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THE NORMAL PLATELET COUNT IN MAN

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A knowledge of the concentration of blood platelets in the circulating blood is essential in the diagnosis of thrombocytopenic states and is of the greatest value in following their response to treatment.

Although the current methods employed for counting blood platelets are acknowledged to be of limited accuracy, the best of them are sufficiently accurate to be of clinical value, since only fairly gross changes in the platelet count can be related to changes in the patient's physical condition. The value of platelet counts would, however, be greatly enhanced if we had more accurate knowledge of what does in fact constitute a normal count, within what limits this is significantly determined, and what does constitute a significant change when successive counts are performed on one individual.

The normal figures for platelet counts given by various authors differ markedly according to the method used, and there is sometimes considerable variation between the results of two investigators using the same method. The methods fall into two main groups: the direct methods, in which the blood platelets are counted directly, after suitable dilution of the blood, on a haemocytometer slide; and the indirect methods, in which the proportion of platelets to erythrocytes is determined and the red cell count also, the absolute value for the platelet count being calculated from these two observations.

The mean platelet count in normal persons, according to Wintrobe (1946), is about 250,000 per c.mm. of blood. The results of some investigators are shown in Table I.

TABLE I

PLATELET COUNTS* IN HEALTHY ADULTS

Year	Author	Method	No. of Subjects	Sex of Subjects	Mean	Range†
1912	Fonio	Indirect	30	♂	234	130–350 a
1922	Kristenson	Direct	18	♂ + ♀	300	188–349 a
1926	Van Goidsenhoven	Direct	x	x	x	300–350 a
1931	MacKay	Indirect	6	x	390	250–450 a
1932	Dameshek	Indirect	52	♂	716	500–900 a
1935	Olef	Indirect	38	♂ + ♀	514	437–586 a
1935	Lempert	Direct	x	x	x	250–350 a
1936	Tocantins	Direct	40	♂	250	243–257 b
1946	Aggeler, Howard, and Lucia	Direct	64	x	409	273–545 b
1947	Masure	Direct	100	♂ + ♀	269	162–376 b

* Counts in thousands of platelets per c.mm. of blood.

† a = actual range; b = physiological range as calculated ($M \pm 2\sigma$); x = not mentioned.

In this investigation platelet counts were performed on a group of healthy young adults under carefully standardized conditions in order to find the mean platelet count and the range. A second group was investigated under less standardized conditions to find whether the mean and the range of this group were significantly different from those of the control series, and to establish a normal standard for healthy young adults.

In an attempt to analyse the variability of successive counts on the same individual, two further groups were investigated in order to find the daily and day-to-day variations respectively in the platelet count.

Finally, in an attempt to assess the observer component of the experimental error, a series of diluted bloods was counted independently by three observers.

Choice of Method

For this investigation a technique was sought which would cause the minimum inconvenience to the patient, would be sufficiently rapid to permit the physician or haematologist to perform it himself, and of an order of accuracy comparable with that of other techniques.

To cause the minimum discomfort to the patient, it was decided to perform the counts on capillary blood. This has the added advantage of not requiring paraffin- or oil-coated apparatus. The capillary blood was obtained from the palmar surface of a finger-tip.

A direct method was chosen in preference to an indirect one, since the indirect methods take longer and are probably less accurate owing to the additional source of error in the red cell count itself (Biggs and Macmillan, 1948).

Three direct methods were compared in preliminary tests; the method of Rees and Ecker (1923) in which the red blood corpuscles are preserved, and the methods of Lempert (1935) and of Baar (1948) in which the red cells are haemolysed.

The method of Rees and Ecker was chosen as the most suitable for clinical use. It has the following advantages: (1) Simplicity, for only one stock solution is required, and this keeps in good condition for at least six months if stored in a refrigerator; (2) rapidity, because a platelet count by this method takes 25 minutes from start to finish (Barr's method is more rapid, but Lempert's takes longer); (3) minimum of artefacts, because there are fewer artefacts than are found with either Baar's or Lempert's methods, or any other methods which involve laking of the red cells and produce highly refractile bodies of the size of small platelets (Rees and Ecker, 1923), a source of error minimized by avoiding haemolysis; (4) other cell counts, for an additional advantage is that a red cell count may be performed on the same preparation during the time allowed for the platelets to settle (Rees and Ecker, 1923). The leucocytes also are preserved and may be counted, but the dilution is too great for this count to be of much clinical value.

