STUDIES IN THE BLOOD CYTOLOGY OF THE RABBIT

I. Blood Counts in Normal Rabbits

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The cells of the peripheral blood in rabbits have been studied in this laboratory in connection with the problem of the constitutional factors related to the occurrence and course of disease, and with especial reference to the influence of environmental conditions (1). Although many investigations have dealt directly or indirectly with the cytology of rabbit blood comparatively little has been published regarding the blood cell picture of normal rabbits from the standpoint of a large material. Furthermore, the possibility of spontaneous variations over periods of time with respect to general environmental conditions such as seasonal change has received but scant attention. These features are among those included in the present study. Observations have also been made on various types of animal material and in connection with studies primarily concerned with factors of an environmental (experimental) nature. Still other observations have been carried out on rabbits inoculated with *Treponema pallidum*, a malignant tumor, and virus III, the first two agents being those with which most of our studies on constitutional and environmental factors have been conducted.

The results of the investigation will be published in a series of papers of which this is the first. It contains the results of a statistical analysis of 1110 blood counts obtained from 174 normal rabbits, with especial attention directed to distribution frequencies. The values obtained from this analysis will be used in subsequent papers as a basis with which to compare the results of other experiments. Preliminary notes on certain phases of the study have already been published (2).

Materials and Methods

The rabbits employed were, as far as could be determined, a fair sample of the animal material available for experimental purposes. For the most part, the ordinary brown and gray types predominated but the type described as the Flemish cross or mixture was also represented, and there were a few black and albino animals. Only male rabbits approximately 6 to 8 months old were used; a few were thought to be slightly younger and a few were probably a month or two older. Each rabbit was caged separately in a well lighted (sunlight), well ventilated room; the diet throughout the period of observation consisted of hay, oats, and cabbage.

The results reported are based upon 1110 blood counts on 174 rabbits during 13 months, from October 20, 1927 to November 22, 1928. The majority of examinations, 1001 in number, were made during the period ending June 20, 1928. The observations were derived from 3 sources of animal material:

I. 10 rabbits; 426 observations. Between October 20, 1927 and June 20, 1928, blood counts on 5 animals were made at weekly intervals. With the other 5 rabbits, biweekly examinations were carried out from October 20, 1927 to February 10, 1928 and thereafter to June 19, 1928 at weekly intervals.

II. 10 rabbits; 130 observations. Weekly counts were made from March 29, to June 19, 1928.

III. 154 rabbits; 554 observations. This mixed group contains, first, single observations on 9 groups of 5 to 10 rabbits each, a total of 75 animals, examined from December 16, 1927 to August 9, 1928, and within a few days from the time of receipt from the dealers. Second, there are 470 observations distributed among 11 groups of 5 to 10 rabbits each, a total of 70 animals; these groups were examined 4 to 11 times during 1 to 8 weeks. Third, a single observation on 9 of the 10 rabbits comprising group I made 4 months after the regular examinations had been discontinued, is included in this group.

A uniform routine with respect to the time and the method of collection and examination of the blood was followed. The rabbits were fed as usual on the day preceding the examination but received no food on that day until after the specimens of blood had been obtained. The majority of observations were made from 9 A.M. to 1 P.M. In the case of 2 groups with a large number of observations, the interval at which examinations were made was largely determined by other experiments in progress; the rabbits were always examined in the same order on the same day of the week and at the same or approximately the same hour.

The blood was obtained from an ear vein without disturbance by simply placing the rabbit upon a rough surface such as a towel stretched tightly across a table top. An assistant supported the head and held the ear forward from its base in a convenient position. The external surface of the ear was moistened with dilute alcohol and a small area was carefully shaved. The shaved skin was dried with gauze and the vessels dilated by means of an electric bulb beneath the ear. A small vessel was punctured with the point of a corneal knife and a few drops of blood allowed to flow freely before any was collected. The specimens for the following

examinations were then obtained in the order designated: the differential white count (supravital technic), the white cell count, the hemoglobin estimation, and the red cell count. All specimens from 4 rabbits were taken one after the other and the counts were then made immediately. The method of Sabin (3) was followed for the supravital preparations, vital neutral red dye[1] being used and 100 white cells in each specimen counted. Standardized pipettes and the usual dilutions of 1:100 of Hayem's solution for the red cells and 1:10 of a 1.0 per cent acetic acid solution for the white cells were employed. The hemoglobin content was determined by the Newcomer hemoglobinometer, using 0.2 cc. blood in 10 cc. of $\frac{N}{10}$ hydrochloric acid.

