A New Coupling Component and Simplified Method for the Estimation of Sulphanilamide Drugs

By F. L. ROSE, *I.C.I. (Dyestuffs) Limited, Research Department, Blackley, Manchester,* and H. G. L. BEVAN, *Biochemical Laboratory, Crumpsall, Manchester*

(*Received* 29 *December* 1943)

The determination of sulphanilamide drugs in biological fluids depends essentially upon the removal of protein and cellular matter, followed by diazotization and development of an azo colour. The choice of coupling component is important, particularly when sulphanilamide drugs of low solubility are involved. The best substance so far recorded appears to be *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Bratton & Marshall, 1939). Following an examination of a large number of dyestuff intermediates, we have been able to select a coupling component, *N*-β-sulphatoethyl-*m*-toluidine

$$(HO_3SOC_2H_4NH.C_6H_4.CH_3),$$

which provides freely soluble colours with sparingly soluble drugs, and which in addition gives no appreciable colour with nitrous acid. As a result, the sulphamate treatment to remove excess of this reagent, which is a step in Bratton & Marshall's method, is avoided, with a consequent simplification in the procedure. We describe here a method suitable for use in hospital practice together with the slight adjustments required for adaptation to a micro-scale with very small quantities of blood.

METHOD

Reagents

0·05% saponin solution.

Trichloroacetic acid, 15 and 5%.

0·1% $NaNO_2$ solution.

Coupling reagent, 1·0% *N*-β-sulphatoethyl-*m*-toluidine solution (stored in an amber bottle and made freshly each month).

N-HCl.

2·5N-NaOH.

Standard solutions of 5, 10 and 20 mg./100 ml. of the test drug.

Procedure

0·2 ml. of capillary blood, 2·8 ml. of saponin solution and 1 ml. of 15% trichloroacetic acid are mixed and immediately centrifuged for 10 min. (delay leads to cloudy solutions). The supernatant fluid is transferred carefully to a clean tube, and the first tube washed out with 0·8 ml. of 5% trichloroacetic acid, which is decanted into the second tube, poured down the same side of the tube as before. 1 ml. of $NaNO_2$ is added and the solutions mixed by shaking. After 2 min., 2 ml. of the coupling reagent are added. The red dye is compared with standards prepared from 0·2 ml. of the standard solutions in place of the blood sample, either in a visual colorimeter or a photoelectric absorptiometer.

For the estimation of total drug, the blood sample is treated as before and transferred to the second tube. The level of the liquid is noted and 1 ml. of N-HCl is added. The tube and contents are heated in a boiling water-bath for 45 min. After cooling, 0·4 ml. of 2·5N-NaOH is added to neutralize the HCl, the volume is made to the mark and the colour developed as before. For blood samples of 0·02–0·03 ml. the quantities of reagent are reduced to one-quarter of those given and the final volume is adjusted to 2 ml. It has been found convenient when working with these small amounts to avoid decantation by using narrow tubes ($5 \times \frac{5}{16}$ in.), effecting all the manipulations in the one tube. The centrifuged blood solids are left in a compact mass at the bottom.

Notes on the method

(*a*) The depth of colour of the dye is directly proportional to the amount of drug present over fairly wide ranges.

(*b*) A blue or green light filter is used with advantage in the colorimeter.

(*c*) The method can be applied direct in most cases to cerebrospinal fluid. Occasionally a light flocculent precipitate cannot be centrifuged down or is so easily disturbed that decanting is impossible. Under these circumstances, 1 ml. of fluid is diluted with 14 ml. of saponin and 5 ml. of 15% trichloroacetic acid, and filtered once, or twice if necessary, through a Whatman no. 42 paper. 4 ml. of filtrate are then treated with 5% trichloroacetic acid, nitrite and coupling reagent as before.

(*d*) Purulent fluids, pleural effusions and other body fluids usually have to be treated as in (*c*).

(*e*) The method is applicable to diluted urine. The colour of the urine does not interfere unless very little drug is present.

(*f*) The method can be used for body tissues by grinding with sand (method of Chinn & Bellows, 1940).

SUMMARY

The use of *N*-β-sulphatoethyl-*m*-toluidine for the estimation of sulphanilamide drugs in biological fluids is described.

One of us (F. L. R.) wishes to thank Imperial Chemical (Pharmaceuticals) Ltd. for permission to publish this work.

REFERENCES

Bratton, A. C. & Marshall, E. K., Jr. (1939). *J. biol. Chem.* **128**, 537.

Chinn, H. & Bellows, J. (1940). *J. Lab. clin. Med.* **25**, 357.