THE EVOLUTION OF BLOOD-COUNTING TECHNIQUES*

by

M. L. VERSO

THE counting of blood-cells was one of the earliest methods of investigation introduced for the quantitative study of the blood, and for many years, of all the tests performed in clinical laboratories, it was probably the one most frequently requested. The story of the evolution of the blood count deserves, therefore, an honoured place in the history of medicine.

The credit for performing the first blood counts, and thereby laying the foundations of the laboratory study of haematology as an aid to clinical diagnosis, goes to Professor Karl Vierordt, of the University of Tübingen. Vierordt was a very great scientist, for besides his work on the blood he made pioneer contributions to many other aspects of physiology. Among his most outstanding achievements was the introduction of a number of instruments for studying the pulse and circulation by graphic methods, including a haemotachometer for measuring circulatory velocity and a tambour for studying the pulse (the latter instrument was later perfected by Marey). He also made contributions to respiratory physiology, the study of sensation and spectrophotometry. An account of his life with an accompanying bibliography has been given by Major.[1]

Vierordt's Technique

Vierordt presented his techniques of blood counting in a series of three papers.[2, 3, 4] In his original technique he drew blood into capillary tubes of known diameter whose capacity could be measured and then blew out a known amount of it on to a slide covered with a thin smear of albumen. After drying had occurred a micrometer was placed over the glass plate and the erythrocytes counted under the microscope. Later he improved the method by first diluting the blood with a solution of gum arabic. By retaining some of the mixture in the receptacle in which he had made the dilutions he was able to repeat the counts readily.

His methods were said to be fairly accurate but too tedious and time-consuming for routine clinical use. Nevertheless, his work drew the attention of other investigators to a valuable diagnostic procedure and stimulated them to devise more convenient techniques. His pupil Welcker developed Vierordt's technique further, but it was soon superseded by one introduced by the Dutch physiologist Cramer.

Cramer's Technique

Cramer[5] in 1855 introduced several important principles into blood counting.

* Read at a meeting of the Section of the History of Medicine, First Australian Medical Congress, Adelaide, South Australia, on Wednesday, 23 May 1962.

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

He used a definite volume of blood of a known dilution and counted it in a capillary space of known dimensions. He also introduced a squared ocular micrometer to aid the counting of the cells. Cramer made his dilutions by pipetting the diluting fluid and blood separately into a mixing vessel. The principle of his capillary space was that on a glass slide two thin strips of glass of equal and uniform thickness were cemented. Then when a second glass slide was cemented over them a capillary space of known depth was formed which could be filled with diluted blood by capillary attraction (Fig. 1).

This method of counting was moderately successful but unfortunately Cramer died before he could develop it further and before it had won general acceptance. As far as can be determined no further work was done on blood counting until 1869 when Potain and Malassez turned their attention to the subject.

Potain and Malassez

The name of Pierre-Carl Joseph Potain needs little introduction to medical readers. It has been perpetuated in the 'Potain's Aspirator', but this was only one of his many contributions to medicine, and it is to him that we owe the introduction of the sphygmomanometer to bedside medicine and it was he who first described 'gallop rhythm'. He was the most outstanding French physician of his day.

Louis-Charles Malassez (1842–1909) was Director of Histology in the laboratory of the College of France and published numerous papers on the blood, particularly the development of the corpuscles, and on the spleen. Though a modest and unobtrusive worker, he was an outstanding investigator.

Potain's contribution to blood-counting technique was the invention of a diluting pipette, or 'melangeur', built on the same principle as those in use today but of different dimensions and design. Malassez's contribution was to devise two methods of counting the cells. His first method, which was the one he introduced at this stage, was to draw the blood which had been diluted in a melangeur into a length of capillary tubing of known dimensions, which had been flattened so that it was elliptical in cross-section, and then to count the cells in a given length of the tubing.[6] His second method was developed many years later and will be considered in its appropriate sequence.

Hayem's Haemocytometer

The next worker to take up the study of blood counting was Georges Hayem, an outstanding physician, to whom Dreyfuss in a eulogistic biographical study[7] has given the title of Father of Haematology. Although pre-eminent among the early French haematologists, he also made valuable contributions to the study of the heart, the gastro-intestinal tract and the central nervous system. His studies of blood covered all aspects of the subject but he is particularly remembered for his work on the blood count and for his recognition of blood platelets as separate elements of blood. Hayem's red-cell diluting fluid is still one of the most commonly used in blood counting.

