STUDIES ON THE BLOOD CYTOLOGY OF THE RABBIT

IX. Blood Platelet Counts on Healthy Male Rabbits

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During a study on the hematological constitution of the rabbit under various conditions and circumstances (1–8), observations were made, as opportunity permitted, on the platelet count of the peripheral blood of healthy rabbits. The animals, as far as could be determined by continued inspection and body weight values, were free from disease conditions, such as snuffles, which may occur in the laboratory stock. Similar observations carried out on other rabbits which could not be considered healthy according to these criteria have been excluded from analysis. Within the prescribed limits, therefore, it may be assumed that the results obtained and herewith presented represent a fair sample of the platelet values of healthy laboratory rabbits.

Material and Methods

This study is based upon 991 platelet determinations made on 148 male rabbits during the 2½ year period between Nov. 8, 1929, and May 15, 1932. All animals were in good physical condition and apparently free from any disease during the entire period of observation which continued for 3 weeks after the last count. The criteria of health included the features of nutrition and weight, as well as vigor, alertness, appetite, and condition of the fur. The occurrence of such conditions as a purulent nasal discharge, ear canker, subcutaneous abscess, or diarrhea was considered sufficient to eliminate the counts on such an animal from the present analysis. The results include all counts made on each animal fulfilling the above requirements.

Rabbits from two sources were employed: those purchased from dealers and those bred in the laboratory. The animals purchased from dealers were representative of the types commonly employed, that is, greys, browns, and Flemish crosses with an occasional black or albino. The rabbits bred in the laboratory consisted of pure and of hybrid types (Table I). The animals were 4 to 12 months of age; 6 were 18 months old.

Each animal was kept in an individual cage in a well ventilated, sunny room.

The diet from Nov., 1929, to Sept., 1930, consisted of hay, oats, and cabbage. In Sept., 1930, the cabbage was replaced by artificial food pellets and a free supply of water.

TABLE I

991 Blood Platelet Counts upon 148 Healthy Male Rabbits Distributed According to Animal Breeds

Breed (pure)	No. of rabbits	No. of counts	Breed (hybrids)	No. of rabbits	No. of counts
Sable	2	8	Albino-Himalayan	17	75
Silver	2	10	Dutch-Himalayan-Albino	5	17
Chinchilla	5	25			
Polish	6	30	Lilac-English	1	2
Havana	13	59	(Purchased from dealers—composition unknown.) Brown, grey, etc.	29	474
English	18	81			
Dutch	10	44			
Beveren	10	48			
Gouda	5	23			
Himalayan	19	68			
Belgian	6	27			
Total.....	96	423	Total.....	52	568

Fifteen groups of rabbits were examined as shown in Table II. Elimination of diseased animals is responsible for the smaller number of animals comprising many of the groups. The actual number of animals eliminated is as follows: Group I, 5; Group II, 6; Group III, 4; Group IV, 8; Group V, 3; Group VII, 3; Group VIII, 6; Group XV, 10 rabbits respectively.

In general, 4 to 5 platelet counts were made on an animal, but the intervals between counts were variable. From the standpoint of the time interval, the animal groups fall into three categories. The first is represented by Group V (Table II) in which regular weekly blood examinations were made over an initial period of 9 months and biweekly over a subsequent period of 8 months. The second is represented by Groups I, II, III, IV, VI, XI, XII, XIII, XIV, and XV in which 2 to 5 counts were spread over a period of 1 to 2 weeks. The third category is represented by Groups VII, VIII, IX, and X, on each animal of which 4 successive platelet counts were made on the same day from the same ear vein incision.

In Table III is shown the distribution of the animals and counts by months. During the 2½ year period between Nov., 1929, and May, 1932, platelet counts were made in the following 21 months: Nov. and Dec., 1929; Jan., Oct., Nov., Dec., 1930; Jan., Feb., Mar., Apr., May, June, Sept., Oct., Nov., Dec., 1931; and in Jan., Feb., Mar., Apr., May, 1932. No counts were made during the months of July and August, and only once in June and September. During these 21 months

