STUDIES IN THE BLOOD CYTOLOGY OF THE RABBIT

VI. Blood Cell Relationships in Groups of Normal Rabbits with Respect to Time

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In previous papers (1, 2, 3, 4, 5) repeated observations were reported on the cells and the hemoglobin content of the peripheral blood of normal rabbits, and attention was drawn both to the numerical variations of these elements and to the nature of the variations. The results which were analyzed from the standpoint of the weekly mean values of each animal group examined over long periods of time, clearly demonstrated that the cells and the hemoglobin fluctuate to a greater or less degree. These results were obtained under as uniform experimental conditions as was possible with the usual laboratory equipment. In many instances, the differences in cell level were found to be statistically significant and the rate of change was often of an orderly nature. It was also shown that the trends in two parallel groups of animals examined over the same months were on the whole similar both as regards time and direction, although the general cell levels themselves were different.

The analysis showed further that variations in the levels of certain classes of cells were accompanied by similar or opposite variations in other classes. Between still other cell types, there was no apparent relationship.

In order to determine the significance of these trends and fluctuations from the standpoint of the association or lack of association in the movements of the various cell levels and of the hemoglobin content, a statistical analysis of the material has been made, the results of which are contained in the present paper.

Materials and Methods

The experimental results used in this analysis were those obtained from the weekly blood examinations on 45 normal rabbits distributed in five groups as follows:

Group	Number of rabbits	Number of examinations	Number of counts	First count	Last count
I	10	35	350	Oct. 24, 1927	June 20, 1928
II	10	13	130	Mar. 29, 1928	June 19, 1928
III	10	8	80	Sept. 20, 1928	Nov. 22, 1928
IV	10	29	283	Nov. 27, 1928	June 18, 1929
V	5	26	130	Dec. 29, 1928	June 21, 1929
	45	111	973		

The conduct of the experiments has already been described (2). The rabbits were young adult male animals approximately 8 months of age and the majority represented browns, greys, and Flemish crosses. At the onset of the experiments all animals were apparently free from disease, but three of Group I and six of Group IV developed clinical snuffles while under observation; of the latter group, two died during the course of the experiment and two others soon after its termination. Groups II and III observed for short periods and Group V were clinically free from intercurrent disease. Two cases of ear canker developed in Group I and there were four cases in Group IV. For the most part, however, the general condition of the rabbits was excellent, and a gain in body weight was the rule.

The method employed in the examination of the blood has been described (2). The differential counts were made with the neutral red supravital technique; the red and white cell counts were made with standardized pipettes, and the hemoglobin content was determined by the Newcomer method. The examinations were carried out on the same day of consecutive weeks with the exception of Group III in which the first four counts were made at irregular intervals.

The results are analyzed upon the basis of the weekly mean group values of the various classes of cells and of the hemoglobin content; these values have already been published (2, 3, 4, 5). The existence of technical errors in the blood examinations and the occurrence of individual physiological as well as possible pathological states which might be reflected in variations of the blood constituents determined the employment of mean values. In the present analysis, both smoothed and unsmoothed values were used; the formula employed for smoothing was $\frac{a + 2b + c}{4}$.

The measure of the degree of relationship which existed between any two blood constituents was determined by the use of correlation coefficients. Simple zero

order correlation coefficients were calculated upon the smoothed weekly means for each of the five animal groups, using the formula $R_{xy} = \frac{S_{xy} - M_x S_y}{N s_x s_y}$ (6). The number of blood elements followed, including the total white blood cells, was eight, thus offering possibilities for 28 coefficients. The significance of each coefficient has here been expressed in terms of probability (7). Any result which might occur from the random association of two variables to the extent of five or more times per hundred chances ($P = .05$) has not been interpreted as significant. A value has been considered as probably significant when the chances of its occurring by accident or random association are between two and five per hundred chances ($P = .02 - .05$). The designation of significant has been assigned a value when the chances of its occurring by accidental relation between two variables is one or less than one per hundred chances ($P = .01$).

Significant differences in the correlation coefficients between the various animal groups were determined by the method of "z," that is, the method of transformed correlations. Published tables are available for these calculations (7). Unless the difference between two transformed correlations was greater than twice its standard error, the two correlation coefficients were assumed to be from the same or similar material. When the coefficients for all five groups were found not to be significantly different from each other, they were said to be homogeneous. If the simple zero order coefficients for the groups examined 26 to 35 weeks, that is, Groups I, IV, and V, were not significantly different from each other, they were considered as probably homogeneous, regardless of the values for Groups II and III, the observations on which were too few to be of much statistical importance. If any significant difference existed between the coefficients of Groups I, IV, V, the coefficients listed for that relationship were said to be non-homogeneous.

By this same method of transformed correlations, homogeneous coefficients were averaged so as to give a general combined expression for any given relationship (7). Such a combined expression was calculated for each of the 28 simple relationships both including and excluding Groups II and III, and it was found that the inclusion or exclusion of these shorter groups made no significant difference in the results. The calculation was carried further by combining the coefficients of correlation for all five groups regardless of their homogeneity in order to compare the averaged relationships with each other on a common basis. Any interpretation of these results, however, must necessarily be made with caution and with reference to the values for individual groups.

The technical accuracy of the calculations was checked through the use of the multiple correlation coefficient (6). The averaged correlation coefficients for all five groups were used as the basis, and a multiple correlation coefficient was calculated in which the total white blood cells was the dependent variable, and the neutrophiles, the eosinophiles, the basophiles, the lymphocytes, and the monocytes, the independent variables. The multiple correlation coefficient was found to be equal to unity ($R = 1.00$), signifying that no errors in calculation had been made.