In the analysis of results, absolute numbers of cells per cmm. of blood have been considered. The distribution curves given in Text-figures 1 to 8 are based upon the use of a group interval of approximately one-tenth of the mean value of each class of cells. The curves obtained by plotting the actual results were smoothed by the formula $\frac{a+2b+c}{4}$; the initial value of each smoothed curve is represented by $\frac{2b+c}{3}$ and the final value by $\frac{a+2b}{3}$. In some instances, the curves are not extended to the upper limits but the values omitted are few and scattered. The limit to which a curve should be extended is indicated by a figure placed at the termination of the curve.

The mode as reported was obtained by the use of King's formula applied to the unsmoothed distribution curve, mode $= 1 + \frac{f2}{f2+f1}c$, in which 1 is the lower limit of the group in which the greatest number of values is massed, f 2 the frequency in the group above, and f 1 the frequency in the group below the modal group, and c the size of the group interval. By varying f 1 and f 2 to represent the combined frequencies of the 2 or 3 groups below and above the modal group, the formula was in reality applied to a smoothed curve. In the case of the hemoglobin in which the modal group is approached by an adjoining group, c represents 2 groups. The empirical formula of Pearson, mode = mean − 3 (mean − median), and the mean of the modal group were also used to check the results of the above method.

The figures as given include all data. No count has been omitted because of the occurrence of such conditions as snuffles or ear canker in occasional rabbits observed for the longer periods. In all other cases, the animals were apparently free from disease. It seemed best to present the material in the present form be-

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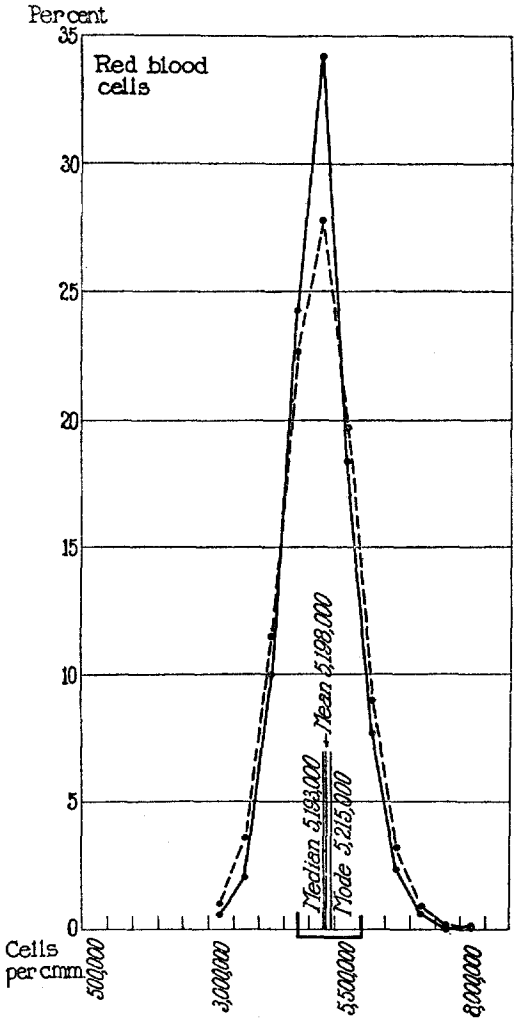
[1] The dye employed was neutral red iodide No. 2 supplied through the kindness of Dr. Barnett Cohen, Hygienic Laboratory, Washington, D. C.

