FIG. 2. Increasing concentrations of FIGLU and glutamic acid plotted against optical densities of eluates from FIGLU and glutamic acid spots.

FIGLU spots were plotted against the concentration. The graph obtained (Fig. 2) shows a straight line except for very low concentration near the limit of sensitivity. Glutamic acid dilutions gave a similar curve.

My thanks are due to Mr. P. H. Motivala for his technical assistance.

REFERENCES

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CORRECTIONS

On page 222 of the paper 'Histology of the aortic media in dissecting aneurysm' by Gerald Manley (*J. clin. Path.*, **17**, 220-224) the two parts of Fig. 2 have been interchanged.

Figure 1 on page 244 of the paper by M. Patricia Jevons and M. T. Parker, 'The evolution of new hospital strains of *Staphylococcus aureus*' (*J. clin. Path.*, **17**, 243-250) has been printed upside down.

On page 301 of the paper 'A shortened automated procedure for the determination of alkaline phosphatase' by Joyce L. Bell and M. Collier (*J. clin. Path.*, **17**, 301-303) under Reagents '1-aminoantipyrine' please read 'Dissolve 1 g. 4-aminoantipyrine in 1 l. distilled water'

A new system for rapid haemoglobin estimations and leucocyte counts

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Blood samples are usually diluted 1 in 200 for the estimation of haemoglobin and 1 in 500 when counting leucocytes in the Coulter counter. These procedures are performed separately and require two tubes and two diluting pipettes. The purpose of this paper is to describe a system which enables the haemoglobin estimation and leucocyte count to be made from the one diluted sample. The system dispenses with the need for pipettes and diluting tubes and enables both examinations to be completed in less than 40 seconds.

PRINCIPLE

To enable both the haemoglobin estimation and leucocyte count to be made from the one dilution it was necessary to increase the dilution for haemoglobinometry to 1 in 500. Compensation for this was made by increasing the light path in the haemoglobinometer from 10 to 25 mm. The sample of blood is first diluted with N/150 ammonia directly into the haemoglobinometer tube using an automatic dispenser. This lyses the red cells and also causes some swelling of the leucocytes. Without delay the sample is further diluted by adding a saline solution of sufficient strength to make the whole mixture isotonic. This saline diluent also contains saponin to ensure adequate stromatolysis. The leucocytes quickly return to their former size and remain stable. The haemoglobin concentration is read in a photoelectric colorimeter as oxyhaemoglobin and the leucocytes are counted in a Coulter automatic cell counter.

APPARATUS

A standard type of rotary specimen mixer is followed by a Trimatic¹ dispenser fitted with pumps suitable for removing a 40 c.mm. sample of blood and delivering 10 ml. of diluent. Next to the dispenser is a 10 ml. semi-automatic pipette² fitted with a small plastic tube in place of the needle thus reducing frothing. It is set to deliver 10 ml. A Spectronic 20 spectrophotometer, fitted with an adaptor to give a 25 mm. light path, is used to measure haemoglobin. This adaptor is a standard accessory. Leucocytes are counted in a Coulter cell counter model A.

REAGENTS

The diluent used in the Trimatic dispenser is N/150 ammonia and in the semi-automatic pipette is 1.7%

¹Manufactured by Research Specialities Corp. U.S.A. Model No. 2642.

²Manufactured by Becton, Dickinson & Co., Cat. No. 1271.

Received for publication 23 October 1963.

sodium chloride with 0.036% of 30% bovine albumin, 2.4% of 2% saponin (Coulter), and 1% formalin. The saponin is added at the start of the day's work and any remaining solution is discarded at the end of the day.

