A Rapid Turbidimetric Method for Heparin Assay

SIR,—During an investigation of the column chromatographic behaviour of several polyanions we felt the need for a procedure for heparin determination which would enable us to deal rapidly and inexpensively with a large number of samples.

Methods already published (Jaques and Bell, 1959) did not meet our requirements and we were compelled to develop a new procedure which might interest other workers in this field.

It is known that the basic antibiotic, streptomycin, forms water-insoluble complexes with high molecular weight polyphosphates (Cohen and Lichtenstein, 1960; Harshaw, Brown and Graham, 1962). Although the molecular weight of heparin is well below the critical size required for the polyanions' reaction with streptomycin, it was thought that the greater degree of ionisation of the heparin-like polysulphates would nevertheless allow the reaction to occur. This was indeed so. In addition, the turbidity produced by the insoluble heparin-streptomycin complex obeys the Beer's relationship within certain concentration limits. This finding served as a basis for the procedure for heparin assay outlined below.

Mix 1·0 ml. of sample containing 15–300 μg./ml. of heparin with 3·0 ml. 0·25 per cent streptomycin sulphate (NBC, diagnostic). Dihydrostreptomycin of reagent grade may also be used. Allow to stand 10 min. at 18–22° and read the extinction in a Pulfrich step photometer with the L_2 filter (maximum transmittance at 480 mμ). If a spectrophotometer is available, readings are preferably taken at 400 mμ against a suitable blank. The turbidity is stable for at least 120 min. at 18–22°. The standard solution (containing 100 μg./ml.) is stabilized by dissolving 10 mg. of sodium heparin (BDH) in 100 ml. of 2 per cent benzoic acid.

The relation between extinction and concentration is linear over the range 0–300 μg. heparin per ml. The standard deviation computed from a series of 10 parallel determinations on the same solution is below 0·01 (Beckman DU spectrophotometer) or 0·03 (Pulfrich step photometer). The method is non-specific, a defect shared by other chemical methods for the determination of heparin.

TABLE I

EFFECT OF IONIC STRENGTH ON THE TURBIDITY PRODUCED BY THE INTERACTION OF HEPARIN WITH STREPTOMYCIN

The reaction mixture contained in 4·0 ml. final volume 200 μg. heparin, 0·0–0·5M halide and 10 mg. streptomycin sulphate. The figures represent the decrease in extinction as per cent of the extinction of the tube containing no halide = 100 per cent. The ionic strength is computed from the formula: $I = 0{\cdot}5\ \Sigma\ [i].Z_i^2$.

Ionic strength	LiCl	NaCl	KCl	$MgCl_2$	$MnCl_2$
0·025	93	98	91	—	—
0·05	89	89	83	83	78
0·10	79	81	78	78	76
0·15	75	70	65	—	—
9·20	64	64	60	67	62
0·25	56	54	55	54	50

All other polyanions so far tested, except some commercial samples of yeast ribonucleic acid gave similar reactions with streptomycin. In this connection it is interesting to note that another basic antibiotic, neomycin sulphate, is

capable of precipitating relatively small molecules of ribonucleic acid of the "soluble" type.

The reaction between heparin and streptomycin is sensitive to the ionic strength of the medium. The interference of some representative halides with this reaction is presented in Table I. It is reasonable to assume that the neutralisation of the sulphate groups of heparin by the guanidine residues in the streptomycin molecule leads to reversible breaking of the hydrogen bonds between the polyanion and water. It thus seems that the interaction between soluble polyelectrolytes does not lead to the formation of firm covalent bonded complexes. Therefore, it is essential to desalt the assayed sample before the addition of streptomycin, and to prepare the latter reagent with deionised water.

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Colomycin and Polymixin E

SIR,—During investigations upon the mode of action of the polypeptide antibiotic colomycin and using *Escherichia coli* we were impressed by the close similarity between colomycin and polymixin E as judged by the effect of inoculum size on minimum inhibitory concentration, the adsorption of the antibiotics from aqueous solution by washed suspensions of the test organism, and the pattern of leakage of cellular material absorbing at 260 mμ.

Subsequent investigations showed that the two samples possessed indistinguishable infra-red spectra when examined in KBr discs. The effects of varying the concentrations of each antibiotic upon the surface tension of water were identical as measured by the De Nouy tensiometer.

R_F values using paper chromatography and two solvent systems are shown in Table I.

TABLE I

R_F VALUES OF COLOMYCIN AND POLYMIXIN E USING TWO SOLVENT SYSTEMS

Solvent system	R_F	
	Colomycin	Polymixin E
n-Butanol : glacial acetic acid : water 4 : 1 : 5	0·398	0·397
n-Butanol : glacial acetic acid : acetone : water 2 : 0·5 : 2·1 : 0·1	0·75	0·74

A mixture of colomycin and polymixin E ran as one spot using the same solvent systems. Both samples were hydrolysed with 5N hydrochloric acid