That $R = 1.00$ when the white blood cells are the dependent variable, is explained by the fact that the value of the white blood cells is equal to the sum of the values for the various classes of white cells, consequently making an excellent check column.

In order to determine to what extent the total shift in a given type of cell was accompanied by or associated with similar shifts in other cells, multiple correlation coefficients were calculated for the eight classes of blood elements studied. Multiple correlations were also calculated upon the simple correlation coefficients of Groups I, II, IV, and V to check the validity of the combined values.

Upon the assumption that the simple correlation coefficients combined for all five groups were representative of our experience with the various intercellular relationships from the standpoint of time trends in group values, it was desirable to know whether these various relationships were independent of each other and to what degree. To this end, partial correlations of various orders up to the fifth were made upon each of the 21 possible relationships (6); the relationships of the total white blood cells to the various classes of white cells could not be included since these values make up the total figure. As a check on the partial correlations of the combined coefficients, partial correlations were made upon the simple coefficients for the individual groups and no striking differences were noted.

The group means were also analyzed from the standpoint of the relationship between short fluctuations holding the long time trends constant. Theoretically, the variations of two classes of cells might be related in trend over longer periods of time but might be unrelated or inversely related as regards short time fluctuations, assuming different causes for the trend and the fluctuation. The variate difference correlation method was employed in analyzing the results along these lines (8, 9, 10). Successive differences to the sixth were determined upon the unsmoothed weekly group means for the various blood elements in the five animal groups, and simple correlations were then calculated upon the sixth differences for the 28 possible combinations. It was found that the significance of the coefficients could be approximately determined for the sixth difference on these short series by making $n = n' - 2 - 6$ and calculating the probability as if it were a simple zero order coefficient or by making it twice the value for the simple zero order coefficients. The standard of comparison was the method for the probable error developed by Anderson (11). Again, in order to check the accuracy of the coefficients listed, multiple R was calculated for these variate sixth difference coefficients with the total white blood cells as the dependent variable and the various classes of white cells as the independent variables; it was found to be equal to unity ($R = 1.00$). In addition, these coefficients were averaged for all five groups by the method of transformed correlations and the multiple correlation coefficient calculated upon these averaged values was still equal to unity. The homogeneity or the lack of it was determined by the use of transformed correlations, making $n = n' - 3 - 6$.

Partial fifth order correlation coefficients were calculated upon the variate

difference coefficients averaged by the method of transformed correlations. No precedent can be given for such a procedure, but if the variate difference values represent any true relationships whatsoever, then such relationships should be susceptible to partial and multiple correlation. The significance was calculated by assuming that for any simple combined variate sixth difference coefficient $n = 67$ where the original number of weekly group means was 111. The significance of the partial fifth order coefficient was read in terms of probability making $n = 67 - 5$.

Recapitulation

This statistical study of the trends and variations in the levels of the cells and the hemoglobin content in the peripheral blood of normal rabbits was carried out for the purpose of determining whether an association or lack of association obtains between the observed fluctuations of these elements. The values employed for the analysis were the unsmoothed weekly means obtained from the observations on five groups of rabbits examined over long periods of time; these values have been published in previous papers (2, 3, 4, 5). The results are contained in a series of tables and charts.

Table I gives the simple trend correlation coefficients for each of the 28 simple relationships of the various cells and of the hemoglobin content. The combined coefficients for the three groups followed for the longest times, that is, Groups I, IV, and V, and for all five groups are listed in the last two columns at the right side of the table.

Table II gives the variate sixth difference correlation coefficients for the 28 simple relationships in four animal groups; Group III contained too few observations to be treated by this method, and although Group II was little better in this respect, it has been included to show the limits of the method. The right hand column of the table contains the coefficients of correlation averaged by the method of "z" for Groups I, IV, V, and also for Groups I, II, IV, and V.

Table III contains a summary of the averaged coefficients obtained by the method of the variate sixth difference and by that of simple trend correlations. Partial correlations of the fifth order (holding all factors constant except total white blood cells) upon the combined coefficients obtained by each of these two methods are given in the column to the right of the zero order coefficients.

Table IV summarizes the multiple correlation coefficients obtained from the combined values by both the variate difference and the simple trend correlation methods. The values for both R and R^2 are given.

The curves of Text-figs. 1, 2, 3, and 4 represent the percentage deviations of the

TABLE I

Simple Zero Order Correlations upon the Smoothed Weekly Means of Blood Constituents in Normal Rabbits

