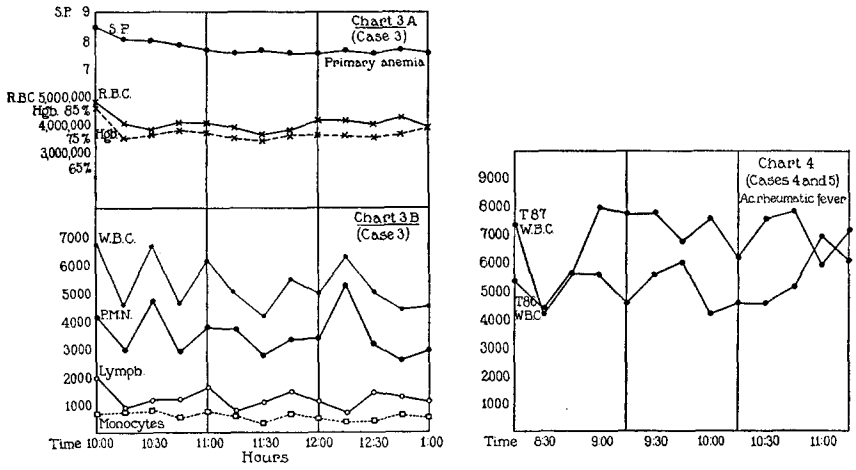
On Chart 1B are outlined the absolute numbers of myeloid, lymphoid, and monocytic cells, based on differential white blood cell counts on fixed preparations stained with Wright's stain, and the average total white cell counts. It will be noted at once that these three types of the white cells vary independently of each other. The increase of lymphocytes at 2.15 is responsible for the peak in the total white cell curve at that moment. The two elevations at 3.30 and 4.00 follow increases in the absolute numbers of myeloid cells, and for the 3 hour period there are four elevations roughly speaking for this group. The monocytes in this case illustrate the tendency to a 45 minute or hourly rhythm which we have noted in a number of cases. Monocytes also vary, rising and falling over periods of several days, as in typhoid fever and tuberculosis. This independent variation in the percentage and total numbers of the three types of white blood cells is the first evidence against a simple dilution explanation for the variations in the total cell count. It will be seen later and more strikingly in other cases. This case may illustrate also the fact that there is a tendency, as found in the normal, to a higher late afternoon white count since the only two counts over 2500 came after 4.00 p.m. The relative proportions of the three types of cells are approximately normal, *i.e.* there is not the relative lymphocytosis frequently seen in leucopenia. However, in so low a total count as is this, it is reasonable to expect the lymphocytes to influence more readily the total count at times. Thus the two groups of cells must be taken into account as mutually potentially influential on the total count in the leucopenic state.

Shaw (2) found, in single determinations in 116 healthy male adults, counted uniformly between 9 and 10 a.m., a percentage of lymphocytes equal to or exceeding the percentage of neutrophils in 10 persons, though subsequent observations in the same individuals usually gave the general excess of neutrophils. An interesting confirmation of the transitory nature of this lymphocyte preponderance was revealed in two of his studies on the diurnal tides, in which the percentage of lymphocytes temporarily exceeded the percentage of neutrophils for some hours. While the lymphocyte curves are not given in the paper, his analysis of the differential counts showed the fluctuations to be largely in the neutrophil group, so that it may be assumed that the transitory lymphocytoses were relative, due

rather to the decrease of granulocytes than to any marked increase in absolute numbers of lymphocytes (10).

Chart 2 (Case 2) is representative of a leucopenic range of from 2900 to 5300 white blood cells in a 3 hour period of observation on a case with the diagnosis of pernicious anemia. In this particular instance the counts were taken at 10 minute intervals to see if any marked difference in the nature and range of the fluctuations could be discerned. Four elevations alternate with lower counts between, two peaks coming within the 2nd hour. This is not an infrequent observation, even though the usual variations at 15 minute intervals over a long period show but one rise on the average each hour.

The second case of pernicious anemia with a relatively low white count showed a fluctuation of from 4000 to 6500 white cells during the 3 hours. Charts 3A and



3B graphically depict the relationship which was found to exist between the total white blood cells, red blood cells, hemoglobin, and serum protein. Two independent series of total white counts were made again for a check on the error of counting, though not charted. It will be seen at once that the white blood cells fluctuated much more widely than, and entirely independently of, the red blood cells, the hemoglobin determinations closely paralleling the latter. The serum protein dropped gradually during the 1st hour, thereafter showing only very slight variations, between 7.55 and 7.67 per cent. On first glance it would appear that the differences in all determinations between the first and second observations, 10.00 and 10.15 a.m., could be accounted for through blood volume increase with dilution and resultant lowering of serum protein, white and red blood cells, and hemoglobin. However, the percentage change when all are reduced to the same common denominator shows the white blood cell change to be 30 per cent, red blood cell 20 per cent, hemoglobin 12 per cent, and serum protein 8.2 per cent.