In place of the 'capillary cell' of Cramer and the capillary tubes of Malassez,

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

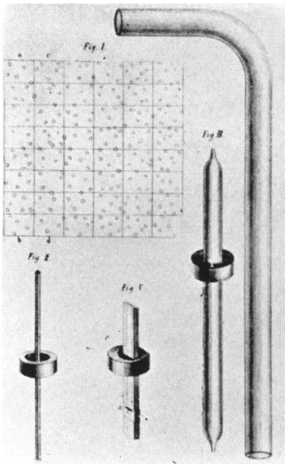

Fig. 1 Cramer's blood-counting apparatus

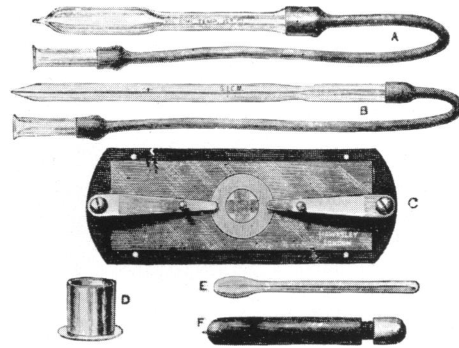

Fig. 3 Gowers' blood-counting apparatus

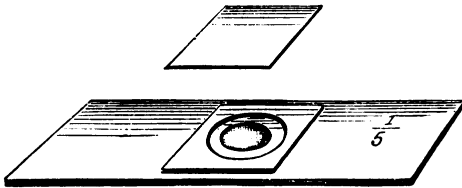

Fig. 2 Hayem's haemocytometer

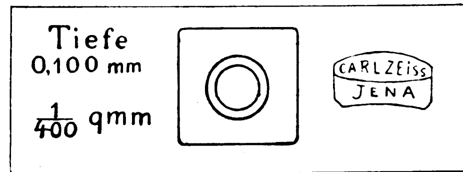

Fig. 4 Thoma's haemocytometer

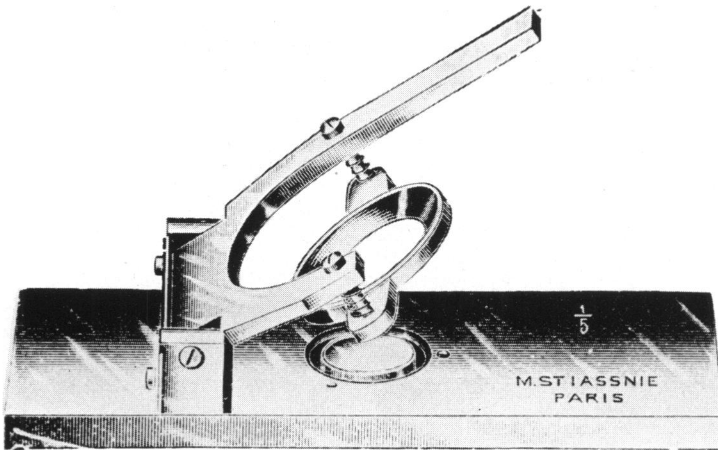

Fig. 5 Malassez's haemocytometer

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

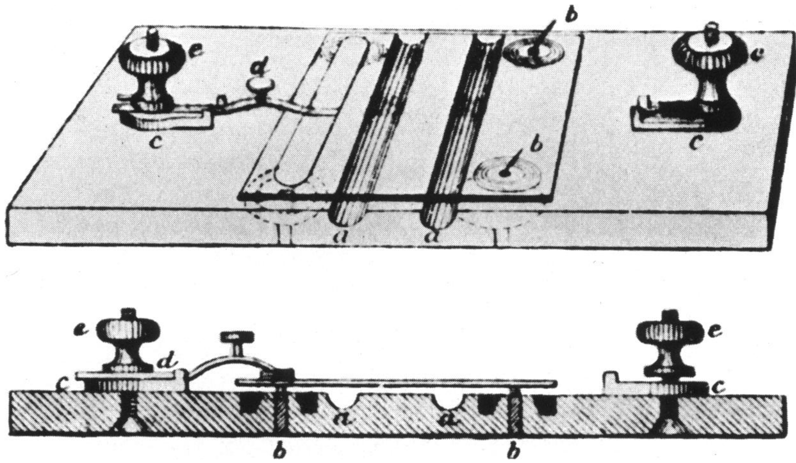

Fig. 6 Alferow's haemocytometer

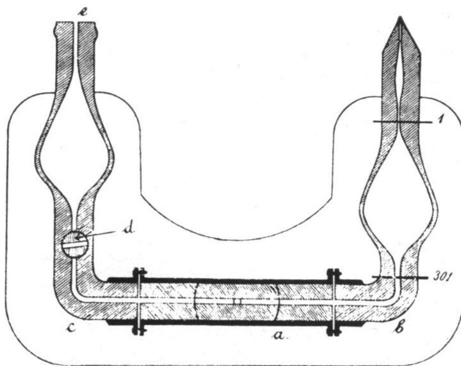

Fig. 7 Bruning's combined diluting pipette and counting chamber

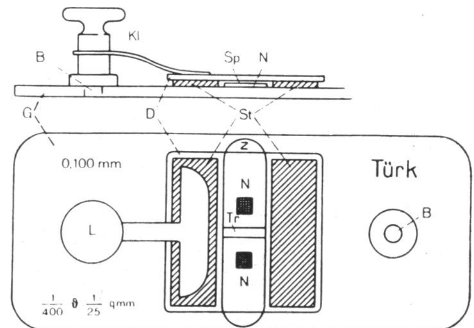

Fig. 8 Bürker's haemocytometer

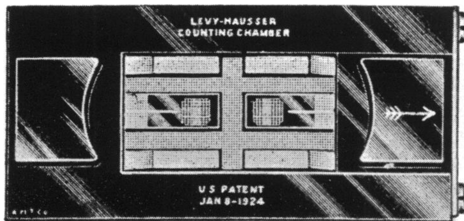

Fig. 9 Levy-Hausser counting chamber

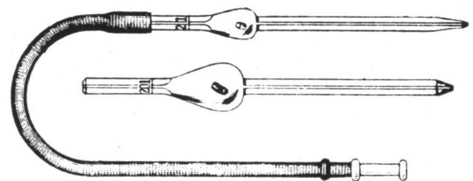

Fig. 10 Trenner automatic diluting pipette

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

Hayem devised a cell consisting of a fine, perfectly smooth, ground glass lamina of precisely determined thickness in whose centre was a hole 1 mm. in diameter. This lamina was cemented to a glass slide so that a small circular trough of known dimensions resulted (Fig. 2). When a drop of blood was placed in this trough and a cover slip applied to it the drop was converted into a sheet of liquid of known depth, with parallel sides. As in the previous methods, the squared ocular micrometer introduced by Cramer was used to count the cells. This design of haemocytometer involved one retrograde step, for in using the apparatus it was impossible to fill the cell by capillary attraction, as had been possible in the methods of Cramer and Malassez.