TABLE II

Data on Animal Material Used for Platelet Counts

Source of animals	Group No.	No. of animals	Date of first count	Date of last count	No. of counts
Purchased from dealers	I	7	Nov. 8, 1929	Nov. 19, 1929	28
	II	6	Dec. 31, 1929	Jan. 14, 1930	30
	III	2	Jan. 15, 1930	Jan. 17, 1930	4
	IV	7	Oct. 30, 1930	Nov. 13, 1930	21
	V	7	Oct. 30, 1930	May 15, 1932	391*
Bred in the laboratory	VI	8	Dec. 31, 1929	Jan. 14, 1930	40
	VII	11	Mar. 24–25, 1931	Mar. 24–25, 1931	36†
	VIII	5	May 19–20, 1931	May 19–20, 1931	20
	IX	10	June 10–12, 1931	June 10–12, 1931	40
	X	10	Sept. 17–18, 1931	Sept. 17–18, 1931	39‡
	XI	6	Apr. 21, 1931	Apr. 28, 1931	13
	XII	10	Nov. 5, 1931	Dec. 3, 1931	50
	XIII	5	Mar. 24, 1932	Mar. 29, 1932	32
	XIV	23	Mar. 22, 1932	Mar. 30, 1932	92
	XV	31	Apr. 12, 1932	Apr. 26, 1932	155
From dealers.....	5	29	Nov. 8, 1929	May 15, 1932	474
Laboratory bred.....	10	119	Dec. 31, 1929	Apr. 26, 1932	517
Total.....	15	148	Nov. 8, 1929	Apr. 26, 1932	991

* One animal died through accident.

† 8 counts lost.

‡ 1 count lost.

TABLE III

991 Blood Platelet Counts upon 148 Healthy Male Rabbits Distributed According to Months (1929–32)

Month	No. of rabbits	No. of counts	Month	No. of rabbits	No. of counts
Sept.	10	39	Mar.	45	207
Oct.	14	38	Apr.	43	229
Nov.	31	127	May	15	57
Dec.	31	83	June	16	52
Jan.	23	119	July	0	0
Feb.	7	40	Aug.	0	0
Total.....				148	991

blood for platelet counting was taken on 90 different days, usually Tuesdays, Thursdays, or Fridays. Approximately 11 platelet counts were made, therefore, on each day on which animals were bled.

The observations were usually made between 9 and 12 a.m., but a large number were also made between 2 and 4 p.m., and the examinations were always carried out on animals which had received no food since the previous day. The platelet values were determined according to the method described by Casey and Helmer

TABLE IV

Blood Platelet Counts on Healthy Rabbits

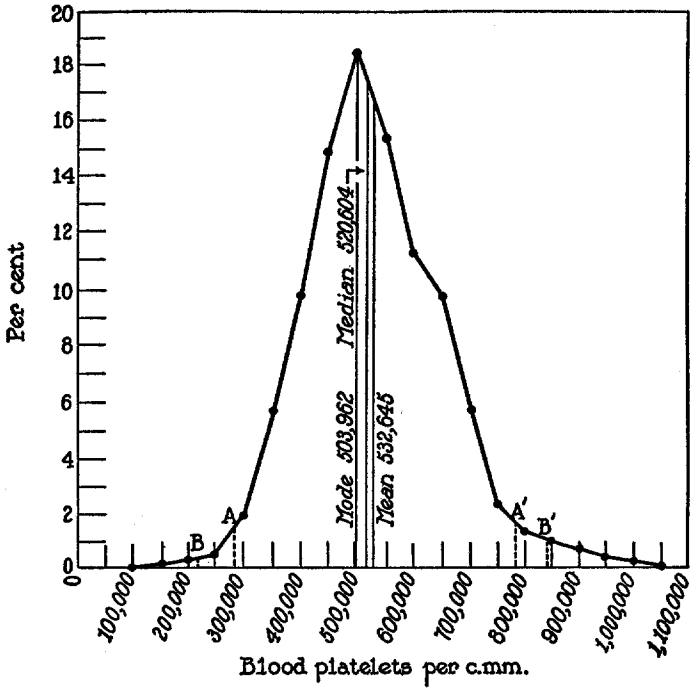
Mean of class	Frequency of counts	Frequency	Summary of observed and estimated values		
		per cent			
150,000	1	0.1	Mean	=	532,645 per c.mm.
200,000	3	0.3	Median	=	520,604 per c.mm.
250,000	5	0.5	Mode	=	503,962 per c.mm.
300,000	20	2.0	Maximum	=	1,120,000 per c.mm.
350,000	56	5.7	Minimum	=	170,000 per c.mm.
400,000	97	9.8	Standard deviation	=	124,960 per c.mm.
450,000	148	14.9	Standard error of		
500,000	182	18.4	the mean	=	3,970 per c.mm.
550,000	153	15.4	Standard error of		
600,000	111	11.2	the standard de-		
650,000	97	9.8	viation	=	2,808 per c.mm.
700,000	56	5.7	γ_1	=	$+0.558 \pm 0.078$
750,000	24	2.4	γ_2	=	$+0.872 \pm 0.156$
800,000	14	1.4	Coefficient of vari-		
850,000	10	1.0	ation	=	23.46 per cent
900,000	7	0.7			
950,000	4	0.4			
1,000,000	2	0.2			
1,050,000	0	0.0			
1,100,000	1	0.1			