Method

The composition of Rees-Ecker diluting fluid as modified by Wintrobe (1946) is shown in Table II. It is fundamentally an isotonic solution of sodium citrate, to which is added fixative and stain. The stock solution is kept in a glass-stoppered bottle in a refrigerator until required, when a small quantity is filtered into another container for current use. This

TABLE II

REES-ECKER (WINTROBE'S MODIFICATION) DILUTING FLUID

Sodium citrate	3.8 g.
Formaldehyde (neutral 40% solution)	0.2 ml.
Brilliant cresyl blue	0.05 g.
Distilled water	100.0 ml.

filtered solution is usable for the next six hours, after which the accumulation of sediment in it makes counting more difficult.

The stock solution keeps for at least six months, but samples should be filtered and examined from time to time to exclude the presence of platelet-like particles.

Pipettes and counting chambers must be scrupulously clean to avoid contact haemolysis.

The technique is as follows. Diluting fluid is drawn up to the mark 0.5 in a red cell haemocytometer pipette. The subject's finger-tip is cleaned and dried with alcohol and pricked with a Hagedorn needle, the puncture being deep enough to permit a free flow of blood. The first drop is wiped away and the second drop is drawn into the pipette until the column of diluent and blood reaches mark 1. More diluting fluid is then drawn in to the mark 101, and the pipette is shaken for three minutes.

In this investigation the further procedure was as follows. About one-third of the contents of the pipette was discarded and both sides of a double Neubauer counting chamber were filled from the remainder. To allow the platelets to settle to the same optical plane, the slide was allowed to stand from 10 to 40 minutes between two pledgets of moist cotton-wool under a petri dish to reduce evaporation. Then all the platelets in both sets of small squares were counted (a total of 800 small squares corresponding to a volume of 0.2 c.mm. of diluted blood). The reading gives the platelet count in thousands per c.mm. of undiluted blood.

The haemocytometer slides used in this investigation were to B.S.I. specification with N.P.L. certificates of accuracy to 1%. The haemocytometer pipettes were calibrated for the dilution factor and the appropriate correction made in each case.

Plan of the Investigation

The subjects for the investigation were students of physiology at Glasgow University. All were European, and their ages ranged from 18 to 29 years inclusive. No subject was included who had any history of illness or loss of blood, apart from normal menstruation, during the preceding month.

The samples were taken under standard conditions to reduce the number of physiological factors which might produce variation in the platelet count. The subject was warm and at rest in the supine position, and the sample of blood was withdrawn after a period of five minutes' recumbency.

All counts were performed by the same observer (A.W.S.), except those where two other observers performed counts on the same blood samples in order to find the difference between observers.

Results

First Series.—For the first series certain additional standard conditions were imposed in order to eliminate as many variable factors as possible. All samples were taken between 4 and 5 p.m., at least two hours after eating, smoking, or active exercise. Female subjects were not taken during menstruation or during the two days before

or after a menstrual period. A haemoglobin estimation was performed on each case, the haemoglobin being estimated as oxyhaemoglobin with a " spekker " photo-electric absorptiometer (Bell, Chambers, and Waddell, 1945), and a Leishman-stained blood film was examined for abnormal cells. Three females with haemoglobin concentrations below 12 g. per 100 ml. of blood were excluded from the series. No abnormal cells were seen in any of the remainder. This " basal " series comprises 15 men and 15 women (Table III).

TABLE III

FIRST (" BASAL ") SERIES

Men*	Women	Total*
Range 140–317	Range 165–351	Range 140–351
Mean 237.9	Mean 245.3	Mean 241.7
n 14.0	n 15.0	n 29.0
σ 53.1	σ 47.1	σ 50.3
v 22.3%	v 19.2%	v 20.8%

Counts in thousands of platelets per c.mm. of capillary blood.

* = excluding 1 case with platelet countof 440,000; n = number of subjects; σ = standard deviation; v = coefficient of variation.

There is no significant difference between the mean platelet counts for men and for women in this series, and, if we exclude one quite exceptional count of 440,000 platelets per c.mm. in one of the men, we find that there is no significant difference between the range for men and for women in this series.

