THE COLOUR REACTION Optimal conditions for the development of the colour are discussed by Giorgio and Plaut (1965) and have been confirmed. Values above 150 mg per litre may be estimated by dilution of a further aliquot of the eluate. Because of the variation of E^{620} due to interfering brown pigments, values below 15 mg/litre are not reported but the levels found in vitamin B₁₂ deficiency have been reported to be above 50 mg after valine loading, and this was so in nine patients with pernicious anaemia whose urine has been tested using this method.

RECOVERIES Recovery of methylmalonic acid from aqueous solutions and from additions to various urine samples by this method is 70-80% (Table), almost all the loss being in the ether/ethanol extraction stage. Recovery may be improved by increasing the number of extractions. Purity of the extract has been confirmed by thin-layer chromatography.

TABLE
RECOVERIES OF METHYLMALONIC ACID
ADDED TO URINE

No. of Estimations	Methylmalonic Acids Added (mg)	Methylmalonic Acid Found Mean $\pm$ 2SD (mg)	Mean Percentage Recovery
3	250	180 $\pm$ 15	72
3	175	130 $\pm$ 10	74
12	100	79 $\pm$ 9	79

SUMMARY

A colorimetric method for the estimation of methylmalonic acid in urine is described. The method is suitable for use in clinical pathology laboratories.

Acidified urine is saturated with ammonium sulphate and extracted with ethanol and ether, and the extract is purified using a strongly basic ion exchange resin. The methylmalonic acid is eluted and a colour developed by coupling with diazotised p-nitroaniline.

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Measurement of mean corpuscular and packed cell volumes with a Coulter cell counter

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The demonstration by Mattern, Brackett, and Olson (1957) that the Coulter cell counter could be used for cell sizing has been confirmed by many others (Douglas and Atkinson, 1960; Grant, Britton, and Kurtz, 1960; Brecher, Jakobek, Schneiderman, Williams, and Schmidt, 1962; Nevius, 1963; Lushbaugh and Lushbaugh, 1965).

A small computer is now available from Coulter Electronics Ltd., Dunstable, Bedfordshire, England, which can be attached to Coulter cell counter models B and F. This summates the size of the pulses produced by the cells over a period during the actual count, divides by the number of cells, including correction for coincidence, and displays the mean corpuscular volume (MCV). An additional computer, used in conjunction with the above, will calculate the packed cell volume (PCV) from the MCV and the total red cell count and display this value. These results are available at the end of the counting period for the total cell count, no additional time or calculations being required.

We have tested these attachments for calculating the MCV and PCV and the purpose of this communication is to record our assessment of their performance.

MATERIALS AND METHODS

The attachments for measurement of the PCV and MCV were loaned by Coulter Electronics Ltd., together with a Coulter cell counter model F. The apparatus was installed by the manufacturers, calibrated and ready for use.

The red cell count was performed using a 1 in 50,000 dilution of the blood in phosphate buffered isotonic saline formulated as follows:

NaCl.....	.8g
KH ₂ PO ₄	0.2g
KCl.....	0.2g
Na ₂ HPO ₄ 12H ₂ O.....	1.15g
Di sod. versenate.....	0.2g
Water to.....	1 litre

The pH of this solution was 6.8

One hundred different samples of blood were counted from patients suffering from a variety of anaemias (megaloblastic, other macrocytic anaemias, haemolytic anaemias, microcytic, hypochromic, and symptomatic anaemias) as well as from patients with polycythaemia and normal persons. The MCV and PCV recorded by the machine were noted.

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In addition, the PCV was determined by the microhaematocrit method after 10 minutes' centrifugation at 6,400 *g*. From this figure and the total red count, the MCV was calculated.

In some cases, the same diluted sample of blood was also counted on a Coulter counter model D, and the results were compared.

RESULTS

After a few days' use, it was clear that the results of the PCV given by the machine were consistently higher than those obtained from the microhaematocrit. The manufacturers came and re-calibrated the apparatus. The results reported below were obtained in the three-week period following this adjustment.