Animal group	Simple values					Combined values	
	I	II	III	IV	V	I, IV, V	I, II, III, IV, V
n'	35	13	8	29	26	84	99
rWN	+.867 S	+.913 S	+.860 S	+.943 S	+.929 S	+.915 S	+.913 S
WB	**+.686** S	+.743 S	−.240	**−.074**	+.012	+.302 S	+.336 S
WE	**−.173**	+.174	−.137	+.465 PS	+.546 S	+.260 PS	+.232 PS
WL	+.918 S	+.929 S	**−.123**	**+.454** PS	+.891 S	+.829 S	+.823 S
WM	+.406 PS	+.642 PS	**−.340**	+.647 S	+.659 S	+.567 S	+.540 S
WR	+.555 S	+.676 PS	−.377	−.253	+.283	+.252 PS	+.276 S
WH	**+.819** S	−.161	+.191	**−.094**	+.527 S	+.531 S	+.456 S
RH	+.227	−.222	−.081	+.474 S	+.497 S	+.390 S	+.309 S
RN	+.380 PS	+.398	−.592	**−.355**	+.220	+.102	+.094
RB	+.503 S	**+.936** S	+.367	+.452 PS	**−.015**	+.354 S	+.470 S
RE	−.166	−.393	−.065	+.017	+.105	−.031	−.073
RL	+.527 S	+.767 S	+.512	+.132	+.350	+.360 S	+.425 S
RM	+.523 S	+.353	**+.929** S	**−.279**	+.110	+.167	+.261 S
HN	**+.908** S	**−.031**	+.116	**−.068**	+.564 S	+.641 S	+.567 S
HB	**+.482** S	−.339	+.048	−.078	+.027	+.188	+.125
HE	−.150	−.249	+.009	−.264	+.096	−.119	−.126
HL	**+.638** S	+.025	+.059	**−.032**	+.487 PS	+.414 S	+.360 S
HM	+.221	−.273	−.058	−.255	+.067	+.023	−.013
NB	+.428 PS	+.467	−.597	−.208	+.009	+.115	+.114
NE	**−.236**	+.481	−.201	+.355	+.447 PS	+.156	+.175
NL	+.645 S	+.752 S	**−.550**	**+.183**	+.696 S	+.541 S	+.523 S
NM	+.250	+.607 PS	**−.632**	+.588 S	+.560 S	+.460 S	+.426 S
BE	−.020	−.339	+.406	+.322	+.193	+.153	+.116
BL	**+.658** S	+.770 S	+.440	**−.143**	**−.118**	+.228 PS	+.316 S
BM	+.203	+.465	+.626	−.099	−.117	+.016	−.077
EL	−.276	−.040	−.146	+.054	+.415 PS	+.031	+.014
EM	+.308	+.149	+.071	+.442 PS	+.449 PS	+.398 S	+.359 S
LM	+.279	+.497	+.423	+.010	+.494 PS	+.264 PS	+.299 S

In this and all other tables the following symbols are used:

n' = Number of observations.
r = Coefficient of correlation.
S = Significant.
PS = Probably significant.
W = Total white blood cells.
N = Neutrophiles.
B = Basophiles.
E = Eosinophiles.
L = Lymphocytes.
M = Monocytes.
R = Total red blood cells.
H = Hemoglobin.

TABLE II

Variate (6th) Difference Correlations upon the Unsmoothed Weekly Means of Blood Constituents in Normal Rabbits

Animal group	Simple values				Combined values	
	I	II	IV	V	I, IV, V	I, II, IV, V
n'	35	13	29	26	66	70
'WN	+.**448** PS	+.948 S	+.890 S	+.807 S	+.745 S	+.763 S
WB	−.135	+.**968** S	+.637 S	−.029	+.173	+.280 PS
WE	+.212	+.405	+.**574** S	−.**669** S	+.078	+.099
WL	+.**042**	+.929 S	+.633 S	+.684 S	+.445 S	+.500 S
WM	+.085	+.**960** S	+.397	+.427	+.284	+.372 S
WR	−.**387** PS	+.**986** S	+.**774** S	+.138	+.195	+.321 S
WH	−.091	+.549	−.471 PS	+.114	−.167	−.122
RH	+.468 S	+.499	−.142	+.471 PS	+.293 PS	+.307 PS
RN	−.**367** PS	+.**969** S	+.**832** S	−.**279**	+.143	+.254 PS
RB	+.454 PS	+.942 S	+.649 S	+.387	+.503 S	+.558 S
RE	−.195	+.329	+.155	+.034	−.022	−.001
RL	+.096	+.874 S	+.310	+.304	+.222	+.285 PS
RM	+.036	+.**917** S	+.251	+.249	+.164	+.244 PS
HN	−.360 PS	+.326	−.101	−.227	−.245	−.212
HB	+.312	+.728	−.**371**	+.247	+.077	+.128
HE	−.179	+.**879** S	−.168	−.310	−.212	−.120
HL	+.359 PS	+.743	−.**688** S	+.441	+.014	+.071
HM	−.608 S	+.506	−.530 PS	−.255	−.500 S	−.450 S
NB	−.**762** S	+.**851** PS	+.**637** S	−.331	−.261	−.174
NE	−.237	+.113	+.451 PS	−.**590** S	−.128	−.114
NL	−.**817** S	+.**764** PS	+.**268**	+.218	−.315 PS	−.242 PS
NM	+.232	+.857 PS	+.141	+.490 PS	+.280 PS	+.333 S
BE	+.487 S	+.612	+.347	+.532 PS	+.459 S	+.468 S
BL	+.567 S	+.**973** S	+.188	−.090	+.293 PS	+.391 S
BM	−.083	+.**935** S	−.009	−.011	−.041	+.063
EL	+.160	+.672	+.243	−.**735** S	−.105	−.053
EM	+.081	+.477	+.533 PS	+.096	+.242	+.256 PS
LM	−.364 PS	+.**948** S	+.172	−.104	−.130	−.014