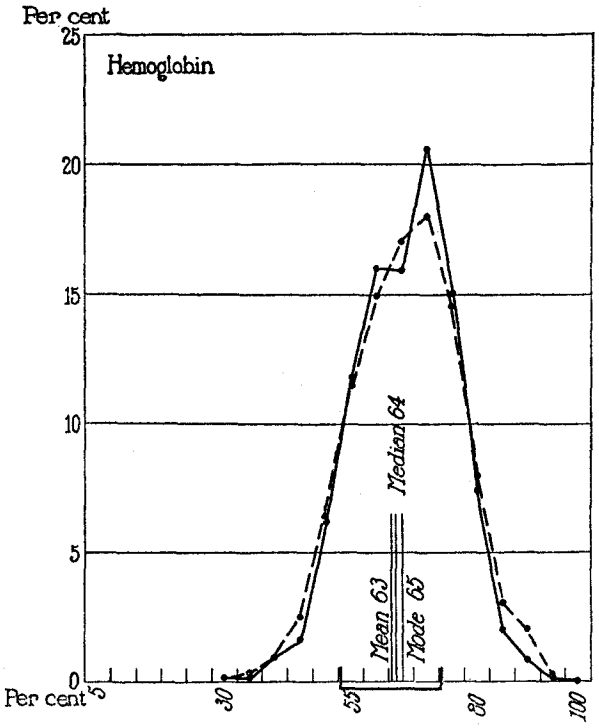
fore attempting to make corrections for disease or any other factor that might be associated with variations in the numbers of cells.



TEXT-FIG. 1. Distribution frequency of numbers of red blood cells per cmm. in per cent. In this and subsequent text-figures, the smoothed curve is graphed as a broken line and the brackets on the base line denote the limits of the standard deviation.

RESULTS

The results obtained in this series of 1110 blood cell determinations on 174 normal rabbits are presented in condensed form in Table I and Text-figures 1 to 8. The analysis of this material has included the determination of the following numerical values for the total red and white cells and the several classes of white cells per cmm., and the hemoglobin content: minimum, maximum, and mean counts, the probable error of the mean, the mode, the median, the standard deviation and the coefficient of variation (Table I). The distribution frequencies of the cells and the hemoglobin have also been determined (Text-figs. 1 to 8).

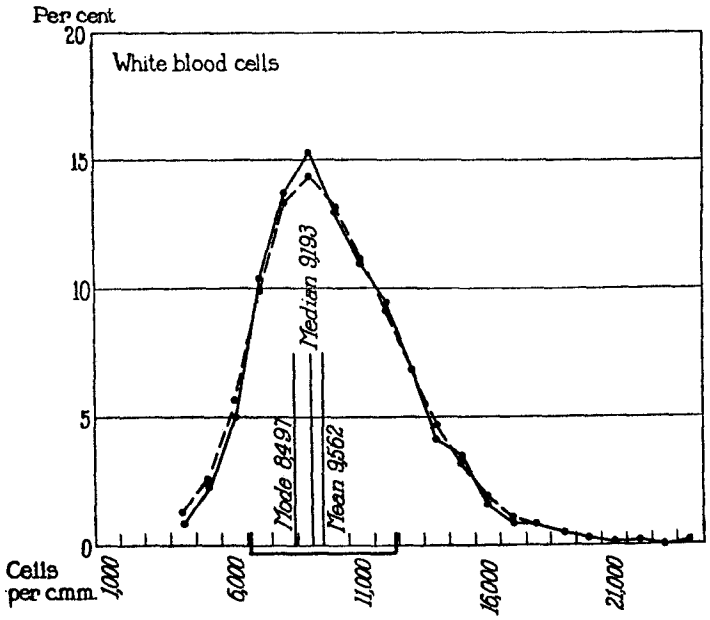


TEXT-FIG. 2. Distribution frequency of percentage of hemoglobin content in per cent.