Hayem abandoned the use of a special 'melangeur' and made his dilutions in a small container using separate pipettes for blood and diluting fluid. An account of his methods and a review of earlier techniques can be found in his classic book on the blood.[8]

Gowers' Haemocytometer

Taking Hayem's basic design Gowers (1877) made an important step forward in the design of the counting chamber. Sir William Richard Gowers (1845–1915) is best remembered as a neurologist. He described the 'tract of Gowers' in the spinal cord and was one of the first to recognize the importance of the ophthalmoscope in neurology. But in addition to the discoveries he made in his own speciality, he also made basic contributions to haematology by the invention of two important pieces of equipment, for he designed the first practical clinical haemoglobinometer as well as the haemocytometer now to be described. An account of his life has been given by Critchley.[9]

In his counting chamber (Fig. 3) Gowers abandoned the ocular micrometer and conceived the principle of ruling the floor of the well of a counting chamber similar to Hayem's into 1/10th mm. squares. The depth of the well was 1/5th mm. When the drop of diluted blood was placed in the cell, the cover slip applied and the corpuscles allowed to settle, the count could be made with much greater ease than with earlier instruments that depended on an ocular micrometer. The cover slip was kept in place by spring clips. Gowers' complete apparatus consisted of a small pipette with a capacity of 995 c.mm., a fine capillary pipette capable of holding 5 c.mm., a small glass jar for diluting the blood and the counting chamber itself.[10]