TABLE III

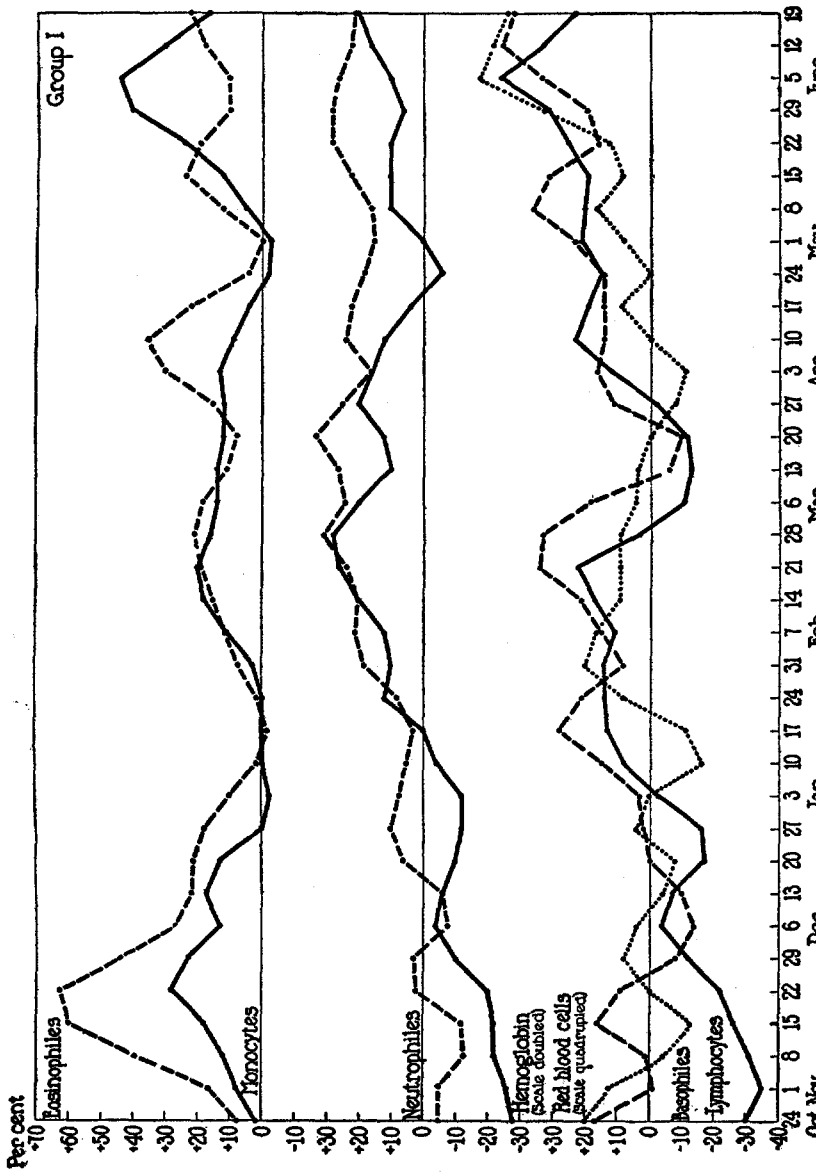
Summary of Analyses of Combined Correlation Coefficients (Method of "Z" Applied to Five Groups of Normal Animals)

Relation	Simple zero order correlation	Partial 5th order correlation	Variate 6th difference correlation	Variate 6th difference, 5th order partial
WN	+.9125 S		+.7630 S	
WB	+.3360 S		+.2800 PS	
WE	+.2317 PS		+.0990	
WL	+.8225 S		+.4996 S	
WM	+.5395 S		+.3720 S	
WR	+.2755 S		+.3214 PS	
WH	+.4560 S		−.1215	
RH	+.3093 S	+.4349 S	+.3065 S	+.5202 S
RN	+.0937	−.4030 S	+.2537 PS	+.4412 S
RB	+.4696 S	+.4281 S	+.5579 S	+.6665 S
RE	−.0726	−.1514	−.0005	−.4050 S
RL	+.4249 S	+.4148 S	+.2848 PS	+.0917
RM	+.2605 S	+.4425 S	+.2440 PS	+.4662 S
HN	+.5670 S	+.6334 S	−.2120	−.3065 PS
HB	+.1254	−.1185	+.1276	−.2241
HE	−.1264	−.1007	−.1197	+.1140
HL	+.3600 S	−.0555	+.0706	−.0903
HM	+.0125	−.3939 S	−.4496 S	−.5230 S
NB	+.1140	+.0857	−.1741	−.2807 PS
NE	+.1746	+.1072	−.1139	+.0030
NL	+.5228 S	+.4215 S	−.2415 PS	−.2594 PS
NM	+.4256 S	+.4667 S	+.3331 S	+.0598
BE	+.1155	+.2006 PS	+.4683 S	+.6347 S
BL	+.3156 S	+.1293	+.3908 S	+.2800 PS
BM	+.0771	−.2122 PS	+.0634	−.3058 PS
EL	+.0144	−.1045	−.0529	−.2612 PS
EM	+.3585 S	+.3114 S	+.2564 PS	+.3840 S
LM	+.2989 S	+.0086	−.0142	+.0454