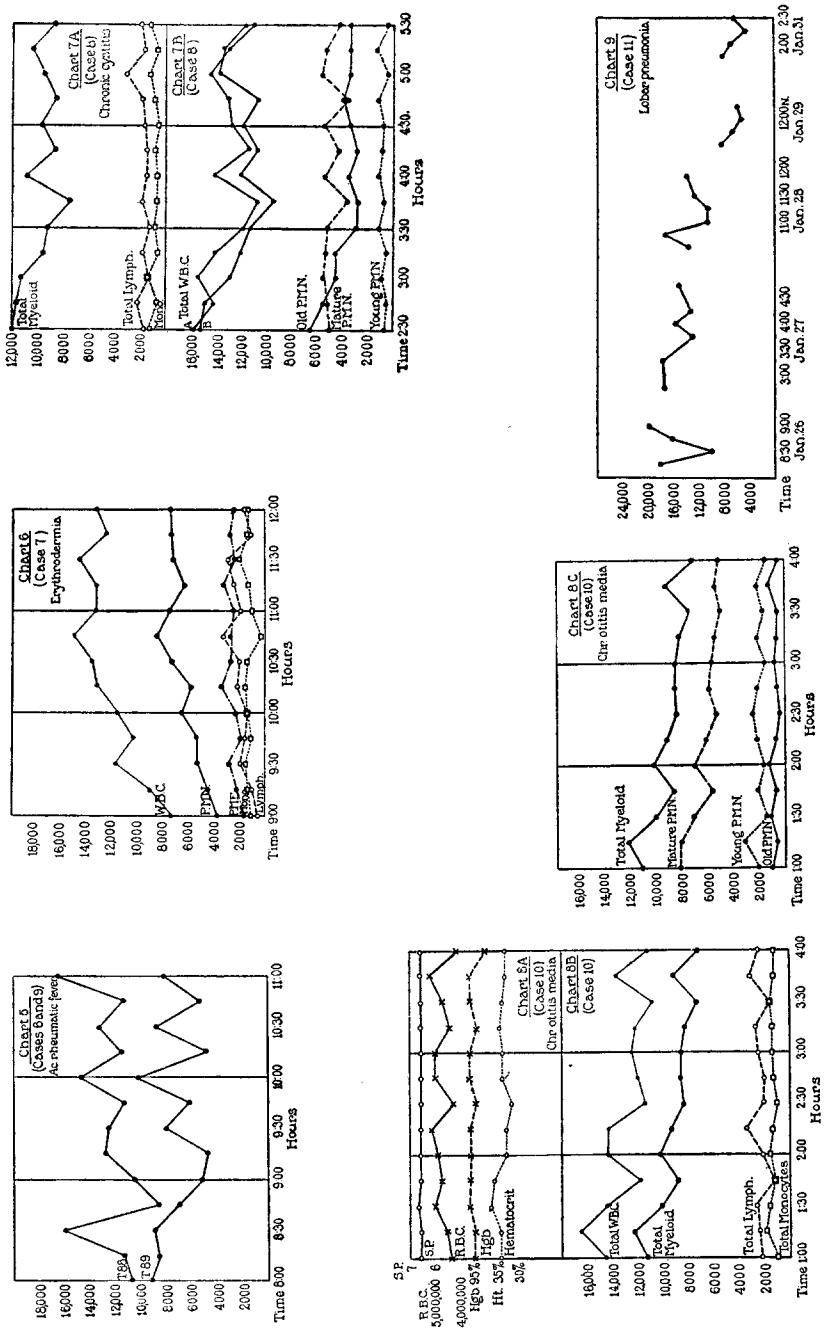


Chart 5
(Cases 6and9)
Ac rheumatic fever
T 99
T 69
Time 8:00 8:30 9:00 9:30 10:00 10:30 11:00
Hours
Chart 6
(Case 7)
Erythrodermia
W.B.C.
P.M.N.
L.
E.
M.
Time 9:00 9:30 10:00 10:30 11:00 11:30 12:00
Hours
Chart 7A
(Case 6)
Chronic gonitis
Total Myeloid
Total Lymph.
Mono.
Chart 7B
(Case 8)
A Total W.B.C.
B
Old P.M.N.
Mature P.M.N.
Young P.M.N.
Time 2:30 3:00 3:30 4:00 4:30 5:00 5:30
Hours
Chart 8A
(Case 10)
Otitis media
S.P.
R.B.C.
Hgb
Hematocrit
Chart 8B
(Case 10)
Total W.B.C.
Total Myeloid
Total Lymph.
Total Monocytes
Time 1:00 1:30 2:00 2:30 3:00 3:30 4:00
Hours
Chart 8C
(Case 10)
Otitis media
Total Myeloid
Mature P.M.N.
Young P.M.N.
Old P.M.N.
Time 1:00 1:30 2:00 2:30 3:00 3:30 4:00
Hours
Chart 9
(Case 11)
Lobar pneumonia
Time 8:30 9:00 3:00 3:30 4:00 4:30 11:00 11:30 12:00 12:00n. 2:00 2:30
Jan. 26 Jan. 27 Jan. 28 Jan. 29 Jan. 31

Nevertheless, should such parallel changes in the same direction always occur in all the fluctuations, it would be justified to attribute small discrepancies in actual identical percentages to the error indisputably a part of each technical procedure. But this has never been found to be consistently the case. As in the instance in point, Chart 3A, the serum protein falls directly to a relatively fixed level and may be correlated with the fact that the patient was not on a fasting base line.

Chart 4 represents two series of white counts which might well serve as an example of the range of variation in individuals with a low normal total count, yet both patients had acute rheumatic fever, not yet treated, Case 4 having two active joints at the time of the count. Three elevations in the total counts may be noted in each series, during the 3 hour period. Chart 5 carries two acute cases under treatment, with the same diagnosis but with the counts in Case 9 definitely above the normal range, and each showing in the first 2 hours two peaks but in the 3rd hour two half-hourly peaks. With the recognition of the physiological fluctuations in the white cells, the influence of treatment (11), but the possible presence of activity without elevation in the total count, prognostic deductions based on small variations in the total white count in acute rheumatic fever should be conservative.

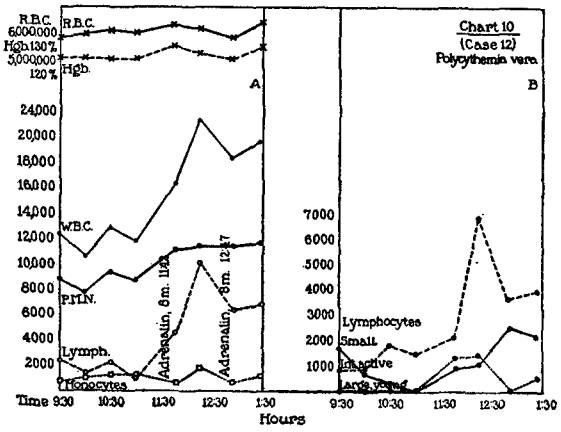
Leucocytoses.

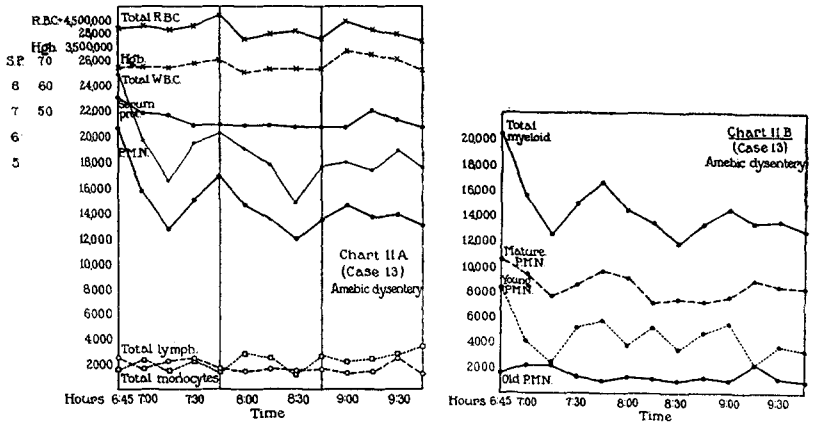
Chronic.—It is of interest to note the differences appearing in the chronic and acute leucocytoses. The chronic state is represented by Cases 8 and 10 as graphed in Charts 7A and B, and 8A, B, and C.