Hayem criticized Gowers' apparatus on the grounds that the technical difficulties involved in making the rulings were likely to introduce errors. However, he approved a modification in his own counting chamber that was inspired by Gowers' design and suggested by one of his own associates, Nachet. In this modification the image of a squared ocular micrometer was recorded on the floor of a Hayem haemocytometer by a photographic process. Thus, as in Gowers' instrument, it was possible to work without using a squared ocular, but at the same time the cell was free from the errors that were likely to arise from the technical difficulties involved in making the rulings mechanically.

The Thoma-Zeiss Haemocytometer

A most important development in the counting chamber was due to Richard

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

Thoma (1847–1923), who at the time he invented his haemocytometer was Assistant at the Pathological Institute at Heidelberg. Later he received a professorship there and at Dorpat. A description of his instrument and methods is contained in a paper he published in collaboration with Lyon[11] in 1881.

For diluting the blood, Thoma used a pipette based on Potain's melangeur but differing in certain details. It is in fact the common diluting pipette used today. His counting chamber (Fig. 4) consisted of an evenly ground slide (*Objektträger*) with a thin glass plate cemented to it. In the centre of this plate was a hole 11 mm. in diameter. The effect up to this point was that of a trough as in Hayem's and Gowers' instruments, but in the centre of the trough was cemented a thin glass plate 5 mm. in diameter, so that the final effect was that of a platform surrounded by a moat. On the free surface of this platform an area 1 sq. mm. was divided into 400 square fields, which, to facilitate counting the cells, were divided further into 25 groups of 16 fields. As the plate that formed the platform was 0·1 mm. thinner than the main plate cemented to the *Objektträger*, a space 0·1 mm. deep was left when a cover slip was placed in position. It can be seen then that the main difference in principle between the Gowers and the Thoma instruments was that the former had the ruling at the bottom of a well and the latter had them on a platform surrounded by a moat.

Thoma's instrument was made by the firm of Carl Zeiss and Company of Jena. In England the firm of Hawksley modified it by making it out of a solid piece of glass instead of relying on separate cemented parts.

Later Thoma,[12] realizing that an accurate count of leucocytes was not possible in a dilution of 1 : 200, particularly in the presence of red cells, devised a leucocyte pipette in which the blood could be diluted 1 : 10 and 1 : 20. He also introduced the idea of using acetic acid as a white-cell diluting fluid to haemolyse the red cells. Up to that time it had been the custom to count the leucocytes in the same dilution, using the same preparation as the red cells, but by using a diluting fluid which haemolysed the red cells, leucocyte counts could now be done much more readily than before.

Malassez introduced his second instrument about this time. It featured a raised platform with a surrounding moat as in the Thoma counting chamber, a cover glass held in a hinged carrier and three metal points on the slide on which the carrier of the cover glass rested and which determined the depth of the drop of diluted blood (Fig. 5).

Alferow's Haemocytometer

The faults of the instruments used up to that time were realized by Sergei Alferow,[13] a Russian working in Paris. In his opinion their main disadvantages were that it was difficult to construct apparatus accurately to any of the designs that had previously been suggested, it was not easy to get a uniform distribution of cells in counting chambers where the drop had to be placed in the cell and then the cover slip applied, and that it was therefore difficult to get valid counts. To overcome these disadvantages he devised a counting chamber which had a detachable cover slip but which was still capable of being filled by capillary attraction. Instead of having a centre platform 0·1 mm. below the

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

level of the main object carrier as in the Thoma instrument, the counting platform was formed by cutting it off from the rest of the slide by two troughs (Fig. 6*a*), and the depth of the cell was determined by resting the cover slip on four metal supports (Fig. 6*b*). When the preparation was set up the cover slip was held in place by clamps (Fig. 6*d*). It is to be noted that by devising a cell that could be filled by capillary attraction, Alferow had reverted to the principles first laid down by Cramer.