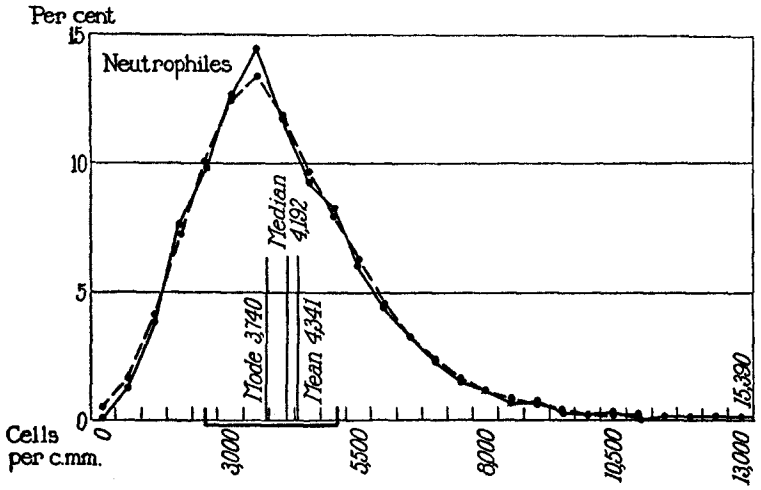
TABLE I

Summary of Numerical Values Obtained from 1110 Blood Counts on 174 Male Rabbits of Various Ages and Breeds—from October 20, 1927 to November 21, 1928

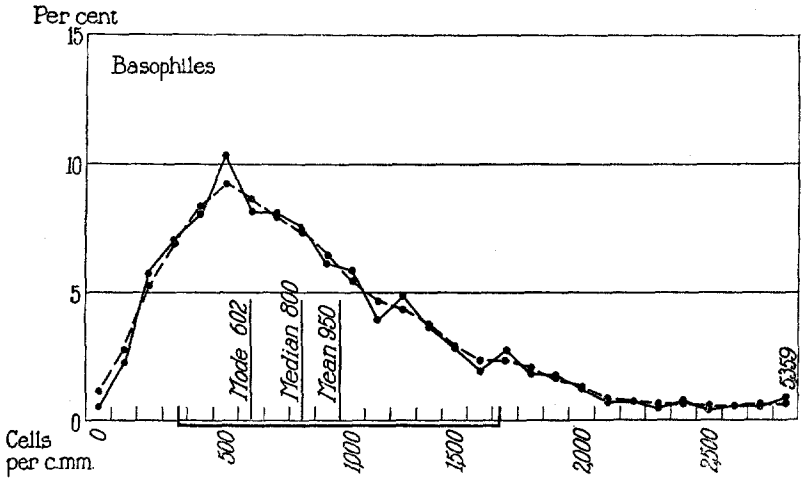
	Mean	Mode	Median	Minimum	Maximum	Standard deviation	Coefficient of variation
	per cmm.	*per cmm.*	*per cmm.*	*per cmm.*	*per cmm.*	*per cmm.*	*per cent*
Red blood cells	5,198,000 ±12,700	5,215,000	5,193,000	3,020,000	8,040,000	628,250	12.09
Hemoglobin	63%± 2%	65%	64%	28%	90%	10%	15.87
White blood cells	9562± 59	8497	9193	3150	23500	2919	30.53
Neutrophiles	4341± 37	3741	4192	1050	15390	1823	41.99
Basophiles	950± 13	602	800	0	5359	635	66.84
Eosinophiles	214± 4	90	159	0	1760	217	101.40
Lymphocytes	3045± 28	2402	2904	630	9900	1366	44.86
Monocytes	1000± 12	750	898	72	5405	571	57.10



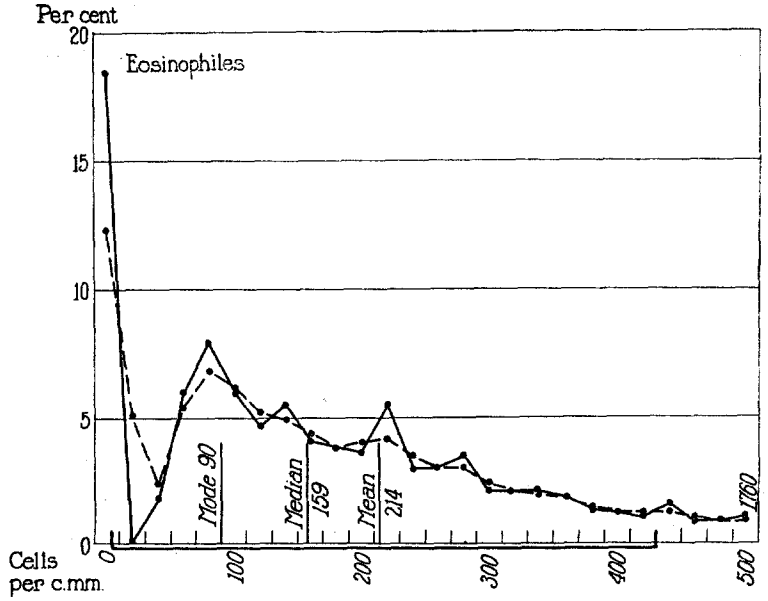
TEXT-FIG. 3. Distribution frequency of total numbers of white blood cells per cmm. in per cent.



