TECHNICAL METHODS

ESTIMATION OF *p*-AMINOSALICYLIC ACID IN BLOOD

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Owing to the increasing use of *p*-aminosalicylic acid in the treatment of tuberculosis, it was considered that a simple method employing an ordinary single-cell type of photoelectric colorimeter for the estimation of the drug in blood was desirable.

Several methods of determining it have been reported. Way and others (1948) use a modification of the Bratton-Marshall (1939) method for determination of sulphonamides. The method of Morris (1941) has been modified by Klyne and Newhouse (1948) who use 0.5 ml. blood, and by Spinks (1948) (using 0.1 ml. blood). These latter procedures involve anil formation with *p*-dimethylaminobenzaldehyde. Although Spinks's method apparently gives satisfactory results when the optical densities are measured on a spectrophotometer, it was found not to be very sensitive when a single cell direct reading type of photoelectric colorimeter was used.

Tennent and Leland (1948) outline a somewhat similar procedure to the method described below, in which they use two reactions. (1) The *p*-aminosalicylic acid in acid solution is coupled with diazotized *p*-nitroaniline, followed by the addition of sodium hydroxide to give a red colour; and (2) a blue colour is produced when both the *p*-aminosalicylic acid and the *p*-nitroaniline are diazotized, followed by coupling in the presence of pyridine and the addition of sodium hydroxide. The effect of interfering materials is eliminated by reading both the red and the blue colours at 620 m μ , using the former as a blank for the latter.

The following method, which appears to be more suitable for use with the

simple photoelectric colorimeter and when only small quantities of blood are available, has been devised.

Method

The proteins of blood are precipitated with trichloroacetic acid. *p*-Nitroaniline is added to the supernatant, and this and the *p*-aminosalicylic acid are diazotized with sodium nitrite. On the addition of sodium hydroxide coupling occurs and a purple dye is produced. Coupling presumably takes place initially at the *p*-position to the hydroxyl group of the *p*-aminosalicylic acid, which is probably followed by further coupling with more of the *p*-nitroaniline.

The stability of the colour is shown in Fig. 1. The intensity is at a maximum immediately after formation and decreases gradually on standing. This decrease, which is only slight after the solution has been

FIG. 1.—Stability of colour.

allowed to stand for 15 minutes, becomes smaller with decreasing concentration of *p*-aminosalicylic acid.

The following reagents were used:

1. 5N-trichloroacetic acid.
2. Sodium nitrite 0.5 per cent (w/v). This solution should be renewed after two weeks.
3. *p*-Nitroaniline 0.1 per cent (w/v) in 0.25 N-hydrochloric acid.
4. 2 N-sodium hydroxide.
5. *p*-Aminosalicylic acid standard. 11.4 mg. of anhydrous sodium *p*-aminosalicylate (or 13.8 mg. of the dihydrate) are dissolved in distilled water and made up to 100 ml. This solution is equivalent to 10 mg. *p*-aminosalicylic acid/100 ml.

0.2 ml. whole blood was measured into 3.2 ml. distilled water in a centrifuge tube. It was mixed well and allowed to stand for 3 minutes for the cells to

lake. Then 0.6 ml. trichloroacetic acid solution was added, mixed thoroughly, allowed to stand for 10 minutes, and centrifuged for 10 minutes. To 3 ml. of the clear supernatant liquid was added 0.3 ml. sodium nitrite solution and then 2 ml. of the *p*-nitroaniline reagent. This was mixed well and allowed to stand for 3 minutes, when 2 ml. sodium hydroxide solution was added. The colour was read after 15 minutes in a photoelectric colorimeter; using a green filter (Ilford No. 404). A reagent blank was prepared by using 0.2 ml. distilled water in place of the blood.

Standards were prepared from suitable dilutions of an aqueous solution of the dihydrate of sodium *p*-aminosalicylate and were used in the construction of a calibration curve (see Fig. 2). Since this curve is rectilinear, a single standard may be employed as an alternative and the following calculation used:

Concentration of unknown =

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times \text{Concentration of standard}$$

A blank of the order of 1 mg./100 ml. is given by normal blood. The recoveries from whole blood are shown in the Table.

TABLE
RECOVERIES FROM WHOLE BLOOD

μg. Added	μg. Found	% Recovery
5	4.6	92
10	9.0	90
20	19.0	95
30	27.0	90
40	37.0	93
Mean recovery		92%

Summary

A simple method for the estimation of *p*-aminosalicylic acid in blood is described.

0.2 ml. of blood is required.

An ordinary single-cell photoelectric colorimeter is used.

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FIG. 2.—Typical calibration curve.