In counting the cells, Alferow thought that the best procedure was to project their images on to the ground-glass plate of a photomicrographic attachment. The image of each cell could then be marked on the plate and the count made at leisure. Although he admitted that this procedure was slower than conventional methods of counting, he considered it more accurate.

Oliver's Turbidometric Method

An entirely new principle in blood-cell counting was proposed by George Oliver.[14] In his Croonian Lectures of 1896 he described an arteriometer, a haemoglobinometer and a haemocytometer. The haemocytometer worked on a turbidometric principle and was based on the fact that when a candle flame is viewed through a flat test-tube containing water, one sees a bright transverse line composed of minute closely packed images of the flame produced by tiny corrugations on the glass. If, instead of water, blood diluted in Hayem's solution is used, a more or less opaque suspension results and the line is not seen until a definite dilution is reached. According to Oliver, the amount of dilution is proportional to the red cell count and, therefore, an accurate measure of it. He claimed an accuracy of 1 per cent, but the method never became popular, and we know today that all turbidometric methods are faced with the difficulty that variations in cell size, shape or haemoglobin content are likely to affect the accuracy of the estimation.

Miscellaneous Methods

Around the turn of the century it was realized that the ruling of the Thoma-Zeiss counting chamber was not really suitable for leucocyte counts and modifications in the ruling were made by several workers, notably Zappert, Elzholz, Türk, Neubauer and Coles, for this purpose. A paper by Robert Breuer,[15] in 1902, pointed out the difficulties of counting leucocytes in the Thoma counting chamber and described the rulings made by Zappert, Elzholz and Türk to overcome them.

A number of people suggested various modifications of blood-counting methods round about that time. Sir Almroth Wright,[16] in 1902, stated that the use of diluting pipettes and of haemocytometers with standard rulings was unnecessary. Blood could be diluted to the appropriate concentration by ordinary pipettes and all that was needed for the actual counting was a plain cell of known depth, provided that the diameter of the field of the microscope was known.

C. A. McMunn,[17] in 1903, advocated photography as an aid to accurate counting.

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

Strong and Seligman,[18] in 1903, counted leucocytes by diluting 5 ml. blood 1:100 in a fixing solution containing methyl violet. They then spread 5 c.mm. of the diluted blood on a slide, allowed it to dry, and then mounted the preparation in Canada Balsam. The white cells were counted over the whole field, and the white-cell count calculated by multiplying this figure by the dilution. The same method could be used for counting red cells by diluting the blood a further 100 times.

A novel modification of the counting chamber was introduced in 1903 by Bruning.[19] In an effort to overcome the errors caused by faulty transfer of the diluted blood from the pipette to the counting chamber he incorporated the mixing pipettes and counting chamber in the same apparatus. His haemocytometer, which is represented in Fig. 7, consisted of a ruling incorporated in the wall (*a*), a mixing pipette (*b*) and the suction portion (*c*) which contained a second mixing chamber and which was separated from the counting portion by a cock (*d*).

The Bürker Haemocytometer and its Modifications

One of the most important developments that took place in blood counting was the development of the Bürker counting chamber and its subsequent modifications. Karl Bürker, whose interest in blood counting was first aroused while investigating the physiological effects of high altitudes, published a series of papers over a period of several years,[20, 21, 22, 23] describing the development of his haemocytometer. He found that the Thoma-Zeiss instrument had the disadvantages that it was difficult to set the counting chamber together satisfactorily, that it was easy to distribute the drop of diluted blood unevenly on the counting surface, and that sudden alterations of air pressure affected the dimensions of the counting chamber.

He first designed a counting chamber which consisted of a ground-glass plate (*Grundplatte*) on which was cemented crosswise a piece of glass 25 mm. long and 5 mm. broad with rounded ends. This piece of glass was divided into two parts by means of a channel 1·5 mm. deep. Further, a channel 1·5 mm. broad separated it on each side from a rectangular piece of glass 21 mm. long, 7·5 mm. broad and of such a thickness that when a cover glass was laid across it a space 0·1 mm. deep was left (Fig. 8).