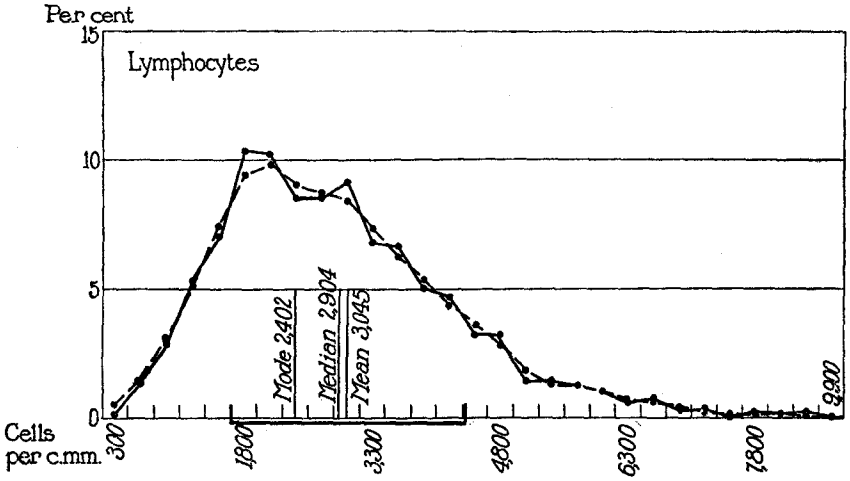
TEXT-FIG. 4. Distribution frequency of numbers of neutrophiles per cmm. in per cent.



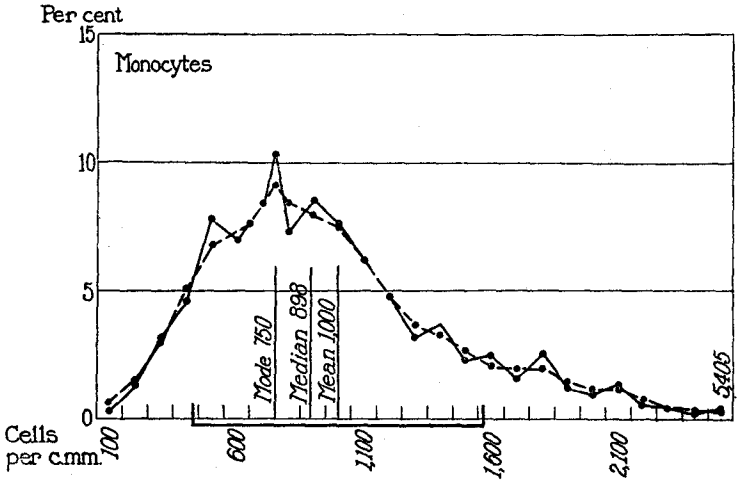
TEXT-FIG. 5. Distribution frequency of numbers of basophiles per cmm. in per cent.



TEXT-FIG. 6. Distribution frequency of numbers of eosinophiles per cmm. in per cent.



TEXT-FIG. 7. Distribution frequency of numbers of lymphocytes per cmm. in per cent.



TEXT-FIG. 8. Distribution frequency of numbers of monocytes per cmm. in per cent.

DISCUSSION AND CONCLUSIONS

In discussing the results of these experiments, it should be borne in mind that no effort was made to maintain uniformity of material other than that the rabbits employed were comparable with those used in other experiments. And the same may be said regarding the conditions of living while the animals were under observation. Various breeds of rabbits were used, certain groups were studied for short and others for long periods of time, while other series were examined but once. These conditions, of course, tend to emphasize the element of variability. One purpose of this study, however, was to ascertain the possible as well as the probable limits of variation in the numbers of cells and in the hemoglobin content. In the present paper, the results are considered primarily from the standpoint of the range of individual counts rather than from that of the various groups of rabbits comprising the entire series.