The case, No. 8, of chronic cystitis was running a white count between 9500 and 15,500, the chronic otitis media, Case 10, 10,800 to 16,500. There is a definite hourly rhythm in both cases, and the low points reached in each had they been single observations would have been equivocal in so far as a leucocytosis is concerned, particularly since they were chronic conditions and the adjustment of the bone marrow had been made on the new level. Charts 7B and 8C indicate the Arneth differentials which show a low level of young and old white cells, with the majority mature forms, even as is the case in the normal; in contrast, in the acute leucocytoses, the Arneth "shift to the left" is constant. Lymphocytes and monocytes are within their normal limits, the neutrophil group being entirely responsible for the elevation in total white count.

It will be seen strikingly again and again that particular uncomplicated pathologies stimulate independently one or another of the cell types and that the response of the organism is either limited entirely to this specific pathognomonic reaction or so predominantly to the one that the slight relative increase in the other types is obviously not a part of the specific response. Hickling (12) has pointed this out

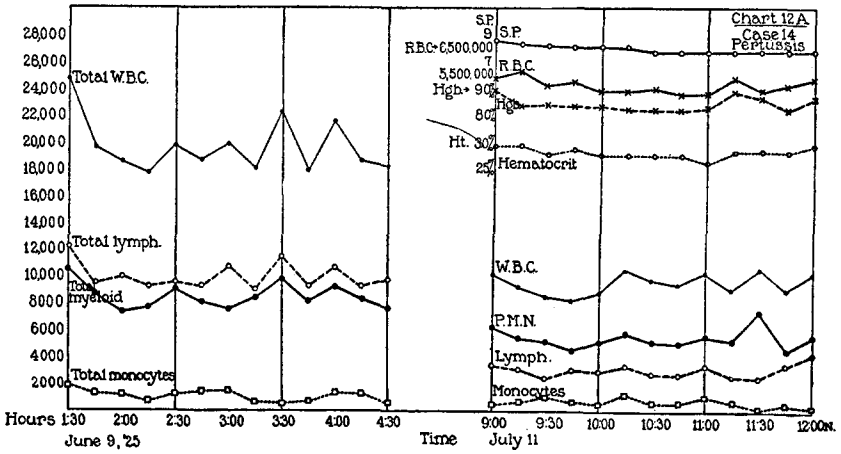
in a clinical and experimental study of toxic and chemical stimuli on the circulating leucocytes. He found a specific neutrophilic response to the presence of bacterial infection with the degree of postinfective lymphocytosis dependent upon the extent of tissue injury. In the

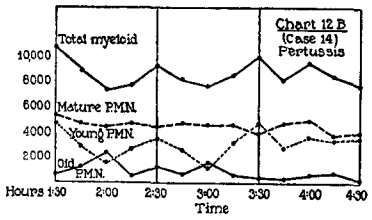




particular response as analyzed in the present series, the specific cell type maintains its original rhythm as will be seen in Chart 10A with lymphocytes elevated after adrenalin, in Chart 12A in which the lymphocytes are elevated in a case of pertussis, in Chart 6 in which there is an eosinophilia with erythrodermia, and in Chart 16A giving a neutrophilia typical of lobar pneumonia.

Acute Leucocytoses.—The group of acute leucocytoses secondary to infection are represented in six cases, two with diagnoses of lobar pneumonia, one with amebic dysentery, one with rheumatic endocarditis, one with lung abscess, and the last with streptococcus septicemia. All show the specific polymorphonuclear neutrophil increase with the "shift to the left" in the Arneth differential pattern, the younger neutrophils both showing a higher percentage than is found



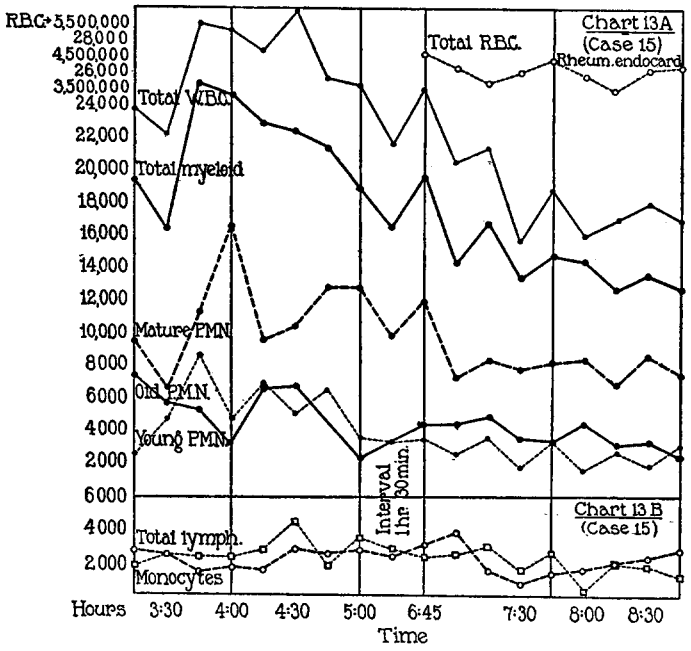


normally and the high points in the consecutive counts being seen to be a reflection of periodic increases in these forms.