One important feature that made this counting chamber superior to that of Thoma, Malassez, Hayem and Gowers was that like Alferow's it could be filled by capillary attraction after a cover slip had been set in place, and one did not have to place the drop of diluted blood on the slide and then apply the cover slip, a procedure fraught with many possibilities of error. In the earlier models the counting was carried out with the aid of an ocular aperture which could be adjusted to give a field of known dimensions by means of a stop, but later the apparatus was modified by having a calibration on the surface of the counting chamber itself.

Later, a number of manufacturers, notably the firm of Max Levy of Philadelphia, modified the Bürker haemocytometer by fashioning it out of a single block of glass and thus avoiding the disadvantages of a cell that had its

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

components cemented together and consequently prone to loosening. A further modification of the Bürker counting chamber is the Levy-Hausser instrument whose distinguishing features are the protective bakelite casing and the sloping platform to facilitate filling of the counting chamber (Fig. 9). The development of the 'bright-line' counting chamber in 1932 by Harry G. Ott, Director of Research and Development of the Spencer Lens Company, was an additional improvement that added to the ease of counting. In that instrument the metallic sheen of the surface of the counting platform allows the rulings to stand out much more clearly than in the conventional instruments.

Bürker, in diluting the blood prior to counting, abandoned the melangeur, and made his dilutions in a small phial using separate pipettes for the blood and for the diluting fluid. However, firms manufacturing instruments based on the principles he laid down have usually supplied diluting pipettes with their haemocytometers. The pipettes supplied are usually of the Thoma type, but an alternative design is the Trenner automatic pipette (Fig. 10) in which the blood comes to rest at the required mark automatically.

Dreyer's Fowl Cell Technique

A novel method of performing blood counts was devised by Dreyer[24] in 1921. He diluted 0·1 ml. blood in 19·9 ml. diluting fluid and, after mixing, 0·1 ml. of the suspension was transferred to a small tube. To the same tube he added 0·1 ml. of a suspension of hen's blood in a corrosive sublimate solution containing 20,000 cells per c.mm. After shaking the mixed suspension thoroughly, a drop of it was placed on a clean slide and examined with a 1/6th objective, using a squared diaphragm in the ocular. Fifty fields were counted and the number of nucleated (hen's) cells found was divided by the number of non-nucleated (human) cells and multiplied by 4,000,000 to give the red-cell count per c.mm.

Photoelectric Turbidometry and Other Recent Developments

In the twenties, as a result of the success of photoelectric methods in counting suspensions of bacteria, a revival of interest in turbidometry in red-cell counting took place. The first paper on this topic was published by Marcandier, Bideau and Dubreuil.[25] In an important paper by Blum[26] certain principles of turbidometry were laid down. These were, that when light passes through a turbid solution a fraction is absorbed, a fraction is transmitted directly forward, a fraction is transmitted forward diffusely and a fraction is reflected by the particles. The amount of light passing through to affect the photo-cell depends on the total surface area of the particles as well as the wave-length of the light. Once the photometer is calibrated the reading of a suspension will be proportional to the number of particles present, but if there is variation in the size, shape or haemoglobin content the reading will no longer be proportional. Errors will also creep in if the suspension is not properly mixed or if it is allowed to stand sufficiently long for such changes as haemolysis or rouleau-formation to occur.

Variations in the readings due to the haemoglobin content of the cells can

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

be overcome readily by using a suitable filter, but problems arising from variations in the size and shape of the cells are not so easily solved. An ingenious attempt to overcome this difficulty was made by Osmond and Robertson,[27] who suspended the cells in a hypertonic solution so that crenation would tend to bring them to a fairly uniform size.

The difficulties inherent in turbidometric methods of cell counting have largely been overcome by the invention of the electro-haemoscope devised by Loeschke and Wever and manufactured by the Shandon Scientific Company. It depends on the principle that the intensity of light passing through a suspension is dependent on cell numbers and cell size, but the amount of light scattered is dependent on cell size alone. By covering a calculated section of the diffraction image, the intensity of the light that is diffracted on the remaining surface of the photo-cell is for all practical purposes independent of the particle size and can be measured photoelectrically.