Second Series.—In an attempt to find the total effect of normal variations and physiological activities on the platelet count, counts were performed on a further series of 80 subjects (45 men and 35 women), the samples being withdrawn under the same conditions of warmth, rest, and posture, but in this random series the samples were taken at any time from 9 a.m. to 5 p.m., irrespective of the time after eating, smoking, or muscular exercise.

Female subjects were taken at any stage of the menstrual cycle, but careful note was taken of the exact time in the menstrual cycle when the sample was taken. In this series no further blood examination was performed, any subject being accepted who conformed to the conditions already mentioned and had no history of blood disease.

TABLE IV

SECOND ("RANDOM") SERIES

Men	Women	Total
Range 127–351	Range 165–359	Range 127–359
Mean 235.8	Mean 247.9	Mean 241.1
*n 45.0	n 35.0	n 80.0
σ 49.0	σ 51.2	σ 50.0
v 20.8%	v 20.6%	v 20.7%

Counts in thousands of platelets per c.mm. of capillary blood.

*n = number of subjects; σ = standard deviation; v = coefficient of variation.

The results of this series are shown in Table IV. No significant difference was found between the mean and range for men and for women, and there was no significant difference between the mean and range of this series and those of the basal series (excluding the one exceptional case in the basal series). The platelet counts on five of the 35 women subjects were performed during menstruation. It is of interest to note that the mean of these five readings (239,000) is not significantly different from the mean for the other 30 women (249,000).

Third Series.—In order to assess the variation in the platelet count during the day, counts were performed at standard times during the day on a series of 12 subjects (five men and seven women). The results are shown in Table V. An irregular

TABLE V

THIRD SERIES: FOUR SAMPLES FROM 12 SUBJECTS TAKEN AT STANDARD TIMES DURING THE COURSE OF ONE DAY

Subject	10 a.m.	Noon	2 p.m.	4 p.m.
1	230	235	185	239
2	213	212	165	249
3	250	308	288	259
4	154	204	202	174
5	283	214	286	236
6	319	266	248	296
7	290	288	314	271
8	131	202	158	173
9	256	237	228	286
10	255	237	259	293
11	236	217	209	192
12	244	282	256	261

Counts in thousands of platelets per c.mm. of blood.

variation was observed during the day, but this was not significant. By analysis of variance the standard error of a single observation of this series was found to be 28,000.

Fourth Series.—In order to assess the variation in the platelet count from day to day, counts were performed at the same time (11 a.m.) each day for four successive days on a series of 12 subjects (six men and six women). The women were not taken during menstruation or during the two days before or after a menstrual period (Table VI). The variation from day to day was not significant. The standard error of a single observation of this series was 15,000.

Fifth Series.—Finally, in order to assess the observer component of the experimental error, a series of 12 blood samples was examined by three observers, all experienced in haematological technique. A preparation of diluted blood was obtained in a haemocytometer pipette and was used to fill three haemocytometer slides. The counts were performed simultaneously by the three observers, each of whom retained the same slide throughout the experiment. In this experiment most variables were eliminated, leaving the distribution of platelets in the counting chambers and the difference between observers, presumably in visual acuity and in interpretation of the objects seen. The difference between observers was not significant.

TABLE VI

FOURTH SERIES: ONE SAMPLE TAKEN AT THE SAME TIME ON FOUR SUCCESSIVE DAYS FROM TWELVE SUBJECTS

Subject	1st day	2nd day	3rd day	4th day
1	306	274	312	274
2	219	199	178	221
3	270	281	282	258
4	380	322	323	334
5	310	279	283	256
6	229	219	221	218
7	270	270	264	254
8	298	311	287	298
9	204	231	202	210
10	176	184	185	163
11	325	307	326	343
12	246	244	254	252

Counts in thousands of platelets per c.mm. of blood.

TABLE VII

FIFTH SERIES: SAME BLOOD SAMPLE COUNTED SIMULTANEOUSLY BY THREE OBSERVERS

Subject	Observer 1 (A.W.S.)	Observer 2 (J.B.C.)	Observer 3 (F.O.B.)
1	181	141	164
2	154	121	155
3	210	227	217
4	217	211	230
5	223	220	183
6	299	273	278
7	293	309	346
8	227	238	238
9	183	211	197
10	268	247	242
11	142	164	166
12	232	268	192

Counts in thousands of platelets per c.mm. of blood.