Charts 11B, 14, and 15 indicate this particularly clearly. Chart 13A which combines an afternoon period with a later evening period of observation on the same day indicates the high late afternoon total count with a marked "shift to the left" of the Arneth differential and an evening count decidedly lower reflecting a lowered percentage of young cells. The lymphocytes and monocytes maintain essentially undisturbed total numbers throughout both series of counts, and the

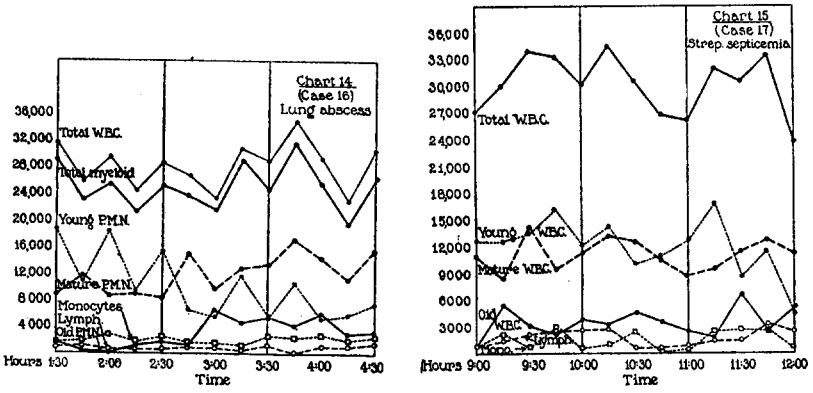
red cells during the later period show no consistent downward trend as do the white cells.

Chart 9 graphically presents the total counts done on consecutive days in a case first seen on the evening of the 3rd day of the disease. The patient gave a history of sudden onset of symptoms typical of lobar pneumonia occurring at 9.30 a.m. January 24. He arrived at the Hospital on the 26th without definite physical signs in the chest, with a temperature of 102.5°, pulse 100, and respirations 35. The white count on admission was 8000. It happened to be a period when influenza, rather than lobar pneumonia, was prevalent, so that the low total white count and physical findings in spite of the history seemed to coincide better with

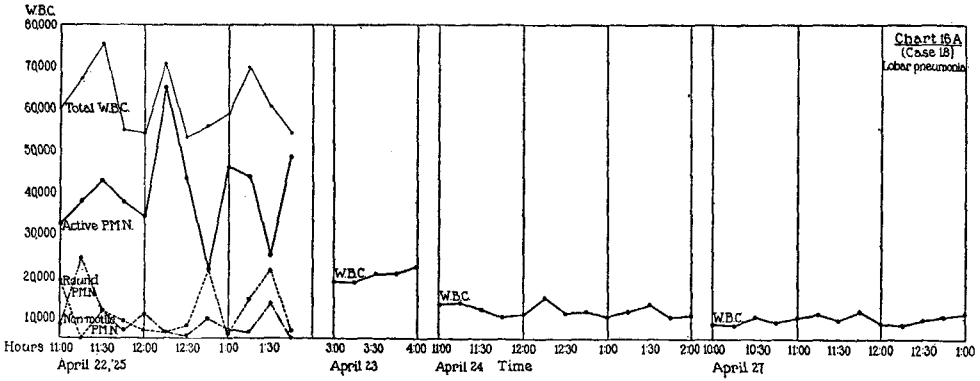


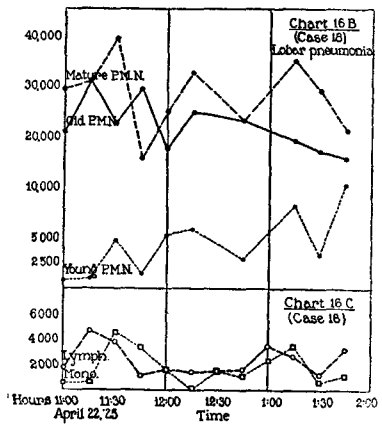
the former diagnosis. Within an hour the four counts taken at 15 minute intervals and recorded on Chart 9 had been taken. It will be seen that one of the four counts was 10,000 but that the other three were all above 16,000, one going to 20,000. Both the 8000 and the 10,000 total white blood cell counts were in all probability accurate determinations of the numbers of these cells in the peripheral blood at the moment those samples were taken, but represented only the lowest swings of an arc, which was periodically at least, indicative of a definite pyogenic stimulation. In this case there was never the marked leucocytosis seen in Case 18, but a lobar consolidation developed and a typical crisis came on the 5th day with the fall to normal in the total white count as shown under date of January 29 on the chart.

Case 18 (Chart 16A) presented the typical history and picture of a severe, uncomplicated lobar pneumonia, with complete consolidation of the right lung, temperature, at 1.00 p.m. April 22, the day of the first count, 106°, pulse 150, respirations 50. The crisis came during this afternoon, the 4.00 p.m. temperature being 100° and the 8.00 p.m. temperature 98°. The first 3 hour count, therefore, represents the maximum mobilization of the cellular defense in this patient just prior to, and almost coincident with, his crisis. It will be seen that there were three high points, one each hour, at which the total count reached or surpassed 70,000 per c.mm., but that seven of the twelve counts were between 50,000 and 60,000. The supravital differential counts revealed a high percentage of actively motile neutrophils, particularly at the moments of the highest counts, with the round and "non-motile" forms, indicating injury and cell death, relatively few in number, except at the periods of lowest ebb in the count. This is in correlation with the periodic "non-motile showers" (1) found in the counts on normal in-



dividuals. The same prognostic significance we believe is probably attached to this observation as Pons and Ward (13) attribute to the leucocyte fragility test in pneumonia, *i.e.* the larger the percentage of living cells the better the prognosis. Mauriac and Moureau (14) also analyze the curves of fragile cells most instructively. Chart 16B shows peaks in the young and mature neutrophils (Arneth differential on fixed films) corresponding with the peaks of total cells and with the high points in numbers of active cells in the supravital preparations. Chart 16C shows a normal number of lymphocytes, the pneumonic process having had no influence on this strain of cells; the monocytes show fluctuations from a normal level to a considerable increase over the usual percentage of these cells in normal blood (15). Chart 16A includes the counts taken on successive days following the crisis, showing totals ranging from 18,000 to 22,000 during only five observations on the 23rd, 10,000 to 14,000 on the 24th, and on the 27th, 6000 to 12,700. The last two periods, as the first, show the tendency toward an identical hourly fluctuation in the total cells. Whereas the polymorphonuclear neutrophils during


WBC.
60,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
Total WBC.
Active PMN.
Rajuid PMN.
Mononuclear PMN
WBC.
WBC.
WBC.
Chart 16A
(Case 18)
Lobar pneumonia
Hours 11:00 11:30 12:00 12:30 1:00 1:30 3:00 3:30 4:00 11:00 11:30 12:00 12:30 1:00 1:30 2:00 10:00 10:30 11:00 11:30 12:00 12:30 1:00
April 22,'25
April 23
April 24
Time
April 27