An examination of the data contained in Table I and in the distribution curves of Text-figs. 1 to 8 shows that with one exception, the numbers of cells in the peripheral blood of rabbits are subject to wide variations. The exception is the erthrocytes, the coefficient of variation of which is 12.09 per cent, a value much lower than those of the various granulocytes, the lymphocytes, and the monocytes. As might be expected in view of this finding, the variation of the hemoglobin content is of a similar order of magnitude, its coefficient of variation being 15.87 per cent. In contrast to these findings, the various white cells are characterized by a high order of variability. The coefficients of variation of the neutrophilic leucocytes and the lymphocytes are 41.99 and 44.86 per cent; those of the basophiles and monocytes are somewhat higher, 66.84 and 57.1 per cent while that of the eosinophiles reaches the high value of 101.4 per cent. The variability of the total white blood cells, on the other hand, occupies a lower level, its coefficient of variation being 30.53 per cent.

It will be seen that the distribution curves for the red blood cells (Text-fig. 1) and the hemoglobin content (Text-fig. 2) have the same general form and are almost symmetrical; they show a relatively narrow range of numerical distribution with a rapid rate of increase and decrease in the percentage of counts whose numbers are close to the mean and mode and have but a slight tendency to prolongation at either

extreme. Both curves, however, show a slight left skew, a feature which is brought out more clearly by the relative positions of the mean, the median, and the mode. The differences between the values for the mean and mode are extremely small, 17,000 cells for the erythrocytes and 2 per cent for the hemoglobin respectively. It is not unlikely that with a larger number of observations the curves would conform to theoretical expectations. In the case of the hemoglobin, the curve in its ascending portion at the 60–65 per cent level shows a peculiar flattening which is reflected in the more rounded shape of the ascending as compared with the descending limb of the smoothed curve. The explanation of this peculiar feature is not entirely clear but it may be a question of the matching of shades at this particular level in making the readings.

The distribution frequencies for the other cells are much more irregular. With the single exception of the eosinophiles (Text-fig. 6), the total white cells, the neutrophiles, the basophiles, the lymphocytes, and the monocytes (Text-figs. 3, 4, 5, 7, 8) have a tendency to follow the same general form of numerical distribution as the red blood cells and the hemoglobin content but the curves are of a much wider range and are decidedly skewed to the right, due to the occurrence of varying counts at levels well above the mean. The form of the curves for the total white cells and the neutrophiles shows that the most frequent values occupy a considerably narrower range than those of the basophiles, lymphocytes, and monocytes. In the case of these 3 classes of cells, the highest portion of the curves shows a fairly wide plateau, the right extreme of which corresponds approximately to the level of the mean while the left falls below the mode. The relative positions of the mode, the median, and the mean conform to theoretical expectations in all cases.

The curves for the total white blood cells and the neutrophiles are quite regular but those of the other classes of white cells show many irregularities chiefly in their descending portions. These conditions extend the zone of high frequency distribution and in addition, are responsible for the high coefficients of variation. The distribution curve for the eosinophiles (Text-fig. 6) differs from all the others with respect to the high level of its initiation, but if this portion of the curve is omitted, its general form resembles the others. It is, however,

somewhat flatter. The peculiar first part of the curve is due to the fact that in many counts no eosinophiles were seen. In order to make the chance of including a fair representation of eosinophiles equal to that of the monocytes and basophiles for example, it would be necessary to count 500 instead of 100 cells upon the basis of the ratio of the respective mean values for these cells which is approximately 5:1 (Table I). As with the other classes of white cells, the curve for the eosinophiles shows a marked skew to the right indicating a wide range of high frequencies. Its irregularities (descending portion), however, are more pronounced than those of the other cells with the possible exception of the monocytes. These conditions make for the high order of magnitude—101.4 per cent—of the coefficient of variation.

The curves for the total white cells and the neutrophiles are very similar and at first glance, it might be supposed that the distribution of neutrophile values was responsible for this similarity. But the neutrophiles represent approximately only half the total number of white cells (Table I) and since the coefficient of variation of the total white cells is less than that of the neutrophiles, it is evident that the other cells, considered collectively, would form a distribution curve not unlike that of the neutrophiles and with a smaller coefficient of variation than those of the individual classes of cells. This would suggest that while these various classes of cells vary widely, they tend to preserve some kind of a relation so that their total numbers, from a collective point of view, are approximately as constant as the numbers of neutrophiles.

