A NOTE ON THE BIOLOGICAL ASSAY OF HEPARIN

by

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B.P.[1] and USP[2] have described different procedures for biologically estimating the potency of samples of Heparin; the method suggested in B.P. employs ox blood and thrombokinase extract from the acetone dried ox brain, while citrated sheep plasma is used in the technique recommended in U.S.P. Birger[3] et al, while critically reviewing the methods used in the assay of Heparin have stated that the method employing sulphated whole blood (ox) and thrombokinase is simple, rapid and accurate, while the USP method gives lower results.

In view of the local difficulties of procuring ox-blood, B.P. method could not be adopted; the USP method could also not be accepted in its entirety due to (*i*) its reported inaccuracy, and (*ii*) the difficulty of procuring pure sheep blood and the separation of plasma promptly after collecting the blood. Consequently investigations were taken up in order to devise a suitable method, so that reliable results could be obtained under local conditions. The following procedure has been found to give satisfactory results:—

(*a*) *Collection of Blood*—The blood of sheep or goat (or mixed) is collected in the slaughter house directly into a wide mouth glass bottle, containing approximately 5·0 cc of 7·5 percent sodium citrate solution for every 100 cc of blood; the bottle is stoppered with a velvet cork, whose lower end is coated with paraffin wax. The bottle is agitated gently and the blood stored at room temperature for 6 to 8 hours; thereafter the blood is centrifuged at 2,500 r.p.m. for 30 minutes and the separated plasma stored at 5°C for use on the following day or even after 48 hours; the plasma was brought to room temperature at the time of the test.

(*b*) *Suitability of Plasma*—We observed that specimens of plasma, used from 24 to 48 hours after storage at 5°C, 1·0 cc of which formed a solid clot at about 25°C within fifteen minutes of addition of 0·2 to 0·3 cc of 1·0 percent calcium chloride solution, were entirely satisfactory for the assay. This procedure helps in determining the quantity of calcium chloride required in the preliminary stage of the test. In the course of further study on the suitability of the plasma, it was observed that specimens of plasma, strictly not conforming to this requirement were also satisfactory for the assay, the concentration of calcium chloride to be ultimately used in the test being determined in the preliminary trial with Heparin standard. However, as data on this aspect is still being collected, the results given in Table I pertain to the experiments inwhich specimens of plasma, satisfying the suitability test were employed.

(*c*) *Performance of the Test and Calculation of the Potency*—This part of the procedure was based on the work of Mangieri and Nutley[4]. The

test was performed in two stages, viz., (*a*) the preliminary stage comprising of determining the quantity of calcium chloride, with which 0·35 cc (0·01 mg/cc) of a standard Heparin solution just prevents clotting of 1·0 cc of the plasma, and (*b*) the Assay proper, comprising of determining the volumes in cc of the test sample (T) and the standard preparation (S) with which 0·2 cc of the calcium chloride solution, corresponding to the quantity, determined at the preliminary stage, just fails to form a clot in 1·0 cc of the plasma ; six different volumes as near 0·35 cc as possible, of 0·01 mg/cc solutions of standard and test preparations of Heparin are put up and the formation of the clot is studied by slightly tilting the test tube stand, care being taken to avoid the breaking of the clot by premature inversion. In the preliminary stage, Mangieri and Nutley have recommended six different volumes of 0·5 percent. solution of calcium chloride (0·16 to 0·22 ml) to be used, while we observed that this range does not always give satisfactory results. Consequently in our experiments the volume range was determined by the requirement of the test, viz. ranges 0·1 to 0·2 cc, 0·2 to 0·3 cc and 0·3 to 0·4 cc were tried by us and found satisfactory. The potency ratio (T/S) was calculated by dividing the volume of the test preparation with that of the standard preparation, which just prevented clot formation in part (*b*) of the test. For further calculations and making dilutions, 1·0 mg of Heparin was taken equivalent to 130 units.

The results on 13 samples (fresh as well as life-expired) are given in Table 1, the pharmacopoeial stipulation in regard to potency is 90 to 110 percent of claim. From this table it will be seen that samples of Heparin stored at a temperature not exceeding 20°C, start deteriorating three years after manufacture. Further work on these lines is in progress to establish the useful life period of this preparation.

TABLE I—RESULTS OF THE ASSAY

Sample No.	Potency		
	Stated units/cc	Estimated units/cc	Estimated %
1.	5,000	5,434	108·7
2.	5,000	5,000	100·0
3.	5,000	4,735	94·7
4.	5,000	4,785	95·7
5.	5,000	5,270	105·4
6.	1,000	1,000	100·0
7.	1,000	1,000	100·0
8.	1,000	950	95·0
9.	1,000	756	75·0
10.	1,000	885	88·5
11.	1,000	910	91·0
12.	1,000	916	91·6
13.	1,000	800	80·0

Samples	1 to 5 were assayed during the useful life period (2 years). Here the estimated potency comes within the range prescribed in the Pharmacopoeias.
Samples	6 to 8 were assayed within one year after the stated date of expiry. These samples also conform to the pharmacopoeial stipulation in respect of potency.
Samples	9 to 13 were assayed one year after the stated date of expiry. Three of the five samples are below the pharmacopocial stipulation in respect of potency.
Storage	The samples were from stocks stored at a temperature not exceeding 20°C. The manufacturers normally assign a life of two years for this material.

References

1. *The British Pharmacopoeia*, 1958.

2. *The Pharmacopoeia of the United States of America*, fifteenth revised edition, 1955.

3. Birger *et al.*, *J. Pharm. Pharmacol.* **5**, 1031 (1953),

4. Mangieri and Nutley, *J. Lab. Clin. Med.* **32**, 901, (1947).