136. NOTES ON THE ESTIMATION OF THE MEMBERS OF THE SULPHANILAMIDE GROUP, WITH SPECIAL REFERENCE TO SULPHATHIAZOLE

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THE control of the commonly prescribed members of the sulphanilamide group of preparations is usually carried out, at the present time, by one of the methods or modifications of the methods devised by Marshall. These methods are based on the diazotization of the free or liberated amino group of the drug and subsequent coupling with a substance which will give a suitable azo-dye.

In Marshall's [1937] first and perhaps least known method, the diazo-compound was coupled with dimethyl- α -naphthylamine in alcohol; in his second method [Bratton & Marshall, 1939] the diazo complex is coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride in aqueous solution. Making use of his own modification of the first method, the difference lying mainly in the substitution of water for alcohol as solvent, Osterheld [1940] of the 'Ciba' Research Laboratories found unexpected difficulties in the estimation of 2-(*p*-aminobenzenesulphamido)thiazole (i.e. sulphathiazole), especially when dealing with urine. We, however, found that if we adhered strictly to the conditions laid down in Marshall's first method, the estimation of sulphathiazole presented no difficulty other than that inherent in the method, namely the final filtration of the solution to remove precipitated fats, lipoids etc.

Osterheld advises the use of acetyl H acid (1-acetamino-8-hydroxy-3-6-naphthalenedisulphonic acid) as a coupling reagent, since the red azo-dye formed is soluble in water, but as he points out, it is necessary to use a very pure specimen of the acetyl H acid; such commercial specimens as we have been able to obtain give dark brown solutions which have a large blank value in the colorimetric estimation. The pure acetyl H acid has the advantage that it couples to give a dye soluble in all concentrations in sodium acetate solution, and moreover it is indifferent to moderate excesses of nitrite since it does not couple on itself. The *N*-(1-naphthyl)-ethylenediamine dihydrochloride used by Marshall in his second method gives a somewhat more intense colour than that obtained with acetyl H acid with all members of the group, but in the case of sulphathiazole the azo-dye is much less soluble and tends to separate in flakes even in dilute solution, and the tendency is much exaggerated in the case of urine. These difficulties mentioned by Osterheld concerning the insolubility of the dye do not appear to such a great extent when sulphathiazole is being estimated in blood filtrates by Marshall's second method, provided that the proper precautions are taken, and on consideration we noted that the main difference between the procedures for blood and urine was the use of trichloroacetic acid in the former; when this reagent was added to the urine, clear solutions were obtained with the highest concentrations usable with either an absorptiometer or a colorimeter, though a certain amount of precipitation did occur when the solutions were allowed to stand for more than half an hour.

In this estimation it is more accurate and convenient to make use of the Spekker photoelectric absorptiometer, or some such instrument, for the comparison of the coloured solutions, rather than the visual colorimeter, since the variation of colour with concentration only obeys Beer's law within quite narrow limits of concentration. Using an absorptiometer, the graphs obtained of concentration against drum reading (a logarithmic scale) produce a picture of which the illustration given below is typical. A glance at the curve shows that, using a

Fig. 1. Colour-production/concentration curves for sulphanilamide (A) and sulphapyridine (B), with the Spekker photoelectric absorptiometer.

colorimeter, the use of a single standard of say 5 mg./100 ml. for comparison would lead to considerable error when the unknown is of any higher concentration than the standard, since above that level the curve does not obey Beer's law, the depth of colour produced dropping steadily with the rise in concentration. In comparing say a 10 mg./100 ml. concentration against a 5 mg./100 ml. standard in a colorimeter, the error may well be as high as 15–20 %.

In compiling the curves, three separate sets of series of standard solutions were taken, but owing to the accuracy of the instrument this is not usually necessary for calibration, provided that sufficient care is taken; our variations were rarely more than 2 %. Owing, however, to the idiosyncrasies of each instrument, it is pointless to give tables of figures, since each instrument must be individually calibrated for the estimation of each substance.

The curve for sulphanilamide is similar to, but different in degree from, that of sulphapyridine, but either may be used for the other with no great error, provided that the concentration read from the graph is corrected for the difference in molecular weight. The curve for sulphathiazole is almost identical with that for sulphapyridine, probably because of the similarity in the molecular weights.

Method

For both the blood and urine estimations two curves should be prepared, one for the free drug, and the other for the hydrolysed material, as the change in *pH* due to added HCl, necessary for the hydrolysis, appears to damp down the colour production.

Reagents.

1. 15 % trichloroacetic acid.
2. 0.1 % sodium nitrite solution, freshly prepared.
3. 0.5 % ammonium sulphamate solution.
4. 0.05 % saponin solution.
5. 4*N* HCl.
6. 0.1 % *N*-(1-naphthyl)-ethylenediamine dihydrochloride in water. This solution should be kept in a brown bottle.
7. A stock solution of sulphanilamide, sulphapyridine or sulphathiazole containing 200 mg./l. for preparation of the calibration curves. 5 ml. of this solution are mixed with 18 ml. of 15 % trichloroacetic acid and the solution is diluted to 100 ml.; 10 standards, containing 1 to 10 ml. of this solution, are each made up to 10 ml. with a solution of 18 ml. of the 15 % trichloroacetic acid per 100 ml., and are treated as described below.

Procedure for blood. 2 ml. of oxalated blood are measured into a flask, diluted with 30 ml. of saponin solution and, after 1–2 min., precipitated with 8 ml. of trichloroacetic acid. After standing for 5 min. the liquid is centrifuged.

The free sulphanilamide in the supernatant liquid is determined as follows: to 10 ml. of the clear liquid is added 1 ml. of sodium nitrite solution, and after 3 min. 1 ml. of ammonium sulphamate is added to destroy excess nitrite; after a further 3 min. 1 ml. of the *N*-(1-naphthyl)-ethylenediamine dihydrochloride is added and the colour produced is either measured in the absorptiometer or against an appropriate standard in a colorimeter.

To determine the total sulphanilamide, 10 ml. of the supernatant liquid are treated with 0.5 ml. of 4*N* HCl and the mixture is heated in a loosely stoppered tube in a boiling water bath for 1 hr. After cooling the volume is made up to 10 ml. and the solution is treated as above.

Procedure for urine. The urine is diluted to contain 1 to 2 g. of sulphanilamide per 100 ml. and 50 ml. of the diluted urine with 5 ml. HCl are made up to 100 ml.; 10 ml. of this dilution are treated as for blood filtrates to determine the free drug, and 10 ml. are heated without further addition for the determination of the total sulphanilamide.

In the case of sulphathiazole, 18 ml. of 15 % trichloroacetic acid are added during the second dilution of the urine, and no trouble should be experienced due to precipitation provided that the coloured solutions are not allowed to stand for more than 20–30 min.

A large number of experiments were also made using a great variety of coupling reagents, in the hope of finding an azo-dye of sufficient colour intensity for use in a micro method, but so far we have found nothing to compare with the *N*-(1-naphthyl)-ethylenediamine dihydrochloride.

SUMMARY

1. The difficulties encountered by Osterheld in the estimation of sulphathiazole in urine have been overcome.

2. The colour-production/concentration curves on an absorptiometer have clearly shown that more than one standard is essential if the comparison is made with a colorimeter.

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REFERENCES

- Bratton & Marshall (1939). *J. biol. Chem.* **128**, 537.
Marshall (1937). *J. biol. Chem.* **122**, 263.
Osterheld (1940). *Schweiz. med. Wschr.* **70**, 21, 459.