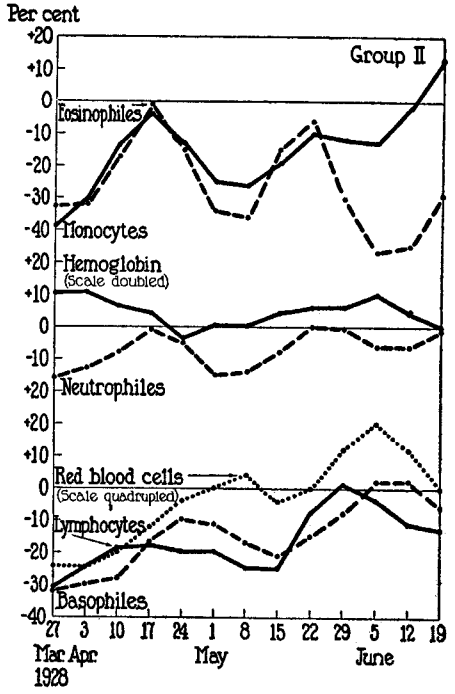
TABLE IV

Multiple Correlation Coefficients upon Combined Simple and Variate 6th Difference (Zero Order) Coefficients (Groups I, II, III, IV, and V)

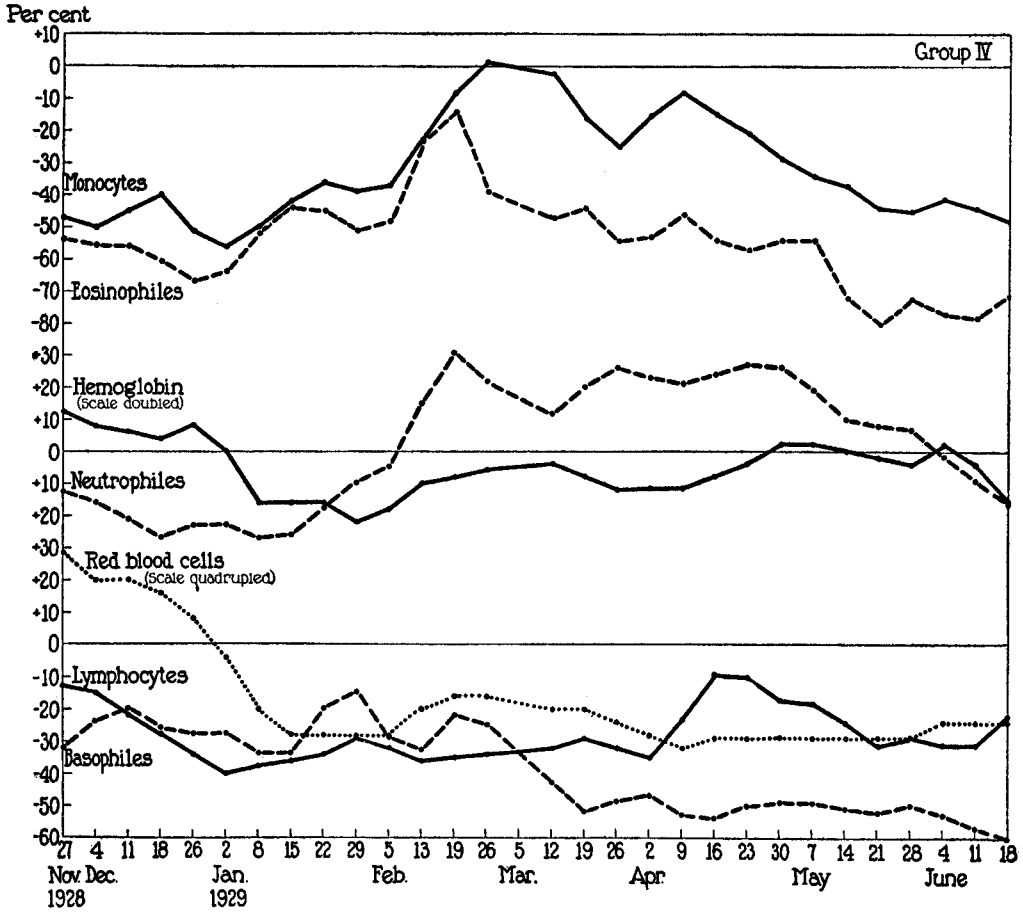
Class of cell	Zero simple correlation		Zero 6th difference correlation	
	R^2	R	R^2	R
Red blood cells	.480	.690	.682	.826
Hemoglobin	.510	.716	.456	.676
White blood cells	1.014	1.007	1.142	1.069
Neutrophiles	.640	.802	.370	.609
Basophiles	.290	.534	.679	.824
Eosinophiles	.210	.453	.470	.686
Lymphocytes	.430	.656	.278	.527
Monocytes	.410	.642	.474	.689



TEXT-FIG. 1. Mean values for consecutive determinations of blood constituents as percentage deviations from standard values. Group I.



