

## THE ENUMERATION OF BLOOD PLATELETS

BY

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The platelets of human blood can, with reference to their fragility, be divided into two groups. The first group comprises the smallest platelets with very high fragility described for the first time by Floessner (1922). In the second group are the medium-sized and large platelets which have a higher degree of resistance and show in normal individuals a typical curve of disintegration (Baar and Szekely, 1929). The existence of the first group was questioned by Hartmann (1932), who regarded the tiny platelets as fragments of normal blood cells. Steinmaurer (1932) was, however, able to demonstrate by a special method this type of platelet in stained films (compare also Juergens, 1934). Apart from these platelets of normal people, there are also pathological giant forms (found especially in thrombocytopenic purpura) and exceptionally highly resistant pathological micro-platelets. The latter form was seen by Baar (1934) for a short period in a case of essential thrombocytopenia.

Corresponding to the two main types of platelets the technical methods for their enumeration may be divided into two groups. Thus the methods of Floessner (1922), Boshammer (1926), Juergens (1934), Steinmaurer (1932), Cumings (1933), and Olef (1935) permit the enumeration of all platelets, and the number found in normal individuals by these methods is 700,000 to 900,000 per c.mm. All these methods, however, are very laborious and unsuitable for routine laboratory work. Moreover, up to date no pathological conditions are known in which only the number of the most fragile platelets is decreased or increased without corresponding changes in the other groups of platelets. The much simpler methods of the second group supply us, therefore, with sufficient information. These are either indirect, in which the number of platelets is calculated from their relation to red blood corpuscles in stained films (Fonio, 1912) or in wet preparations (Cramer and Bannerman, 1929; Damashek, 1932; Leitner, 1935; Ladewig, 1935); or direct, in which the platelets are counted in a counting chamber (Spitz, 1921; Langmeyer, 1918; Kristenson, 1924; Tocantins, 1937; Wintrobe, 1946). The enumeration of platelets in Giemsa-stained films has the advantage that

the morphology of platelets can be studied at the same time; it is, however, too time-consuming for routine work. The counting chamber methods have, on the other hand, various drawbacks. In some (Gutstein, 1932; Lenggenger, 1936; Tocantins, 1937; Wintrobe, 1946) the platelets are counted in the presence of red blood corpuscles, which necessitates a comparatively high dilution; in others, agglutination and disintegration of platelets are not sufficiently suppressed. The latter objection applies also to a method described by the present author (1928) which is a combination of the methods of Kristenson and of Langmeyer. Reliable results are obtained only if the sample is counted immediately after collection. In addition, the diluting fluid deteriorates rapidly in spite of the presence of mercuric chloride, and loses its haemolytic property. Attempts were therefore made to replace urea by another more stable haemolytic substance, and at the same time to inhibit the disintegration of platelets. Saponin was found suitable for the first purpose. It has high erythrocytolytic action even in the smallest concentrations. An alteration of platelets, however, can also be noticed. In high concentrations they become dissolved in a short time. In medium concentrations the red cells are dissolved immediately; at first the platelets remain preserved, but after some minutes they lose their high refraction of light, become shadow-like, and finally disappear. In a concentration of 0.025 g. per 100 ml. the erythrocytes are quickly haemolysed while the platelets remain practically unaffected. A simple substitution of saponin for urea in Kristenson's fluid proved, however, impracticable because of the formation of numerous precipitates in the presence of saponin, mercuric chloride, and brilliant cresyl blue. Some of these precipitates can easily be differentiated from platelets and removed by filtration. Others, however, are spherical, pale blue, highly refractile, and can easily be mistaken for platelets; and it was impossible to remove these precipitates by filtration. This difficulty was overcome by the use of formaldehyde as preservative and fixative. Formaldehyde has a "tanning" action on the cell membranes. It thus prevents the disintegration of platelets, but owing to the

same property it counteracts the erythrocytolytic effect of saponin.

#### Method

A concentration of saponin and formaldehyde was finally found in which the red blood corpuscles are promptly haemolysed without the platelets being affected.