PROCEDURE

Blood samples are collected into plastic vials and mixed on the rotary mixer. Samples are withdrawn from the mixer and placed under the automatic dispenser which removes a 40 c.mm. sample. The sample vial is removed and the outside of the dispenser tube wiped free of blood. The haemoglobinometer tube is now held under the dispenser tip and both the blood sample and 10 ml. of diluent are delivered into the tube. (It is important at this point to hold the tube in a perpendicular position to ensure adequate mixing of the sample and diluent.) Without delay the tube is then held under the nozzle of the semi-automatic pipette and 10 ml. of leucocyte diluent is delivered into the tube.

The tube with its contents is now placed in the Spectronic 20 and the oxyhaemoglobin concentration is measured in the usual way. The contents of the tube are then poured into the Coulter counter sample beaker and the leucocytes counted.

ACCURACY

Haemoglobin estimations and leucocyte counts were made on 50 aliquots of the same blood sample using both the standard technique with tubes and pipettes and the automated technique described in this paper. The standard haemoglobin estimations had a range of 13.5 to 14.2 g./100 ml. (mean 13.9 g./100 ml., standard deviation 0.21). The same sample treated in the manner described had a range of 13.7 to 14.0 g./100 ml. (mean 13.9 g./100 ml., standard deviation 0.13). The standard error of difference between the means was 0.035, therefore the difference between the methods is not significant.

The standard leucocyte estimations had a range of 15,200 to 16,100 (mean 15,550 per c.mm., standard deviation 0.43). The same sample tested in the manner described had a range of 15,300 to 16,000 (mean 15,600 per c.mm., standard deviation 0.98). The standard error of difference between the means was 0.05, thus the difference between the methods is not significant. To test the accuracy of the method at various haemoglobin and white cell levels, estimations were made on 20 separate samples of blood with haemoglobin concentrations of 5.0 to 13.8 g./100 ml. and leucocyte counts of 3,900 to 14,000 per c.mm. The results are shown in Tables I and II. There does not appear to be any significant difference between the two sets of results.

TABLE I

HAEMOGLOBIN ESTIMATIONS

Sample	Standard Method (g./100 ml.)	New Method (g./100 ml.)	% Difference
1	4.9	5.0	+2.0
2	9.1	9.2	+1.1
3	6.2	5.9	-4.8
4	6.4	6.5	+1.6
5	6.5	6.5	0
6	7.2	7.1	-1.4
7	7.3	7.1	-2.7
8	7.7	7.4	-3.9
9	8.8	8.7	-1.1
10	6.9	6.9	0
11	10.8	10.6	-1.9
12	9.9	10.0	+1.0
13	10.8	10.9	+0.9
14	11.6	11.8	+1.7
15	11.7	12.0	+2.6
16	11.4	11.5	+0.9
17	12.1	12.1	0
18	12.1	12.4	+2.5
19	13.4	13.3	-0.7
20	13.7	13.8	+0.7

Average difference between estimates for individual bloods 1.6%.
Mean difference from standard method +0.1%.

TABLE II

LEUCOCYTE COUNTS

Sample	Standard Method (cells per c.mm.)	New Method (cells per c.mm.)	% Difference
1	3,900	3,900	0
2	5,300	5,200	-1.9
3	4,400	4,200	-4.5
4	5,000	5,100	+2.0
5	4,600	5,000	+8.7
6	4,400	4,000	-9.1
7	4,900	4,800	-2.1
8	4,300	5,000	+16.3
9	6,700	6,900	+3.0
10	7,000	7,100	+1.4
11	7,000	7,400	+5.7
12	7,300	7,700	+5.5
13	4,800	5,100	+6.2
14	10,200	10,200	0
15	10,400	10,200	-1.9
16	7,800	8,100	+3.8
17	12,500	12,800	+2.4
18	12,800	12,900	+0.8
19	13,600	14,000	+2.9
20	5,100	5,100	0

Average difference between estimates for individual bloods 3.9%.
Mean difference from standard method +1.9%.

SUMMARY

A rapid method for haemoglobin estimations and leucocyte counts is described. The method is accurate and quick and results in a considerable financial saving as it requires no pipettes or tubes.