THE ASSAY OF PROTAMINE SULPHATE FOR ITS CAPACITY TO NEUTRALISE HEPARIN

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PROTAMINE sulphate is used clinically to neutralise the anticoagulant effect of heparin, and the B.P.C. includes an *in vitro* method for the assay of Injection of Protamine Sulphate: this is based on the assumption that 1 mg. of protamine sulphate will neutralise 86 units of heparin. Excess heparin, in the mixture of heparin and protamine sulphate used, is determined biochemically, but any physical property of heparin will serve equally well.

Excess heparin or protamine sulphate in mixtures of the two may be demonstrated by the extent to which spots of the mixtures spread when applied to Whatman No. 1 filter-paper as shown by staining with bromocresol green. When protamine is in excess the stained spot is compact and well within the zone wetted during application; when heparin is in excess the staining extends to the limit of the wetted area.

TABLE I

REACTIONS NOTED WITH 1 MG. OF PROTAMINE SULPHATE BATCH 269 WITH VARYING QUANTITIES OF HEPARIN BATCH 3338

Units of heparin	64, 66, or 68	70, 72, or 74
Nature of the spot	Compact	Diffuse
Protamine reaction in supernatant fluid	Positive	Negative

The sensitivity and specificity of this change from a compact to a diffuse spot have been examined (Table I). To tubes containing 1 ml. of 0·1 per cent aqueous solution of protamine sulphate, quantities of heparin in aqueous solution were added in increments of 2 units. Drops of these mixtures (0·01 ml.) were applied to Whatman No. 1 paper, the area of wetting being outlined in pencil. The spots were allowed to dry, then stained by immersion in 0·02 per cent bromocresol green for 5 min. followed by washing in 2·0 per cent w/v acetic acid. The specificity of the change was checked by applying the method used by Godal (1960). The tubes containing protamine and heparin were centrifuged at about 16,000 r.p.m. (20 min.) and the supernatant fluids spotted as above. This procedure will detect protamine sulphate in aqueous solution at a level of 5 μg./ml.

A comparison of the amounts of heparin neutralised by 1 mg. of protamine sulphate as determined by this method, and by the method of the B.P.C., is shown in Table II. The values obtained by the two methods do not agree; they also depend on the sample of heparin used. This

illustrates a weakness of the B.P.C. test, which does not call for the use of a specific heparin standard. Although the absolute neutralisation values differ, if one batch of protamine is used as a standard (arbitrarily 100 per cent) and the potency values of the others expressed in terms of this, then the values are roughly equal irrespective of the method or the heparin used. The figures in brackets (Table II) are derived thus.

TABLE II

UNITS OF HEPARIN NEUTRALISED BY 1 MG. PROTAMINE SULPHATE

Protamine Batch	Heparin batch			
	3338		9788	
	B.P.C.	Spot	B.P.C.	Spot
S	82 (100 per cent)	70 (100 per cent)	116 (100 per cent)	102 (100 per cent)
269	80 (97·6)	68 (97·1)	118 (103·4)	98 (96·1)
12,753	80 (97·6)	70 (100)	116 (100)	100 (98·0)
13,897	80 (97·6)	68 (97·6)	114 (98·3)	98 (96·1)

For routine assay both methods can be satisfactorily applied using either a reference standard for protamine sulphate or for heparin. If a heparin standard is used, its capacity to neutralise protamine sulphate will have to be specified for the method which is to be used.

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REFERENCE

Godal, H. C. (1960). *Scand. J. clin. Lab. Invest.*, **12**, 446.

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