(9) and by Casey (10) in which Ringer's solution, containing a small amount of heparin, is used as the diluent. The blood is taken up in an ordinary red blood cell pipette and a red blood cell count made in the usual manner with a Neubauer chamber. After the red blood cells have been counted, the blood platelets are counted in the same preparation with the low power dry lens; the platelets appear as black refractile bodies about one-tenth to one-half the size of a red blood cell. The technical error[1] in this procedure has been found to be 8 per cent which is

[1] The coefficient of variation due solely to technique is called the technical error in this instance.

smaller than those found in any of the other methods tested and compares with a 5 per cent error for red cell and an 11 per cent error for white cell determinations.

The usual statistical methods were employed in analyzing the results obtained (11–13). A difference was considered to be significant when it was more than $2\frac{1}{2}$ times its standard error; that is, when the probability of its occurrence by chance was less than 1 in 100. The mode was calculated by an application of King's formula.



TEXT-FIG. 1

RESULTS

The results of the statistical analysis of 991 blood platelet counts on 148 healthy male rabbits include estimated values for the mean, the mode, the median, the maximum, the minimum, the standard deviation, the standard error of the mean and standard deviation, and the coefficient of variation (Table IV, Text-fig. 1). The value γ_1 indicates that the distribution curve resulting from the particular determinations selected is significantly skewed to the right. The value γ_2 indicates that this distribution does not follow the normal curve of

error, being heaped up too high on the top and cut too thin at the shoulders.

The lines marked A and A′ in Text-fig. 1 are separated from the mean value of 533,000 by an interval equal to twice the standard deviation. The lines marked B and B′ are separated from the mean by an interval equal to $2\frac{1}{2}$ times the standard deviation. This signifies that counts less than the value represented by A or greater than the value represented by A′ should not be expected to occur among healthy animals (if the animals selected constitute a fair sample and the real distribution is normal) more often than once in 22 counts. In like manner, values less than B or greater than B′ should not be expected to occur more often than once in 100 counts. On this basis one might expect a platelet count greater than 1,000,000 per c.mm. or less than 100,000 to occur but once in 1,000 counts among healthy male rabbits.

The results of the present study indicate that a platelet count of more than 280,000 and less than 780,000 per c.mm. should be considered as normal while values of less than 220,000 or more than 820,000 per c.mm. should be designated as abnormal, regardless of the breed, time of the year, or other factors.

DISCUSSION

If platelet counts were made on representative types from all the standard breeds of rabbits and their hybrids, such counts being done at intervals of the day, week, month, year, and era; under various conditions of diet and housing; in different localities in the world, with various technical methods, a relatively accurate analysis of the blood platelet count in healthy adult male rabbits might be attempted. Since such extensive information is difficult to obtain, any one group of workers must strike some sort of compromise and then strive to evaluate the dependability of the results obtained.

Under Material and Methods some pains were taken to describe fully just what sort of compromise was entered into in collecting the present series of counts. It is now necessary to attempt an evaluation of these points of compromise.

Time.—The time of the day, week, month, and year in which the platelet counts were made might affect the results obtained. As to time of the day, the blood was taken between 9 and 12 a.m. and be-

tween 2 and 4 p.m. In an investigation of the material two groups of rabbits with 13 and 11 animals respectively were counted on 4 days distributed over a period of 2 weeks. It so happened that Group I was counted between 9 and 12 a.m. on the 1st and 3rd days and between 2 and 4 p.m. on the 2nd and 4th days; Group II, on the other hand, was bled between 2 and 4 p.m. on the 1st and 3rd days and between 9 and 12 a.m. on the 2nd and 4th days. When all counts (48) made in the morning were averaged and compared with all counts made in the afternoon (48), a difference of 3,917 ± 12,768 existed between the respective means. Since the difference between morning and afternoon counts was less than twice its standard error, it would not seem likely that such variations as might occur during the remaining 12 hours of the day would materially alter the population figures for platelet counts obtained.