40,000
30,000
20,000
10,000
5 000
2 500
Mature PMN.
Old PMN.
Young PMN.
Chart 16 B
(Case 18)
Lobar pneumonia
6000
4 000
2000
Lymph.
Mono.
Chart 16 C
(Case 18)
Hours 11:00 11:30 12:00 12:30 1:00 1:30 2:00
April 22,'25
Time

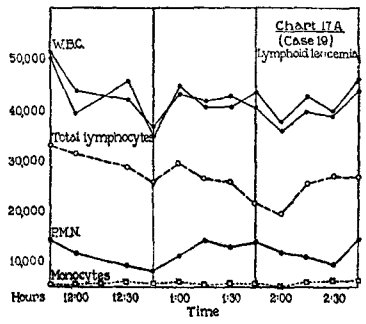
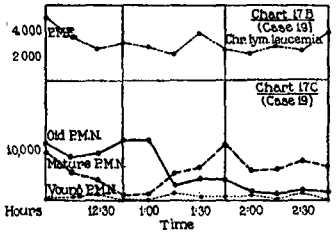
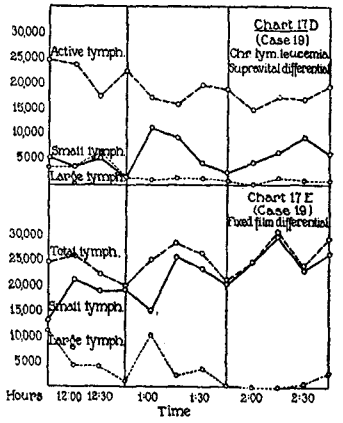


Chart 17A
(Case 19)
Lymphoid leucemia
WBC.
50,000
40,000
30,000
20,000
10,000
Total lymphocytes
PMN.
Monocytes
Hours 12:00 12:30 1:00 1:30 2:00 2:30
Time


4,000
2 000
10,000
PMN.
Chart 17B
(Case 19)
Chr lym leucemia
Chart 17C
(Case 19)
Old PMN.
Mature PMN.
Young PMN.
Hours 12:30 1:00 1:30 2:00 2:30
Time


30,000
25,000
20,000
15,000
10,000
5000
Active lymph.
Small lymph.
Large lymph.
Chart 17D
(Case 19)
Chr lym leucemia
Supravital differential
30,000
25,000
20,000
15,000
10,000
5000
Total lymph.
Small lymph.
Large lymph.
Chart 17 E
(Case 19)
Fixed film differential
Hours 12:00 12:30 1:00 1:30 2:00 2:30
Time

the period of the crisis were averaging from 89 to 98 per cent of the total count, on the 2nd and 3rd days thereafter they were 77 to 90 per cent, and on the fourth count 60 to 75 per cent. Thus the last counts made on the 5th day after the crisis showed the return to an entirely normal range of total white cells with the normal differential both as to supravital and Arneth classifications except for some increase in the monocytes, 8 to 14 per cent. Hickling (15) has just made a very interesting analysis of the monocytes in pneumonia, correlating their late rise in the course of the disease with resolution, and drawing certain prognostic conclusions from their curve from day to day.

Such an analysis, as here attempted, of successive white blood cell counts during a leucocytosis might indicate a periodic delivery of new cells from the hemopoietic organs, the intervening intervals of decreasing total circulating neutrophils being representative of their constant loss to the tissues at the site of the disease in their functional capacity of local cellular defense. The importance to the surgeon of the recognition of these rapid fluctuations in cases of an acute abdominal condition, with the advisability of frequently repeated counts when the diagnosis is in doubt, is evident. The degree to which the multiplication and maturation, of the particular cell type required for the body defense in a specific disease, may be speeded up is an approximate index of the cellular factor in the complex of the "resistance" of that individual (16, 17).

Lymphocytosis.

Case 14 (Chart 12A) presented an equivocal picture on admission to the Hospital with few symptoms, a low grade fever, a cervical adenopathy, and a moderate leucocytosis. The differential count showing a decided lymphocytosis was suggestive and the eventual development of the characteristic cough established the diagnosis. The 3 hour period of 15 minute counts in this case of pertussis reveals a curve of fluctuations in the lymphocytes quite distinct from that of the leucocytes. Because the lymphocytes are in total numbers actually in excess of the neutrophils the fluctuations in the former tend to be reflected in the total count. It will be seen that the usual hourly rhythm of the myeloid cells is maintained, that the peaks are made largely by the influx of young cells (Chart 12B), and that the lymphocytes fluctuate with each count, or on a half hourly swing, as was found for the lymphocytes in normal counts (1). The monocytes, eosinophils, and basophils are all within normal limits. 1 month after the first series of counts a second period of observation was made just prior to the patient's discharge from the Hospital. It will be noted that the total count had subsided to within normal limits, the lymphocytes having returned to normal percentage and number, and

the neutrophils once more reflect their normal predominance in the curve of the total cells.

Chart 10 is presented in further support of the independence, observed so frequently, in the specific response of different cell strains. The leucocytosis following the administration of adrenalin is essentially a lymphocytosis in which the rise is first of the large young forms and then in the mature intermediate and small lymphocytes (10B). This curve is representative of three similar cases, one being a normal individual. It would appear that the change in the white cells under these conditions is not the reflection primarily of a blood volume or redistribution phenomenon, but rather the result of a direct influence on the lymphocyte supply, or its physiological control. Rous (10) found in dogs that the tissues producing lymphocytes are "set" at a rate of activity definite for the individual but subject to wide variations under the influence of changing physiological conditions. Inasmuch as such physiological stimuli are always potentially interactive, in the normally functioning individual periodicity in the delivery of lymphocytes may be assumed on the basis of the experimental work.

It is difficult to escape the impression that there are independent physiological controls for maintaining the relative, normal levels of the different types of white blood cells (14, 18), just as there is a body, growth-limiting, mechanism, a heat-regulating center or mechanism, and a cardiac regulatory mechanism. The readjustment of the control of the normal level for a specific cell strain, while not influenced appreciably by body posture, vasomotor instability, or variation in local distribution (19, 20), nevertheless must be subject to many chemical and toxic influences (12, 16, 21, 22, 23), and may be independent of the actual supply of the particular cells, as was indicated in the case of Minot and Isaacs (24) in which, following a large transfusion of lymphoid leucemic blood into a patient with marked lymphopenia, the original low level of lymphocytes was regained within $2\frac{1}{2}$ hours without evidence of cell destruction and without constitutional reaction. This observation indicates an altered regulatory mechanism in this patient for maintaining a low level of circulating lymphocytes independent of the supply or of a primary lymphocytolytic factor. However, in the individual physiological mechanism, it is probable that the altered control factors are

usually interactive with the formation and maturation processes for the particular cells affected and respond to the general law of supply and demand. Obviously the above discussion does not relate to true cytolytic effects on the mature circulating cells of any strain, the reaction to which is usually a compensatory hyperplasia in the attempt to establish an equilibrium at the new level of turnover in the particular cell type, and is simply the effort to maintain the normal established level for the physiological functioning of that cell.