Saponin (B.D.H.)	...	...	0.25 g.
Sodium citrate	...	...	3.5 g.
Concentrated solution of formaldehyde (40%)	...	...	1.0 ml.
Brilliant cresyl blue	...	...	0.1 g.
Distilled water	to	...	100.0 ml.

The solution must be filtered once after preparation and remains unchanged for many months. The above formula is valid for the B.D.H. saponin only. For other saponins the proper concentration of saponin and formaldehyde can easily be found empirically, bearing in mind the above-mentioned action of these two substances.

For the enumeration of platelets a white cell pipette is used. The counting fluid is first sucked up to the mark 0.6, followed by blood from a finger-prick up to the mark 1.0 and counting fluid again up to the mark 11. The pipette is vigorously shaken for about 3 minutes immediately after collection and again before the counting chamber is filled. The enumeration can be done either immediately after collection or even several hours later without a change in the result. The platelets are easily recognized in the counting chamber by their light refraction and faint blue colour. No red blood corpuscles should be seen in the chamber. A high-power dry objective is used. When counting, the fine adjustment of the microscope must be continuously moved with wide excursions because the platelets are evenly distributed throughout the whole depth of the chamber (0.1 mm.). In cases

with normal numbers of platelets it is sufficient to count half a square millimetre, but with thrombocytopenia the whole chamber or even both sides of a Buerker chamber should be counted. The dilution is 1 in 25, and the number of platelets in one square millimetre has therefore to be multiplied by 1,000 and divided by 4. The white blood cells are well stained and can be enumerated at the same time. Heparinized blood cannot be used.

Although no known method for enumeration of blood platelets can claim to be ideal, the present method has proved to compare favourably with many others which have been tried, so far as quickness and reliability are concerned. It has now been used for many years by junior technicians at the Children's Hospital, Birmingham.

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#### REFERENCES

- Baar, H. S. (1928). In Baar and Stransky: "Klinische Haematologie des Kindesalters." Deuticke. Leipzig and Vienna.  
 Baar, H. S. (1934). *Wien. klin. Wschr.*, **47**, 1039.  
 Baar, H. S., and Szekely, L. (1929). *Z. Kinderheilk.*, **48**, 31.  
 Boshammer, K. (1926). *Fol. Haemat.*, **33**, 105.  
 Cramer, W., and Bannerman, R. G. (1929). *Lancet*, **1**, 992.  
 Cumings, J. N. (1933). *Lancet*, **1**, 1230.  
 Damashek, W. (1932). *Arch. intern. Med.*, **50**, 579.  
 Floessner, O. (1922). *Z. Biol.*, **77**, 113.  
 Fonio, A. (1912). *Dtsch. Z. Chir.*, **117**, 176.  
 Gutstein, M. (1932). *Med. Klin.*, **28**, 1277.  
 Hartmann, E. (1932). *Klin. Wschr.*, **11**, 1828.  
 Juergens, R. (1934). *Klin. Wschr.*, **13**, 97.  
 Kristenson, A. (1924). "Studien über die Anzahl der Blutplättchen beim Menschen." Akademische Abhandlung. Upsala.  
 Ladewig, P. (1935). *Klin. Wschr.*, **14**, 983.  
 Langmeyer, L. (1918). *Zbl. inn. Med.*, **6**, 92.  
 Leitner, J. (1935). *Zbl. inn. Med.*, **56**, 433.  
 Lenggenhager, K. (1936). *Schweiz. med. Wschr.*, **66**, 1289.  
 Olf, I. (1935). *J. Lab. clin. Med.*, **20**, 416.  
 Spitz, S. (1921). *Berl. Klin. Wschr.*, **58**, 1073.  
 Steinmaurer, H. J. (1932). *Folia haemat.*, **Lpz.**, **46**, 269.  
 Tocantins, L. M. (1937). *Arch. Path.*, **23**, 850.  
 Wintrobe, M. W. (1946). "Clinical Haematology," p. 193. Lea and Febiger. Philadelphia.