TEXT-FIG. 2

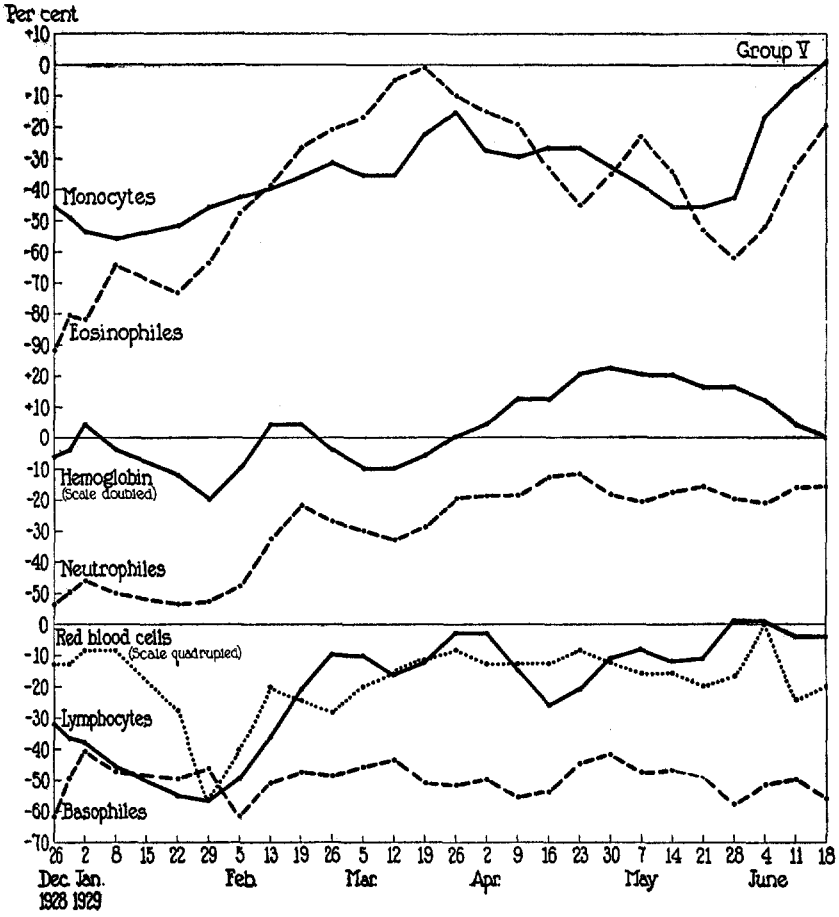


TEXT-FIG. 3

TEXT-FIG. 2. Mean values for consecutive determinations of blood constituents as percentage deviations from standard values. Group II

TEXT-FIG. 3. Mean values for consecutive determinations of blood constituents as percentage deviations from standard values. Group IV

smoothed weekly means from so called standard values[1] for four of the five groups of rabbits; Group III is omitted because it was irregularly followed and contains only eight observations. No curve for the total white blood cells is given because this value does not represent an individual cell entity. The arrangement of



TEXT-FIG. 4. Mean values for consecutive determinations of blood constituents as percentage deviations from standard values. Group V.

charting monocytes with eosinophiles, neutrophiles with hemoglobin, and red blood cells with lymphocytes and basophiles, illustrates certain associations to which attention will later be directed.

[1] The standard values employed were obtained from 1110 counts on 176 normal male rabbits carried out in this laboratory (1).

DISCUSSION

In this paper an attempt is made to evaluate comparatively small numerical variations of the blood cells which are ordinarily ascribed to technical error and assumed to be within the limits of "normal" variation. The particular object of the analysis was to ascertain whether any relationships in the movements of cell levels could be demonstrated. The data were obtained from four groups of ten and one group of five normal rabbits examined for prolonged periods of time; weekly mean values have been used as the basis of the study. Although larger groups and parallel observations would have been desirable, it is believed that the available material suffices for the present purpose.

It is conceivable that the group means of any two blood constituents may vary with time in a variety of ways. For example, the general numerical level of two classes of cells might increase or decrease over 7 or 8 months so that a coefficient of correlation upon their smoothed curves would be very high. Regardless of this general trend, however, there may be a number of abrupt oscillations of short duration in which the two levels fluctuate in the same or opposite direction, or bear no apparent relation to each other. This short period change we have called "fluctuation or oscillation" in contradiction to the term "trend" which is used to describe a progressive change over a period of months.

Table I contains the simple zero order coefficients of correlation which bring out the feature of cell relationship from the standpoint of trends in values over periods of months. All five groups are included although the eight observations of Group III at irregular intervals do not offer the same opportunity for comparison afforded by the other groups. Group II was examined regularly but only for 13 weeks, and Group IV suffered more heavily than the others from spontaneous disease. Bearing these points in mind, it will be noted that Groups I and V contain 16 and 14, and Groups II, III, and IV, 10, 8, and 2 significant coefficients respectively. The inclusion or exclusion of Groups II and III, however, makes no appreciable difference in the combined correlation coefficients as will be seen by comparing the figures in the last column of the table.

Only a tentative value can be assigned to the combined correlation coefficients for all five groups (Table I) inasmuch as Group I had six coefficients significantly higher and Group IV, ten coefficients significantly lower than the combined values. What effect spontaneous disease conditions had upon the blood cell values of Group IV is hypothetical, so that this group cannot fairly be eliminated. The chronic basopenia in Group V with the lack of any trend whatsoever is responsible for the absence of conformity of these cells in the relationships of this animal group. In general, the various relationships were most similar in Groups I, II, and V, and coefficients averaged from this material would show only two or three significantly different values.

Table II represents an attempt to measure the relations between the cells of the various animal groups as regards short term fluctuations or oscillations by means of the variable sixth difference method. In this analysis, trend is practically eliminated. Since at least 10, and preferably more than 25 observations are necessary for dependable coefficients of correlation, Groups II and III were too short to be treated by this method; Group II is included in the table to show that certain spurious results can be obtained by using so short a group. Inclusion or exclusion of Group II in the combined coefficients, however, makes no significant difference in the results. The results of the analysis show that Group I had 4, Group IV, 6, and Group V, 5 coefficients significantly different from the combined values.