The results of this study with respect to the mean values for the red and white blood cells and for the neutrophiles are of the same general order as those reported by others, as for example Bushnell and Bangs (4), Scott and Simon (5), and Cunningham, Sabin, Sugiyama, and Kindwall (6) (Table II). With the other classes of white cells, however, there is less agreement except in the case of the last named authors whose mean values for the lymphocytes and monocytes differ but little from those here reported. It should be noted that the differential counts of Bushnell and Bangs, and presumably those of Scott and Simon, were made from fixed preparations while Cunningham, Sabin, Sugiyama, and Kindwall used the supravital technic.

TABLE II

Comparison of Blood Cell Values as Given by Various Authors

	Number of counts	*Number of rabbits*
Bushnell and Bangs	100	100
Scott and Simon	100	100
Cunningham et al.	217*	53
Pearce and Casey	1110	174

	Mean	Standard deviation	Coefficient of variation	Percentage of white blood cells
	per cmm.	*per cmm.*	*per cent*	*per cent*
Red blood cells				
Bushnell and Bangs	5,989,500	779,358	13.01	
Pearce and Casey	5,198,000	628,000	12.09	
White blood cells				
Bushnell and Bangs	10,675	2224	20.83	
Scott and Simon	11,105			
Cunningham et al.	11,281			
Pearce and Casey	9,562	2919	30.53	
Neutrophiles				
Bushnell and Bangs	4174	1161	27.57	39.1
Scott and Simon	3825			34.4
Pearce and Casey	4341	1823	41.99	45.4
Basophiles				
Bushnell and Bangs	382	232	60.61	3.4
Scott and Simon	135			1.2
Pearce and Casey	950	635	66.84	9.9
Eosinophiles				
Bushnell and Bangs	120	90	75.00	1.1
Scott and Simon	374			3.3
Pearce and Casey	214	217	101.40	2.2
Lymphocytes				
Bushnell and Bangs	5754†	1196	20.77	53.9
Scott and Simon	6297			56.7
Cunningham et al.	2805			24.9
Pearce and Casey	3045	1366	44.86	31.8
Large mononuclears				
Bushnell and Bangs	49	54	118.60	0.43
Scott and Simon	532			4.7
Transitionals				
Bushnell and Bangs	114	96	84.11	1.07
Monocytes				
Cunningham et al.	943			8.43
Pearce and Casey	1000	571	57.1	10.5

* This value which does not appear in their publication, was supplied by the authors.

† Small lymphocytes only.

In regard to the coefficient of variation, the values of Bushnell and Bangs and those reported here are in close agreement for the red blood cells but with the other cells the differences range from 6.23 in the case of the basophiles to 26.4 for the eosinophiles. Similar differences are also found in the standard deviation values of the two series. In view of the experimental and technical circumstances, however, it is not surprising that the results of several series of observations, such as those contained in Table II, do not agree more closely. The relative numbers of counts and of animals employed, the method used for the differential white cell counts, and the nature of the animal material are among the factors which obviously affect the nature of results of this character.

Although the present study is based upon a large number of observations, a comparatively large number of normal stock rabbits and an acceptable method of white cell differentiation, the values submitted for the total red and white cells counts, for the numbers of the various classes of white cells, and for the hemoglobin content in the peripheral blood should be accepted as approximate values of normality. They serve the useful purpose of orientation and in addition, may be employed as a basis of comparison for the results of other experiments. But it will become evident when the various small groups comprising the series and the factors affecting them are considered, that the influence of existing conditions must be taken into account before any value be accepted as a standard of normality.

SUMMARY

A study of the blood cytology of normal male rabbits was carried out from October 20, 1927 to November 22, 1928 in connection with an investigation of constitutional and environmental factors related to the occurrence and course of disease. In 1110 observations on 174 animals, total red and white cells counts, differential white cell counts by the supravital method, and hemoglobin estimations were made.

A statistical analysis of the results obtained is presented. Attention is directed to the occurrence of wide variations in the numbers of the various white cells as contrasted with comparatively small variations in the numbers of red cells and of hemoglobin content.

The results recorded are regarded as representing approximate rather than fixed values of normality.

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