Of recent years, several different types of apparatus have been developed for the electronic counting of microscopic particles, including blood-cells. In one type the particles are counted by a cathode-ray scanning device and the principles involved in this procedure have been discussed by Walton.[28] Another type of electronic counter depends on the fact that cells are poor electrical conductors compared with a saline solution. In such apparatus a dilute suspension of cells in physiological saline is drawn through a minute aperture between platinum electrodes and in so doing modulates an electric current. The Coulter counter depends on this principle and a good account of its use has been given by Brecher, Schneiderman and Williams.[29]

One other method of estimating the red-cell count that has been used is to calculate it from the haematocrit value. As long ago as 1896 Oliver in his Croonian Lectures, mentioned above, said that the volume of packed cells could be used for calculating the red-cell count. More recently Freedman and Mirsky[30] have given a formula to make this calculation. They point out that the only advantage in estimating the count in this way, rather than simply giving the haematocrit value in the report, is that many clinicians have been brought up to think in terms of the red-cell count. Like the turbidometric methods, this method of estimating the red-cell count is only accurate if the cells are of normal size and shape.

The many errors involved in blood-cell counting have been discussed by many workers, including Abbe,[31] Lyon and Thoma,[11] Berkson, Magath and Hurn,[32] Nouvel,[33] Lavergne,[34] Biggs and McMillan,[35] Lancaster,[36] and White.[37] To discuss these errors is beyond the scope of the present study, except to point out that all methods are subject to errors involved in sampling, diluting and pipetting. In addition, haemocytometer methods are subject to errors arising from the distribution of cells in the counting chamber.

An outline of the history of blood counting has been given by Plum,[38] who also discussed the errors involved.

With the development of accurate methods of haemoglobin estimation for the diagnosis of anaemia and with an increased tendency among haematologists to form their opinions as to the nature of an anaemia on the appearances of a

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

blood smear rather than on numerical indices, the counting of erythrocytes is not as prominent a part of laboratory work today as in earlier years. Nevertheless, it is still required from time to time, and so, for that reason and because of its other applications, the haemocytometer is a piece of apparatus that is unlikely to become obsolete in the foreseeable future.

In the foregoing account I have tried to show how workers in many countries each contributed advances in technique, which, though often small in themselves, often had far-reaching consequences in the evolution of an important laboratory investigation.

ACKNOWLEDGMENTS

My thanks are due to Sir Edward Ford, Dean of the Faculty of Medicine, University of Sydney and the staff of the School of Tropical Medicine, Sydney, for advice in preparing early drafts of this paper and assistance in obtaining a number of obscure references.

I am also pleased to acknowledge the help of the American Optical Company, Massachusetts, U.S.A., in supplying information and granting permission to use illustrations appearing in their literature.

REFERENCES

1. MAJOR, R. H., Karl Vierordt, *Ann. med. Hist.*, 1938, n.s. **10**, 463.
2. VIERORDT, K., Neue Methode der quantitativen mikroskopischen Analyse des Blutes, *Arch. f. physiol. Heilk.*, 1852, **26**, 9.
3. —— Zählungen der Blutkörperchen des Menschen, *ibid.*, 1852, **26**, 327.
4. ——Untersuchungen über die Fehlerquellen bei der Zählung der Blutkörperchen, *ibid.*, 1852, **26**. (These articles not seen. Quoted by Major, *op. cit.*)
5. CRAMER, A., Bijdrage tot de quantitative mikroskopische analyse van het bloed. Het tellen der bloedligchaampjes, *Ned. Lancet*, 1855, **4**, 453.
6. MALASSEZ, LOUIS-CHARLES, De la numération des globules rouges du sang, *C.R. Acad. Sci., Paris*, 1872, **75**, 1528.
7. DREYFUSS, C., *Some Milestones in the History of Haematology*, New York and London, Grune & Stratton Inc., 1957.
8. HAYEM, G., *Du Sang et des Altérations Anatomiques*, Paris, 1889.
9. CRITCHLEY, MACDONALD, *Sir William Gowers, 1845–1915. A Biographical Appreciation*, London, William Heinemann, 1949.
10. GOWERS, W. R., On the numeration of blood corpuscles, *Lancet*, 1877, **ii**, 797.
11. LYON, J. F., and THOMA, R., Ueber die Methode der Blutkörperzählung, *Virchows Arch. path. Anat*, 1881, **84**, 131.
12. THOMA, RICHARD, Die Zählung der weissen Zellen des Blutes, *Virchows Arch. path. Anat.*, 1882, **87**, 201.
13. ALFEROW, S., Nouvel appareil servant à compter exactement les globules sanguins, *Arch. Physiol. Norm. et Path.*, 1894, **3**, 269.
14. OLIVER, G., A contribution to the study of the blood and the circulation, *Lancet*, 1896, **i**, 1699.
15. BREUER, R., Zur Technik der Leucocytenzählung, *Berlin klin. Wschr.*, 1902, **39**, 953.
16. WRIGHT, A., On some new procedures for the examination of the blood and bacterial cultures, *Lancet*, 1902, **ii**, 11.