With time trend correlations such as those of the present study, two values may seemingly be related to each other in time, due to a common influence, although actually they may be unrelated. To eliminate this possibility, a third factor or factors should be found which are also similarly affected by this common influence. In this series, it has been assumed that for any simple time relationship between two variables, the remaining variables constitute to a varying degree such a third factor or factors. We have taken the combined correlation coefficients for both trend and short time fluctuations in each of the 21 simple relationships and computed the partial correlations of the highest order possible, that is, the fifth order. Partial correlations with total white blood cells as a separate entity would be inaccurate since this total value is a summation of the values for the

neutrophiles, the basophiles, the eosinophiles, the lymphocytes, and the monocytes. Since there are seven variables under consideration, the relation between any two can be determined with the other five factors held constant.

In Table III, the combined zero order trend correlations for all five groups are given in the first column; the partial fifth order correlations upon these appear in the second column; the combined variate sixth difference correlations for all groups with the exception of the too short third group are given in the third column; and the partial fifth order correlations upon these last values comprise the fourth column. Since the combined correlation coefficients do not represent in every case an homogeneous material, too much emphasis should not be placed upon either these values or their derived partial coefficients. Partial correlations upon the individual groups, however, give approximately the same values as those based upon the combined values.

With the results of these analyses at hand (Tables I, II, and III), the relationships of the several types of cells may now be described. Among the 28 possible relationships, one finds that the white blood cells are highly associated with the neutrophiles and lymphocytes, both as regards general trend and oscillation; and that they are also related with the movements in level of the monocytes, the red blood cells, and the basophiles, in the order named. From the standpoint of cell number alone, such an arrangement of correlations would be expected as far as the white blood cells are concerned although the significant relation of the white blood cells with the red blood cells introduces the question of blood volume change.

The red blood cell levels varied from week to week and from month to month in all groups of animals. In these variations, they were accompanied most closely by the basophiles, the lymphocytes, the hemoglobin, and the monocytes in the order named; no significant homogeneous relation to the neutrophiles or the eosinophiles was found. The combined coefficients representing all groups were the same both for trend and for oscillations. Partial correlation affected very little the combined values of the simple or variate difference correlations, except that the neutrophiles and the eosinophiles showed a possibly significant negative relation, though of an irregular nature, and the relation with the lymphocytes was somewhat reduced. Even

when the question of lag was considered, the neutrophiles and the eosinophiles did not seem to shift with the red blood cells. However, by neither method was the value for the neutrophiles homogeneous or consistent among the component groups. Perhaps one of the most striking points brought out by the analyses is the fact that the hemoglobin content did not always follow the values of the red blood cells.

The hemoglobin content varied in all groups. It was significantly and homogeneously related to the red blood cells both as to trend and fluctuation. In the short term fluctuations or oscillations, a significant negative relation to both monocytes and neutrophiles occurred which was independent of other intercellular relationships. In respect to trend, however, the mean percentage of hemoglobin was directly related to the neutrophiles and inversely related to the monocytes. It was never significantly related to the basophiles, the eosinophiles, or the lymphocytes.

The neutrophiles largely determined both the trends and the fluctuations of the total white blood cells. With regard to trend, the neutrophiles were directly and significantly related to the mean values for the hemoglobin, the lymphocytes, and the monocytes in the order named, but were seemingly unrelated to the eosinophiles and basophiles, and inversely related to the red blood cells when other blood factors were held constant. As to fluctuation or oscillation, the neutrophiles were significantly but not always related to the red blood cells, and bore insignificant negative relations to the hemoglobin, the basophiles, and the lymphocytes; there was no relation whatsoever to the eosinophiles. Although the relation with the monocytes was homogeneous among the component groups and significant for the zero order values, this significance disappeared upon partial correlation.

The basophiles were fairly homogeneously, significantly, and positively related both as to trend and oscillation with the red blood cells, the eosinophiles, and the lymphocytes. Opposing this were small negative relations with the hemoglobin, the neutrophiles, and the monocytes.

The eosinophiles were found to be directly and most closely related, first, to the basophiles and second, to the monocytes. A small negative relationship to the red blood cells and lymphocytes was in

evidence while no apparent relation to the neutrophiles and hemoglobin was detected.

The lymphocytes were not significantly related to any of the other blood elements in regard to short term fluctuations, except perhaps to the basophiles and the red blood cells. However, there was a tendency toward a negative relationship with the neutrophiles and the eosinophiles but none whatever with the hemoglobin and the monocytes. With regard to trend, the lymphocytes were positively and significantly related to the red blood cells, the neutrophiles, and the basophiles, but not at all to the hemoglobin and the monocytes; there was a slight negative relation with the eosinophiles. The only significant difference in the intercellular relationships of the lymphocytes both as to trend and oscillation occurred with the neutrophiles.

The monocytes were found to be positively related both as to trend and oscillation to the red blood cells, the neutrophiles, and the eosinophiles; they were significantly inversely related to the hemoglobin and insignificantly inversely related to the basophiles. No relationship of the monocytes and lymphocytes with respect to either trend or oscillation was found.