Discussion

The average platelet count and the range of normal counts have been estimated by many authors, the results varying considerably with the method adopted, and in some cases there has been considerable variation between the results of two observers using the same method. Indirect methods tend to give higher counts than direct methods, but Wintrobe (1946) has pointed out that the highest counts are not necessarily the most accurate.

The finger-tip was chosen as the standard site of puncture for this investigation. Olef (1935) has pointed out that the ear is an unsatisfactory site for platelet counts, since platelets may adhere to the hairs.

A variation in platelet count with changes in posture has been claimed by von Horvath (1928) and by Steinmaurer (1932).

According to von Horvath, the platelet count falls within 15 seconds of standing up and rises on lying down. He recommended that counts should be performed from three to five minutes after the subject had assumed either the horizontal or the vertical position. Steinmaurer stated that the platelet count rose with change of posture, the greatest rise occurring when the subject stands up from lying down. For the present investigation the standard technique adopted was to take the blood sample five minutes after the subject had assumed the supine position.

Rees and Ecker (1923) recommended that the platelets should be counted 10 minutes after filling the counting chamber, since evaporation from the counting chamber alters the count after about 20 minutes. Since it is inconvenient for the clinician to be tied to a precise time, Lempert's suggestion for delaying evaporation from the counting chamber was adopted in the present investigation (Lempert, 1935). A small series of preparations, counted at 10, 30, and 50 minutes after the slide had been filled, showed at the most a quite negligible variation during this time. It was therefore assumed that, if evaporation from the slide were delayed in this fashion, the actual counting could be performed any time from 10 to 40 minutes after the slide was filled.

Rees and Ecker (1923) did not state how many squares should be counted on the haemocytometer slide. Aggeler, Howard, and Lucia (1946), using a modified Rees-Ecker technique, counted the platelets on 80 small squares of the haemocytometer chamber as for a red cell count, the figure obtained being multiplied by 10,000 to give the platelet count per c.mm. of undiluted blood. At least two chambers were counted in each case and the results averaged. A preliminary survey in the present investigation showed that, if only 80 small squares were counted in each chamber, the discrepancy between the counts on the two chambers was considerable. It was therefore considered advisable to count all the platelets in two sets of 400 small squares. The theoretical justification for counting the greater number of cells is dealt with below.

No significant difference was found between platelet counts for men and for women. This is in agreement with the conclusions of Tocantins (1938) and Wintrobe (1946) that no significant sex difference in platelet counts has ever been conclusively demonstrated.

Other physiological factors which have been claimed to influence platelet counts are race, age, exercise, alimentation, temperature, altitude, menstruation, and pregnancy. In this investigation the subjects were all European, in the same broad age group (18–29 years), and all counts were performed in the same laboratory and at a comfortable room temperature. None of the female subjects was pregnant.

For the first, or basal, series an attempt was made to eliminate the remaining known causes of variation: exercise, alimentation, time of day, and, in the female subjects, menstruation. Any immediate effect of smoking was also eliminated.

For the second, or random, series these additional criteria were not applied, it being assumed that any significant changes in the platelet counts of the group as a result of additional activities or normal daily variations would result in a difference between the mean or the scatter of this series and those of the basal series. No significant difference was found, which suggests that the platelet counts performed at

any time of the normal working day (9 a.m. to 5 p.m.) are equally valid for comparative purposes.

Taking the largest series of observations (Series 2) as the standard, the average platelet count in the 80 young adults tested by this method was found to be 241,000, and the standard deviation 50,000. This gives us a " normal range," calculated according to the usual convention as the mean $\pm$ twice the standard deviation, of approximately 140,000–340,000, and 95% of the readings did in fact fall within these limits.

Changes in the platelet count have been related to the time of day (Degkwitz) 1920; Kristenson, 1924; Kranzfeld, 1925), but Zeller (1921) and Otsuka (1933, found little difference between counts taken at different times during the day. In this investigation no significant variation was noted during the day (Table V), and supports the impression gained from comparison of the first and second series that counts performed at any time during the normal working day are equally valid for comparative purposes. The standard error of a single observation of this series (28,000) is the standard error of a single platelet count taken at any time between 9 a.m. and 5 p.m. A single platelet count performed during this period may therefore be regarded as significantly determined to $\pm$56,000 ($\pm$23%), and a difference of 80,000 (30%) between two successive counts on the same subject would be significant.