The Leucemias.

When a study of the variations in the peripheral blood in the leucemias is attempted, however, it appears that the laws which seem to govern formation, maturation, and delivery of the cells involved, under normal and other pathological conditions, no longer hold. No evidence in Case 19 (lymphoid leucemia), or in Case 20 (myeloid leucemia), of the periodic rhythmicity more or less characteristic of the curves of lymphocytic and myeloid cells discussed above, can be found, as perhaps could hardly be expected when the presence of large numbers of immature forms in the peripheral blood indicates an entirely disordered mechanism of maturation and delivery.

The 3 hour period of observation in the case of chronic lymphoid leucemia cutis gave a range of fluctuation in the total count from 35,000 to 52,000, and the lack of conformity in the curves of lymphocytes and neutrophils and the unaffected status of the monocytes can be seen (Chart 17A). However, it is impossible to establish any periodicity in the total lymphocyte curve within the time period recorded. Charts 17D and E indicate a relative relationship between the young immature cells and the total lymphocyte curve, both falling during the first 2 hours, and Chart 17D, compiled from the supravital differential counts, shows the large number of lymphocytes showing normal motility. The neutrophils maintain an undisturbed hourly elevation and fall (Chart 17A), and show an Arneth differential count within normal limits (Chart 17C). The increased eosinophils in this case are shown on Chart 17B, the rhythm being an hourly one though independent of the neutrophil fluctuations. The eosinophilia in the case of erythrodermia (Chart 6) is quite comparable and probably this specific response in both cases is related to the respective skin involvements. Cioni (25) discusses the mechanism of eosinophilia relative to the nature of the specific chemical stimuli altering the bone marrow supply of these cells, and believes that in the case of infectious skin conditions it may be due either to the metabolic products of the parasite or to products of the destruction of the epithelium, or to both.

The striking thing in the analysis of the fluctuations in the case of myeloid leucemia, with a range in the total count from 200,000 to 284,000 during the 3 hour period of observation, was the inverse relationship between the mature neutrophils and the myelocytes (Chart 18A). Since it was a chronic case, the myeloblasts were relatively few in all counts. With every increase in the number of mature leucocytes there was a corresponding fall in the myelocyte level, and *vice versa* (26). This was particularly striking in the curves of neutrophilic myelocytes and the young mature neutrophils (Chart 18B), an increasing divergence in these two curves being manifest toward the end of the period, the later total counts reflecting this rise in the myelocytes directly. The independent fluctuations of eosinophil, basophil, and neutrophil myelocytes show only the disordered chaos of the myeloid hyperplasia of the bone marrow, though the mature cells still show some semblance of a periodic fluctuation with three peaks during the 3 hours. In the leucemias it would seem that all regulation both as to primary formation and delivery and as to concentration of the cells in the peripheral blood was gone.

The Bone Marrow.

Any attempt at a quantitative estimation of the various cellular elements making up the bone marrow must, in the very nature of the survey, always be open to many questionings: the limitations inherent in any technic used, the fallacies inescapable in trying to draw any general deductions from even 1000 or 2000 cells counted out of the multiple millions present in any functioning bone marrow, the much debated questions of identification and classification of immature forms, the many factors known and unknown affecting hemopoiesis in any particular individual, all of these, and more, make it necessary to be very conservative in the drawing of deductions from any one limited series of observations or in trying to compare the figures obtained from different investigators.

Using the supravital and fixed technic as outlined at the beginning of the paper, we have found a reasonable degree of conformity in our counts, and in so far as any constant procedure can assure comparable results, the findings, as contained in Table III, may be so analyzed. Only very general indications, which might be most readily admitted as based on indisputable cellular identifications, will be attempted.

In the six cases under 8 years of age the relatively high percentage of lymphocytes will be noted, with none or very few mature polymorphonuclear leucocytes. This is in conformity with the well recognized relative lymphocytosis in children. In the presence of infection

TABLE III.

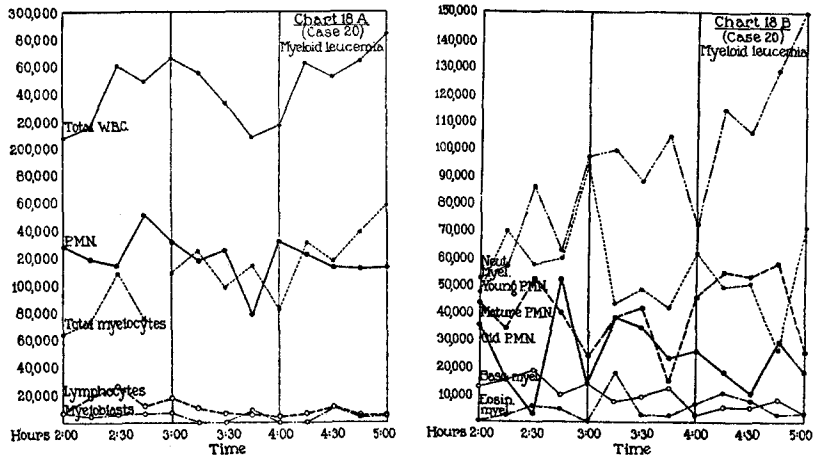
Human Bone Marrow Differentials Based on Count of 1000 to 2000 Cells.