The multiple correlation coefficients in Table IV furnish information on two important points, first, the total association of a blood cell type with other given blood cell types, and second, the total association due to unaccounted for factors. The value R^2 is a rough estimate of the total association of any one cell with other cells, the relationships being considered in percentage values. It will be seen, as was to be expected, that all the total white cell variations both as regards trend and fluctuation, can be attributed to variations in the component cell types. About 50 per cent of the variations in the red blood cells and hemoglobin, and from 21 to 47 per cent in the case of the eosinophiles, lymphocytes, and monocytes are explained by this relation to other cells; in the case of the neutrophiles and basophiles, depending upon the type of fluctuation considered, an irregular amount is similarly accounted for. It should be noted that with the basophiles and the eosinophiles, the total associations as shown by the variate difference method are twice as large as those shown by the simple correlations. Conversely, the values for neutrophiles and lymphocytes are one-third less by the variate difference method. The nature of the material

and the methods of analysis employed justify only the most general interpretations, but it may be stated that from 40 to 65 per cent of the variations in all the blood constituents here considered seem unaccounted for on the basis of common associations or as caused by the operation of a common factor.

The curves of the text-figures which represent the percentage deviations of the smoothed weekly means from standard values have been arranged so as to show the largest number of significant cell relationships. It should be pointed out, however, that several high associations are not well shown by this arrangement, as for example, the red blood cells and the hemoglobin, the neutrophiles and the monocytes, the monocytes and the hemoglobin, and the basophiles and the eosinophiles. In Group I (Text-fig. 1) the eosinophile and monocyte curves are above the standard values for the entire period of observation while in Groups IV and V of the following year (Text-figs. 3 and 4) they are consistently below these values. In Group II (Text-fig. 2) which paralleled Group I in its latter half, the eosinophiles and the monocytes were also below the standard values but intermediate between the findings in Group I and Groups IV and V. But irrespective of these wide variations in general level during the 2 years' observations, the curves illustrate in a striking manner the close relationships between monocytes and eosinophiles, not only in respect to the general trend of mean values, but also as regards the shorter fluctuations, a fact which was brought out by the correlation coefficients already discussed. The same conclusions may be drawn from the curves illustrating the red blood cells, the lymphocytes, and the basophiles on the one hand, and those representing the neutrophiles and the hemoglobin on the other. During the late winter and spring and early summer months of 1929, there was a chronic basopenia in both animal groups examined over this period, and this shift in level was perhaps the most striking one observed. In contrast to these changes in cell levels, the hemoglobin and the neutrophiles vaccilated about the standard level during the entire period. From the standpoint of general trends or shifts in the numbers of cells during the two year period from October, 1927, to June, 1929, the eosinophiles and the monocytes and the lymphocytes, the basophiles, and the red blood cells of successive animal groups sought progressively lower levels

while the neutrophiles and especially the hemoglobin were maintained at comparatively constant levels.

The various classes of cells and the hemoglobin in the several animal groups seem to bear a general similarity of relationship to the standard mean values, that is, all seven elements were either above or below the standard value at the same time. The most frequent exceptions occurred in the case of the neutrophiles, the red blood cells, and especially, of the hemoglobin. This fact would seem to indicate that the hemoglobin, the neutrophiles, and the red blood cells were either less disturbed by common influences affecting shifts in the other cells, or that a compensatory mechanism exists for these blood constituents which operated in such a manner that less marked deviation from the level of these standard values occurred. Successive examinations of the fluid volume of the blood might throw light on these points.

At the present time it seems undesirable to attempt to estimate the significance of these results as they represent only a 2 year period in one species in a single laboratory. It is evident that before any interpretation of the results can be attempted with any general conclusions, supplementary evidence of a similar sort should be obtained.

SUMMARY

1. Statistical analyses have been made of the weekly variations in the blood counts of groups of normal rabbits to find whether there exists any relationship between the numerical changes occurring in the various cell types.

Consecutive blood counts and differential white cell determinations on five groups of normal male rabbits comprising 45 animals in all were made at weekly intervals from October, 1927, to June, 1929, the number of observations on each group varying from eight to thirty-five.

2. The following relationships between the varying group means were found to be consistent and significant:—The number of the red blood cells varied with the amount of hemoglobin per cubic millimeter and with the number of lymphocytes. There was an inverse relationship between the amount of hemoglobin and the number of monocytes. The neutrophiles varied in number with the monocytes; the basophiles with the eosinophiles; and the eosinophiles with the monocytes.

Other associations not always similar but of high significance as far as the combined values were concerned, were the relations of the red blood cells with the basophiles and the monocytes. The relations of the neutrophiles with the red blood cells and the hemoglobin were very irregular.

3. Significant association of the white blood cells with variations in the red blood cells and the hemoglobin content were observed. The numerical variations in the group means of the total white cells were associated with similar variations in the group means of the neutrophiles, the lymphocytes, the monocytes, the basophiles, and the eosinophiles almost to the degree of their numerical occurrence in the peripheral blood.

4. With the exception of the total white cells, approximately only half the variations in the group levels of the various cells and of the hemoglobin content can be accounted for on the basis of simultaneous associations with each other.

5. The red blood cells, the lymphocytes, and the basophiles as one group, the eosinophiles and the monocytes as another group, and the hemoglobin content and the neutrophiles as a third group, described a definite shift from a high to a low numerical value during the 2 year observation period. From the standpoint of the magnitude of the shift, the basophiles, the eosinophiles, the monocytes, the lymphocytes, and the red blood cells participated in the order mentioned. The neutrophiles were only slightly affected and the hemoglobin content relatively not at all.

6. No significant relationship was ever found, even in the component groups, between the weekly mean values of the following: the hemoglobin with the basophiles, the eosinophiles, or the lymphocytes; the neutrophiles with the basophiles or the eosinophiles; and the lymphocytes with the eosinophiles or the monocytes.

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