As to day of the week, counts were made on 90 different days during this $2\frac{1}{2}$ year period particularly on Tuesday, Wednesday, Thursday, and Friday, but also to some extent on Monday and Saturday. The day of the week, therefore, would not be expected to offer particular bias in the results obtained.

As to the month of the year, means were calculated of all counts made in each of the 10 months followed. These ten monthly means were added and a mean for the entire 10 months of 524,759 ± 12,348 obtained. This differs from the general means of 532,645 by 7,876 ± 13,524. Since the difference was less than its standard error, even with the addition of the estimated values for July and August, the general mean if calculated by months would not be significantly different from the mean obtained upon the 991 counts.

Breed.—Of the 30 or more recognized standard breeds of rabbits about 15 are of medium or small body build and most of these are represented in our series. Counts were made also upon the heavier breeds such as New Zealand, American Blue, Flemish Giant, but these animals adapted poorly to cage life and were not in good physical condition. Their counts were eliminated from this series.

The means of all the counts made on each breed were calculated and from these 15 breed means a grand mean of 559,000 was obtained. This differed from the general mean by 26,995 ± 13,524. Since it is not quite twice the standard error of the difference, the disproportion-

ate number of counts on certain breeds did not seem to significantly affect the results obtained. Also the breeds of animals showing highest platelet counts were nearly all counted in the months of the year when the blood platelets are highest; therefore, the blood platelet level for breeds is possibly higher than it might be if seasons of the year were held constant.

The possible difference between pure breeds and hybrids was also considered. Analysis of mean platelet counts upon 96 pure breed rabbits was 545,687 ± 8,564 and upon the 23 laboratory bred hybrids 549,551 ± 17,876. The difference of 3,864 ± 19,821 did not indicate a difference in pure breeds and hybrids which would materially alter the values obtained.

Source of Animals.—It was stated that 29 animals were purchased from dealers whereas the remaining animals were bred in the laboratory. The mean platelet counts in the 29 rabbits obtained from dealers was 523,517 ± 17,243. This differed from the mean for the 119 laboratory bred rabbits (546,434 ± 7,622) by 22,917 ± 18,830 and was not significant.

Means for Individual Animals.—Since a large number of counts were made upon each of a single group of 6 animals, the question arose as to how far the mean and standard deviation might be affected by this type of bias. To this end the mean platelet count was obtained for each of the 148 rabbits in this series. A general mean was then obtained for these 148 means and found to be 543,245 ± 6,915. This differed by 10,000 ± 7,973 from the mean of the 991 counts in the same animals and was not significantly different. Again the standard deviation of the 517 counts made on laboratory bred rabbits did not differ from the standard deviation obtained for the entire 991 counts. It was concluded, therefore, that the large number of counts upon the one group did not materially affect the mean and standard deviation obtained.

The rabbits were selected on the basis of physical fitness and apparent freedom from disease. Our ability to select appropriate animals, therefore, entered into the results obtained. It is possible and also probable that some animals with internal disease conditions may have been included. The question arises as to whether the skew to the right in the frequency curve is due to the inclusion of such individuals.

The only available information on this point is indirect. The mean of all platelet counts on the 48 rabbits eliminated from this series because of visible disease or malnutrition was 655,188 per c.mm., that is, a value which exceeds by 122,543 the mean of the apparently normal animals. This difference which is more than 6 times the standard error of the difference is, therefore, highly significant. When the platelet counts on these 48 externally diseased rabbits were included in the frequency curve, together with those of the normal rabbits, the mode was not affected (503,962) but the mean was significantly higher (560,471) and the skew to the right in the curve was marked. The removal of the counts on the diseased animals from the frequency curve greatly reduced the skew, and it is possible, therefore, that the skew would have been further reduced if the animal material could have been more rigorously selected.

Diet.—It was mentioned in the section on Material and Methods that the diet was altered in September, 1930. The mean of all platelet counts (102) on normal animals (23) made prior to the change was 526,400 ± 12,727 and of (205) counts on (24) rabbits made after the change in the same 3 months of November, December, and January, 521,465 ± 8,474 per c.mm. respectively. The difference between the two means, 4,935 ± 15,290, is less than twice the standard error of the difference, so that the change in the diet cannot be said to have significantly affected the mean values of the platelet counts.