Diagnosis	Polys.	Myelocytes			Myeloblasts	Prim. cells	Clasmatocytes	Monocytes	Megacaryocytes	Lymphocytes	Lymphoblasts	Eosin.	Basoph.	Megaloblasts	Normoblasts	Unclass.	Age
		Neut.	Eosin.	Basoph.													*mos.*
1. Acute dehydration	0.	35.2	1.4	0.	5.9	0.	0.3	9.4	0.4	38.9	0.	2.4	0.	0.1	5.2	0.8	4
2. " "	0.	31.1	2.2	0.6	5.	0.4	1.2	4.2	0.2	44.9	0.	0.	0.	0.	9.1	1.1	5
																	yrs.
3. Normal (accident)	15.3	31.	0.37	0.	1.5	0.81	0.27	4.5	0.45	37.3	0.	0.54	0.	0.	5.7	1.1	4
4. Diphtheria	4.8	56.4	1.6	0.9	1.5	0.2	0.9	3.1	0.6	27.7	0.	0.	0.	0.	1.5	0.8	6
5. Pertussis-bronchopneumonia	0.6	61.8	0.6	0.	1.	0.	0.3	2.1	0.4	28.3	0.	0.	0.	0.1	3.5	1.3	8
6. Bronchopneumonia (vertebral bone marrow)	0.9	56.9	3.	0.1	4.2	0.5	0.6	3.	0.1	29.1	0.	1.	0.	0.	0.6	0.	7
7. Normal (accident)	55.4	18.8	4.1	0.	1.1	1.1	0.	1.6	0.1	10.8	0.	0.6	0.1	0.1	5.4	0.8	28
8. " "	10.5	53.1	0.8	0.	11.9	0.4	0.1	3.5	0.	15.	0.	0.2	0.	0.	4.1	0.4	29
9. " "	13.7	54.9	0.1	0.	1.7	1.	0.	0.5	0.1	14.7	0.	0.	0.	0.	13.	0.3	30
10. Acute cerebral hemorrhage	19.7	40.5	0.9	0.3	5.	0.3	1.	5.2	0.5	9.1	0.	0.7	0.	0.	14.2	2.6	72
11. Lobar pneumonia	19.2	63.5	0.6	0.	4.6	0.2	0.3	1.1	0.	8.8	0.	0.	0.	0.	1.7	0.	28
12. " "	90.	5.	0.1	0.	1.9	0.	0.1	2.	0.1	0.	0.	0.8	0.	0.	0.	0.	60
13. Empyema (biopsy rib)	47.7	30.3	0.8	0.	8.5	0.7	0.2	0.8	0.4	6.3	0.	0.3	0.	0.6	3.	0.4	42
14. Lung abscess	46.7	33.3	1.	0.6	2.7	0.4	0.	2.	0.	6.2	0.	0.2	0.	0.8	3.8	0.7	32
15. Subdiaphragmatic abscess	65.4	15.	1.7	0.	2.6	0.3	0.	0.9	1.1	7.4	0.	1.7	0.	0.1	3.1	0.7	38
16. Empyema (biopsy rib)	46.	30.6	1.9	0.	2.6	1.4	0.	1.1	0.2	10.1	0.1	0.3	0.	0.4	4.7	0.6	38
17. Postpartum infection	27.8	37.8	0.3	0.	3.5	0.1	0.	6.8	0.4	15.	0.	1.1	0.	0.	6.8	0.4	44
18. General sepsis	5.7	65.1	0.3	0.	1.4	0.1	0.1	13.8	0.2	8.3	0.	0.	0.	0.2	4.1	0.3	36
19. Cardiovascular lens. Pneumonia	9.3	71.	0.4	0.2	1.1	0.2	0.1	4.7	0.2	9.3	0.	0.	0.	0.	2.1	1.4	59
20. Cirrhosis of liver	4.4	75.1	0.5	0.2	2.5	0.6	5.2	3.4	0.7	5.	0.	0.5	0.	0.	1.7	0.2	61
21. " " "	1.6	52.7	1.6	0.5	8.	3.1	0.6	8.1	0.1	3.6	0.	0.1	0.	0.	17.7	3.3	46

22. Cirrhosis of liver	6.	58.7	1.1	0.	0.	1.3	0.7	8.7	0.4	20.3	0.	0.1	0.	0.	0.8	0.	57
23. " " " (infection)	26.3	50.1	0.6	0.1	2.1	1.	1.4	1.3	0.	13.1	0.	0.6	0.	0.	3.	0.4	49
24. Pernicious anemia	7.6	62.9	1.7	0.1	4.7	0.6	5.5	6.8	0.6	5.8	0.	0.5	0.	0.6	1.	1.6	62
25. " "	2.9	54.6	4.1	2.6	0.3	0.7	5.5	4.3	0.	20.1	0.	1.5	0.2	1.9	1.	0.5	61
26. " "	0.5	14.8	30.3	0.9	5.3	0.	6.7	12.6	0.	10.3	0.	0.	0.	10.6	3.2	0.8	56
27. " "	6.8	56.	1.6	0.2	1.6	0.5	5.6	7.1	0.	16.8	0.	0.7	0.	1.5	1	0.6	58
28. " "	8.1	12.8	10.6	1.6	19.7	0.8	3.4	13.1	0.	11.6	0.	0.6	0.2	14.4	2.1	1.	46
29. Acute miliary tuberculosis	31.1	9.42	0.38	0.09	2.66	0.19	0.	21.9	0.	32.	0.	0.66	0.	0.	1.42	0.57	36
30. Lymphatic leucemia. Cirrhosis of liver	0.	0.	0.	0.	0.	0.	0.	4.8	0.	0.	93.	0.	0.	0.	0.4	1.8	48
31. Banti's disease	5.1	42.4	2.2	0.	2.	0.3	8.2	5.6	0.3	27.6	1.3	0.8	0.	0.3	2.8	1.1	48
32. Carcinoma of pleura	7.3	42.	14.7	0.9	3.	0.3	5.2	1.3	0.5	13.6	0.	1.8	0.	0.	5.4	3.	52

(Cases 4, 5, and 6) the myeloid cells were increased though the response was not to the extent seen in adult cases (27).

