THE USE OF DEXTRAN SULPHATE AS A BLOOD ANTICOAGULANT IN BIOLOGICAL RESEARCH

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(RECEIVED FOR PUBLICATION APRIL 9, 1952)

Several well-known anticoagulants are in use for blood sample tubes, each having some special application, but there is little doubt that the naturally occurring anticoagulant, heparin, would find wider application were it not so difficult to prepare and consequently expensive. Chemically prepared sulphuric esters of polysaccharides are known to have anticoagulant action. One of these, dextran sulphate, has recently been prepared (Ricketts, 1952) in a form suitable for therapeutic use (Walton, 1951). This material, which is readily prepared and therefore cheaper than heparin, may also find application in biological laboratory work.

Some experiments have been conducted to ascertain whether dextran sulphate will give results comparable with the usual anticoagulants in determinations commonly performed in the clinical pathology laboratory. While not comprehensive, these experiments indicate the general suitability of the material for this purpose.

Technique

For the preparation of blood-sample tubes and Pasteur pipettes it has been found convenient in this laboratory to maintain a stock solution of 2% sodium dextran sulphate in aqueous alcohol, prepared by dissolving 2 g. sodium dextran sulphate* in 66 ml. water and adding 33 ml. absolute ethyl alcohol. This solution maintains its potency for several months of storage at room temperature and contains sufficient alcohol to prevent the growth of moulds.

Blood-sample tubes are prepared by moistening the walls of the tube with the dextran sulphate solution, 0.05 ml. of solution per millilitre of blood, and allowing them to dry in an incubator or oven at 37° C. A maximum temperature of 50° C. is recommended for drying. At higher temperatures—for example, 100° C.—the dextran sulphate will char and liberate sulphuric acid. Considerable inconvenience may arise if the anticoagulant in sample tubes is faulty.

Dextran sulphate tubes may be tested for neutrality with an indicator, and for the presence of readily soluble dextran sulphate by adding a little of a weak (5 mg. per 100 ml.) solution of toluidine blue which changes to the metachromatic purple form in the

presence of dextran sulphate. The amount of dextran sulphate suggested for sample tubes, 1 mg. per ml. of blood, is considerably more than the minimum required, and corresponds to about 15 international heparin units per ml. of sample.

Many biochemical estimations involve the precipitation of the plasma proteins. When less than 40 mg. of dextran sulphate is present per millilitre of plasma, and precipitation is effected by any of the well-known methods, the dextran sulphate remains adsorbed on the protein precipitate and there is no subsequent interference with chemical determinations. Dextran sulphate does not inhibit urease, so the determination of blood urea is unaffected. No turbidity or difference in reading was observed with Nessler's reagent. Experiments showed that dextran sulphate does not inhibit glycolysis by red cells.

In the field of haematology the requirements of an anticoagulant are somewhat more critical. It was quickly established that there was no effect on the haematocrit determination, but there appears to be a slight though probably negligible effect on the erythrocyte sedimentation rate. Walton (1951) reports that the ordinary haematological determinations, haemoglobin, red cell and white cell counts, are not interfered with.

In the field of physiological research there appears to be scope for an anticoagulant of the heparin type in experiments involving the perfusion of tissue with blood. Since it is not prepared from tissue, dextran sulphate is not contaminated with pharmacologically active substances, e.g., histamine. No doubt other specialized applications will suggest themselves to the research workers concerned. Where a sodium salt is unsuitable it is a comparatively simple matter to exchange the sodium for hydrogen by passage through a column of ion exchange resin. The acid solution of dextran sulphate may then be neutralized with any selected base.

REFERENCES

* Dextran sulphate is available from Messrs. Dextran, Ltd., Aycliffe, Darlington.

Ricketts, C. R. (1952). *Biochem. J.*, **51**, 129.
Walton, K. (1951). *Proc. roy. Soc. Med.*, **44**, 563.