A further series (Table VI) showed no significant difference between counts performed on successive days on each subject. The day-by-day variation was even less than the daily variation, presumably because variations caused by physiological activities such as alimentation and muscular exercise were minimized. In this series (Table VI) the blood samples were all taken at 11 a.m., after the student had been sitting for one hour at a lecture. The standard error of a single observation of this series is 15,000, which, as is noted below, is approximately the theoretical minimum error for the method employed.

" Student " (1907) showed mathematically the minimum error which must be present in all counts performed with a haemocytometer, owing to the random distribution of the particles over the field. Later he indicated (" Student," 1919) that the distribution follows Poisson's law and that the probable error of a haemocytometer count is $0.6745 \sqrt{N}$, where N is the total number of cells counted. This will only be true if the presence of particles in a certain division of the field does not appreciably affect the probability of other particles entering that division. When the particles are blood cells and the suspending medium blood, diluted 1 in 200, this is believed to be the case.

Berkson, Magath, and Hurn (1939) divided the minimum error of the haemocytometer count into three parts: " field error," which depends on the Poisson distribution; " chamber error," which depends on inaccuracy of calibration of the chamber and differences in the procedure of filling the chamber; and " pipette error," which depends on inaccurate calibration of the pipette and on differences in the manipulations of filling, diluting, and mixing. They found that under the most favourable conditions, with careful technique and standardized apparatus, the field error might be practically the only effective error. Biggs and Macmillan (1948), from a series of platelet counts on one subject by five observers using five pipette and counting

chamber combinations, calculated the error due to observers, calibration of pipettes and counting chambers, and the time of taking the sample. The main sources of error were found to be the observer component and the random variation, and, with a direct method (Lempert, 1935), the random variation approached the Poisson distribution.

It is obviously important to know the field error of the haemocytometer technique in use, since this is the minimum error which would remain under ideal conditions, and the error of any method involving the use of a haemocytometer cannot be less than this. The field error depends on the Poisson distribution and is proportional to the square root of the number of cells counted. The percentage field error therefore varies inversely as the square root of the number of cells counted. The percentage field error of a count can therefore only be reduced by counting more cells. In this investigation (Series 2) an average number of 241 cells was counted (on 2 sq. mm. of haemocytometer field). For this number, the field error of the platelet count from the Poisson distribution would have a standard deviation of approximately 15,000, and this is the minimum which can be hoped for by this method. The error could, however, be reduced by performing more than one count on each occasion.

By calibration of pipettes and counting chambers and standardization of technique an attempt was made to reduce pipette and chamber error to the minimum. The observed variations from day to day (Series 4) approximate to the inevitable variation dependent on the Poisson distribution, and support the view that this is a good method.

Finally the difference between observers was investigated. Some authors (Ivanitzky-Vasilenko and Klimova, 1937) find no significant difference between counts performed on the same subject by two observers, whereas others (Aggeler *et al.*, 1946) maintain that the difference is so great that it is impossible to obtain similar results from different technicians and that consequently each must establish his own " normal range." As observers can be taught to withdraw blood, fill the pipette and mix the contents, and fill the counting chamber according to a standardized technique, it was felt that the most important factors in determining the observer component of the error must be the visual acuity of the observers and their interpretation of the objects seen. This was accordingly investigated, and no significant difference was found between counts made by different trained observers on different portions of the same sample of diluted blood. From this it is assumed that reasonable agreement can be found between platelet counts performed by different trained observers, using the same technique and working under similar conditions.

Summary

By a modification of the method of Rees and Ecker (1923) the mean platelet count on 80 healthy young adults was found to be 241,000 and the " normal range " ($M \pm 2\sigma$), approximately 140,000–340,000.

Between these limits a count is significantly determined to $\pm 56{,}000$ ($\pm 23\%$).

No significant difference was found between the platelet counts on men and those on women.

The variation in the platelet count of individuals during the day and from day to day was not significant.

In successive samples from one subject in this range, taken at any time between 9 a.m. and 5 p.m., a change of not less than 80,000 (30%) in the platelet count is significant, whether the counts have been performed by the same observer or by different trained observers.

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