It should be pointed out that although no significant changes occurred in platelet values of the present series which could be ascribed to differences in source of animals, diet, or method of counting, it is possible or perhaps probable that such related changes could occur. The experiments were not arranged to bring out such differences, but, on the contrary, to minimize them if they were present. As far as can be ascertained at present, all that one can say is that in this series no individual factor appeared to exert a significant bias in the results.

Other features of the technique employed which might affect the results were the fact that the counts were made on fasting animals and that the procedure of platelet enumeration was different from that commonly used. Conditions of housing and climate and the years in which the study was made are other factors which theoretically might

be considered. The results of other workers using different methods of platelet counting as well as different experimental conditions are, however, comparable to ours, and it would appear, therefore, that in the usual circumstances of the laboratory and with the usual rabbit stock, no striking differences in mean platelet values are to be expected. The values compiled by Scarborough (14) in 1926 from the results of ten authors, most of whom used different methods and made counts on fewer than 20 animals, averaged 540,000 platelets per c.mm. with limits of from 200,000 to 1,000,000. Dudgeon and Goadby's (15) recent platelet values for 3 normal rabbits were 503,400, 489,000, and 1,009,200 per c.mm.; Ecker and Rees (16) gave platelet values for 8 normal rabbits which averaged 559,000 and ranged from 368,000 to 853,000 per c.mm. respectively. It may be mentioned in this connection that the selection of rabbits from the standpoint of freedom of disease or of age or sex did not appear to have been specifically considered. It is of additional interest, therefore, that the results obtained differ so little from those of the present experiments in which these factors were controlled.

It has been stated above that counts of less than 175,000 or more than 875,000 per c.mm. should be considered definitely abnormal. It should be pointed out, however, that the rabbit in which an abnormal platelet value occurs need not necessarily be an abnormal animal. The classification of abnormality must obviously be made on more inclusive grounds.

SUMMARY AND CONCLUSIONS

1. Repeated platelet counts, 991 in number, were made on 148 adult male rabbits of various breeds and types which were specifically selected on the basis of physical fitness and continued freedom from disease. The observations extended over a $2\frac{1}{2}$ year period (1929–32).

2. A unimodal, peaked, moderately asymmetrical distribution curve was obtained and an analysis of the results gave the following values: the mean, 532,645 platelets per c.mm.; the median, 520,604 per c.mm.; the mode, 503,962 platelets per c.mm. respectively.

3. The extent of the variation among the counts is shown by the standard deviation of 124,960. High platelet counts were found to occur more frequently than low counts, a result that is reflected in a significant skew to the right in the frequency curve.

4. It was calculated that in healthy male rabbits a platelet count of less than 220,000 or more than 845,000 per c.mm. should be considered abnormal.

BIBLIOGRAPHY

1. Brown, W. H., *Harvey Lectures*, 1928–29, **24,** 106; *Arch. Int. Med.*, 1929, **44,** 625.
2. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51,** 83.
3. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52,** 23, 39, 145, 167.
4. Casey, A. E., *J. Exp. Med.*, 1931, **53,** 695.
5. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1931, **53,** 895.
6. Pearce, L., and Casey, A. E., *Proc. Soc. Exp. Biol. Med.*, 1929, **26,** 666.
7. Casey, A. E., *Proc. Soc. Exp. Biol. Med.*, 1929, **26,** 670.
8. Casey, A. E., and Pearce, L., *J. Exp. Med.*, 1931, **54,** 475.
9. Casey, A. E., and Helmer, O. H., *Proc. Soc. Exp. Biol. Med.*, 1930, **27,** 655.
10. Casey, A. E., *Proc. Soc. Exp. Biol. Med.*, 1931, **28,** 523.
11. Fischer, R. A., Statistical methods for research workers, London, Oliver and Boyd, 3rd edition, 1930.
12. Pearl, R., Medical biometry and statistics, Philadelphia and London, W. B. Saunders Co., 1923.
13. Chaddock, R. E., Principles and methods of statistics, Boston, Houghton Mifflin Co., 1925.
14. Scarborough, R. A., *Yale J. Biol. Med.*, 1930, **3,** 63.
15. Dudgeon, L. S., and Goadby, H. K., *J. Hyg.*, 1931, **31,** 247.
16. Ecker, E. E., and Rees, H. M., *J. Infect. Dis.*, 1922, **31,** 361.