There are four cases in the series (Cases 3, 7, 8, and 9) which have been considered to present normal bone marrow pictures, inasmuch as they were medicolegal cases of sudden accidental death in supposed healthy young adults (one child of 4 years). It is only desired to call attention to the relationships found between mature polymorphonuclear neutrophilic leucocytes and the neutrophilic myelocytes. It may be granted that the distinction between cells with characteristic multilobed nuclei and their full complement of specific granules and those with oval single nuclei and relatively fewer specific granules is



possible of ready appraisal by any worker familiar with the blood. Thus while the total myeloid percentage in the three adult cases showed close agreement, 74 per cent, 64 per cent, and 68 per cent, in Case 7 there were 55 per cent segmented forms and 19 per cent myelocytes, in contrast to Cases 8 and 9 with only 10 and 13 per cent mature cells and 53 and 55 per cent myelocytes, respectively. Schilling (28) and Benzler report a cytological study of the bone marrow from ten normal healthy men, just shot, in which the myeloid elements ranged from 39 to 43 per cent, eosinophils from 1.1 to 4.6 per cent, and normoblasts from 31 to 42 per cent. The peripheral blood in these cases showed neutrophils ranging from 32 to 74.5 per cent, and

in the bone marrow differentials, the myelocytes varied between 34 and 47 per cent, the metamyelocytes between 34 and 56 per cent, and the segmented nuclear cells between 5 and 22 per cent, but there is no relationship expressed in the individual cases. In the present series, with the technics used, normoblasts were found in much smaller relative percentages than those given above by Schilling, *viz.* 4 to 13 per cent in the normal cases and from 0 to 17.7 per cent in the pathological cases. The latter figures are more in accord with those reported in a small series of pathological cases by Doan (7) with the supravital technic, in which the normoblasts were found in percentages of from 4 to 22. A study of the fixed sections of bone marrow, except perhaps in certain severe secondary anemias and pernicious anemia, gives a definite impression of the predominance of myeloid metaplasia over erythroid foci in contrast to the relative numbers of the respective definitive cells in the peripheral blood. This must be related to the more rapid turnover in the white cells, the red cells under most conditions probably surviving for a much longer period; also the entire functional activity of the red cells is dependent upon their circulation within the vascular bed, whereas the circulation is simply the means of communication for the white cells from source of supply to the local points of activity in the body outside the blood stream. Certainly with the supravital technic, which has now been used in many scores of surveys of bone marrow from experimental animals (29, 30), and with the fixed technic as here used, the nucleated red cells have been far more often below 20 per cent of the differential than above.

In two instances of lobar pneumonia (Cases 11 and 12) the total myeloid cells in the bone marrow were 82.7 and 95 per cent respectively, but in the former there were 19.2 per cent mature cells and 63.5 per cent myelocytes, whereas in the latter the mature cells were 90 per cent and myelocytes 5 per cent.

A comparison of the first two columns of Table III straight through shows the reciprocal relationship maintained between the percentages of mature and immature neutrophils. When taken into account in the presence of the observed periodic fluctuations in the total white blood count in the peripheral blood, as presented in the first part of this paper, a tenable hypothesis of the periodic maturation of myelo-

cytes into polylobed, motile leucocytes may be conceived. The particular moment at which the process of manufacture is stopped would give for a given area either a majority of mature neutrophils or the reverse according to the phase of maturation at that point at that time. This has been particularly striking in the experimental study of the bone marrow after specific stimulation of the maturation of the neutrophil group by sodium nucleinate and similar substances producing a peripheral leucocytosis (29). In bone marrows relatively hypoplastic, so that the two processes of red and white cell formation may be analyzed separately, it is striking to find always the cells in any one focus or area all at the same stage of maturation (30, 31, 32). Thus, it would seem from all the evidence at hand that the white cells, leucocytes, lymphocytes (33), and monocytes (34, Simpson "macrophage showers") are constantly leaving the blood stream, and that the supply is primarily dependent upon a more or less periodic maturation and delivery of granulocytes from the bone marrow, lymphocytes from the thoracic duct (10, 35), and monocytes and clasmatocytes possibly from the spleen. It is not believed that other factors are negligible as affecting the total numbers of circulating cells, but that the factors mentioned are important in the cellular economy of the body seems probable.

In view of the recent attention attracted to the cellular aspects of experimental and clinical tuberculosis (36, 37) it is of interest to note the one case (No. 29) of miliary tuberculosis in which the monocytes in the bone marrow were 21.9 per cent and the lymphocytes 32 per cent. It has been shown (36) that the typical epithelioid cell of tuberculosis, when stained with one of the Romanowski stains, reacts with a characteristic faint eosin tint in the cytoplasm near the *Hof* of the nucleus where the rosette of neutral red bodies is found in the supravital preparation. In this case these cells could not be mistaken, and though listed as monocytes the majority of the cells were true epithelioids. In no other case were monocytes found in as high percentages in the bone marrow, and the lymphocytes only in the cases within the first decade of life.

The five cases of pernicious anemia, Nos. 24, 25, 26, 27, and 28, show the well recognized (7, 38) increased percentages of clasmato-

cytes, again a cell type easily recognized through its marked capacity for phagocytosis.

SUMMARY.

In a study of twenty clinical cases with a wide range of diagnoses, repeated total counts of the white cells at 15 minute intervals reveal a large fluctuation at various levels comparable to that found for the normal (1, 2). The granulocytes seem to follow a more or less hourly rhythm, the most marked shift to the left in the Arneth pattern and the moment of greatest percentage of motility coinciding with the peaks.

The independence found existing between the peripheral blood concentrations of individual strains of white cells and the red cells, as determined by total and differential counts, their differential response to pathological and pharmacological stimuli, and their normal relative relations, all indicate some separate physiological mechanism of control for each type of cell, either working through, or independently of, their sources of origin.

The many factors to which the circulation of the blood, as such, is subject, the complexity of the influences on origin, maturation, delivery, longevity, and destruction of each cell group, the limitations inherent in the present involved, indirect technics of counting, combine to make any single observation subject to grave misinterpretation. The value to the clinician must come in repeated observations, at times when the diagnosis or a therapeutic procedure is in doubt, at frequent intervals, at other times over longer or shorter periods, but always with the relation between consecutive counts, rather than the absolute values, the important point for consideration.

Both the red and the white cells probably change their relative concentrations in the peripheral blood from time to time over a considerable range that is quite within normal physiological limits, so that, in theoretical considerations and in practical functional estimations, a zonal concept with adequate individual extremes should always be kept in mind for both physiological and pathological states.

A cytological analysis of thirty-two bone marrows from human biopsy and autopsy material shows the striking reciprocity found to

exist between the myelocytes and the mature polymorphonuclear leucocytes. This, together with the observed focal uniformity of maturation found in bone marrow, and the periodicity of the fluctuations of the neutrophils in the peripheral blood, leads to the formulation of the hypothesis of a constant functional withdrawal of granulocytes from the peripheral blood with a periodic delivery of new cells from the marrow, which in leucopenia and in leucocytosis represents a depression or a stimulation, respectively, of the normal mechanism. The nature and degree of the response are an approximate index of the cellular factor in the complex of the "resistance" of the particular individual.

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