Ten dilutions from the same sample of normal blood were prepared so as to give results on the Coulter cell counter corresponding to values ranging from 1 to 10 M/cmm. The results are shown in Figure 1. This demonstrates that the haematocrit reading shown on the Coulter is strictly proportional to the dilution, and errors in dilution will result in errors of both red cell count and PCV as determined by this apparatus. On the other hand, the MCV shown by the apparatus on these 10 dilutions only varied between 91.5 and 94 $\text{cu}\cdot\mu$. This demonstrates that computation of the MCV was independent of the dilution. We did notice, however, that with the MCV displayed by the apparatus in this experiment, there was a definite association between the lower MCV values and the higher counts.

The mean corpuscular volume measurements on 100 different samples of blood were determined by the Coulter computer attachment and from the microhaematocrit reading and red cell counts. These results are shown in Figure 2. The standard deviation of the differences was 4.1 $\text{cu}\cdot\mu$. Seventy-two of the hundred

FIG. 2.

observations agreed within these limits and in only four instances did the difference exceed 8 $\text{cu}\cdot\mu$. It was possible to repeat the observations on three of the four latter specimens. In two of the three specimens the results given by the Coulter counter were unaltered but the microhaematocrit reading was different and now gave results similar to the Coulter counter. In the third specimen both the red cell count and the PCV indicated by the Coulter counter altered and agreed with the microhaematocrit reading. The original divergence was therefore due to an error of dilution.

The relation of the PCV determined by the microhae-

FIG. 1.

FIG. 3.

matocrit to that displayed by the Coulter counter is shown in Figure 3. The standard deviation of the difference was 1.7 divisions. Seventy-four of the hundred observations agreed within two divisions and all but four agreed within three divisions. Of these four, the observations were repeated on only two and, as mentioned earlier, the results of the microhaematocrit reading were incorrect in the first instance.

CONCLUSIONS

During the period of use we found the most valuable features of the computer attachments were the saving of time normally required to obtain the PCV and MCV, and the knowledge that the computer result of the MCV was independent of errors often associated with two sequential dilution processes. We had previously compared the mean cell volume with the median cell volume derived from the median threshold on a model A cell counter (standard deviation of the difference $3.8 \text{ cu} \cdot \mu$) and the standard deviation of the computer attachment was no different ($SD = 4.1 \text{ cu} \cdot \mu$). However, unlike previous experience in comparing the median and mean cell volumes, there were no examples of consistent differences between the two methods of computing the MCV and it seems probable that the difference is almost wholly due to errors in reading the microhaematocrit and preparing two sequential dilutions for the red cell count.

The PCV recorded by the Coulter attachment is, of course, dilution dependent, but the errors associated do not seem any greater than those of reading a microhaematocrit.

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Electrically-heated inoculating loop

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Many anaerobic bacteria are sensitive to oxygen and the use of an anaerobic cabinet for handling the specimens is almost essential if all the anaerobic bacteria in a specimen are to be isolated (Drasar, 1967). The cabinet used here is filled with nitrogen so that a gas burner cannot be employed for sterilizing the bacteriological loop. An electrically heated loop was therefore developed and, though this was devised for anaerobic work, it seems to have wider applications.

THE LOOP

The loop is a cautery burner (Allen and Hanburys Ltd. No. 3, type A) mounted in an insulated holder moulded from silicone rubber (Fig. 1); it is connected to a power supply by either insulated braid, permanently attached to the copper handle (Fig. 1), or plugs and sockets. Since for some applications, the platinum tip of a standard cautery burner is too short, a loop with a longer ($1\frac{1}{2}$ in.) tip has been developed.

ELECTRICAL CIRCUIT

The loop is heated by the power obtained from the secondary of a tapped transformer; an ac current meter, a 1 ohm resistance and a fuse, all in series with the loop, complete the secondary circuit. The apparatus also contains appropriate warning lights and a simple control device that automatically switches off the power at the end of a predetermined, but variable, time: the operator has only to initiate the process by momentarily pressing

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FIG. 1. *Top. The complete loop in its moulded holder. Bottom. A standard cautery burner, with insulated braid permanently attached, before the moulding procedure.*