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press

17. McMunn, C. A., Photography as an aid to counting red blood corpuscles, *Brit. Med. J.*, 1903, **i**, 274. (Not an original article but an annotation reporting a demonstration at the Physiological Society held at King's College.)
18. Strong, W., and Seligman, C. J., Report of a Communication to the Pathological Society of London, *Lancet*, 1903, **i**, 1446.
19. Bruning, W., Ein neuer Apparat für Blutkörperchenzählung, *Pflug. Arch. ges. Physiol.*, 1903, **93**, 377.
20. Bürker, K., Eine neue Form der Zählkammer, *Pflug. Arch. ges. Physiol.*, 1905, **107**, 426.
21. —— Erfahrungen mit der neuen Zählkammer nebst einer weiteren Verbesserung derselben, *ibid.*, 1907, **118**, 460.
22. —— Ueber weitere Verbesserung der Methoden zur Zählung roter Blutkörperchen, *ibid.*, 1911, **142**, 337.
23. —— Das Grundübel der älteren Zählmethoden für Erythrocyten und seine Beseitigen, *ibid.*, 1913, **152**, 271.
24. Dreyer, G., A simple procedure for the accurate enumeration of blood cells and bacteria without the use of a counting chamber, *Lancet*, 1921, **i**, 219.
25. Marcandier, M., Bideau, L., and Dubreuil, Y., Applications de la photométrie à la numération des hémities, *C.R. Soc. Biol., Paris*, 1928, **99**, 741.
26. Blum, L., The photoelectric determination of the erythrocyte count. I. Basic principles and technic, *Amer. J. clin. Pathol.*, 1945, Technical Supplement, **9**, p. 85.
27. Osmond, R., and Robertson, J. F., A photoelectric method for the determination of red cell numbers and haemoglobin in peripheral blood, *Med. J. Aust.*, 1950, **2**, 36.
28. Walton, W. H., Automatic counting of microscopic particles, *Nature* (*Lond.*), 1952, **169**, 518.
29. Brecher, G., Schneiderman, M., and Williams, G. Z., Evaluation of the electronic red blood cell counter, *Amer. J. clin. Pathol.*, 1956, **26**, 1439.
30. Freedman, A. M., and Mirsky, T. A., Estimation of red blood cells from the haematocrit, *Amer. J. clin. Pathol.*, 1946, Technical Supplement, **10**, p. 104.
31. Abbe, E., Ueber Blutkörperzählung, *Jena. Z. Naturw.*, 1879, **6**, 98.
32. Berkson, J., Magath, T. B., and Hurn, M., The error of estimate of the blood cell count as made with the haemocytometer, *Amer. J. Physiol.*, 1940, **128**, 309.
33. Nouvel, M. J., L'erreur dans la numération des hémities (Premier note: L'erreur instrumentale, Deuxieme note: L'erreur experimental), *Sang*, 1944, **16**, 574, 579.
34. Lavergne, G. H., Numérations des hémities—concordance et discordance des résultats fournis par differents types d'hématimètres, *Sang*, 1944, **16**, 204.
35. Biggs, R., and McMillan, R. L., Errors in the red cell count, *J. clin. Pathol.*, 1948, **1**, 288.
36. Lancaster, H. O., Statistical control in haematology, *J. Hyg., Camb.*, 1950, **48**, 402.
37. White, C., Errors involved in the counting of blood cells, *Med. J. Aust.*, 1950, **11**, 434.
38. Plum, P., Accuracy of haematological counting methods, *Acta med. scand.*, 1936, **90**, 342.

https://doi.org/10.1017/S0025727300029392 Published online by Cambridge University Press