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Stability of heparin solutions

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Heparin injection B.P., adjusted to a pH between 7·0 and 8·0, preserved with 0·15% chlorocresol, is a relatively stable biological product and may be expected to retain its potency for up to 15 years if stored at 4°, over 7 years at room temperature and 6–8 years at 37°. The stability is markedly reduced if the pH of the solution falls below 6·0, so that a rapid and marked loss in potency can be expected if heparin is sterilised in, or mixed for any length of time with, solutions of dextrose, owing to the low pH of these solutions.

TO determine the stability of solutions of heparin, now increasingly used in open heart surgery and artificial kidney treatment in which exact dosage is important, reference samples from batches manufactured over the past 15 years have been retested for potency.

As heparin is sometimes added to saline and glucose solutions before these solutions are sterilised the stability of heparin over a range of pH values at temperatures of 100° and 115° has been determined.

Experimental methods

The heparin solutions were batches of commercial origin (Pularin, Evans), a sterile solution of sodium heparin in distilled water, adjusted to a pH between 7·0 and 8·0 and preserved with 0·15% chlorocresol. The strength of the solutions tested varied from 10 to 35,000 units/ml.

Storage was at 4°, room temperature (approx. 18°) and at 37° for periods between 3 and 15 years.

Heating to 100° was in a boiling water-bath and to 115° by autoclaving in a small bench autoclave to allow a minimal time for raising and lowering the temperature.

The potencies were determined by the method of Pritchard (1956) with which, with samples of commercial grade heparin, the fiducial limits of error ($P = 0.95$) of a single assay are given as $\pm 1{\cdot}7\%$ and for the mean of a duplicate assay as $\pm 1{\cdot}2\%$.

Results

Table 1 shows the change in potency of batches stored at the 3 temperatures. If changes in potency within the limits of error of the test are excluded, it was found that at 4°, 2 batches lost potency, one by 10·7%, the other by 3·8%, while two batches apparently gained in potency by 7·4% and 3·1% respectively.

At room temperature, 4 batches showed slight gains and 2 slight losses, while at 37° there was one loss and one gain in potency. Thus of 29 batches tested, 12 show a significant change, 7 showing an apparent increased potency and 5 a decrease in potency.

Fig. 1 shows the change in potency of a solution of heparin standardised to contain 660 units of heparin per ml when autoclaved for 10 min at 115° over a range of pH values from 3·0 to 10·0. Between pH 5·5 and 8·5

From The Evans Biological Institute, Runcorn.

there is no significant change but below pH 5 there is a sharp fall-off in unitage; above 8·5 there is also some loss of activity but this is less marked than at acid pH values.

TABLE 1. CHANGES IN POTENCY OF BATCHES STORED AT THREE TEMPERATURES

Batch No.	Manufactured	Original potency	Storage period		Change in potency %
			Years	months	
Temperature 4° C					
24	July 1947	1,000 u/ml	15	2	+ 0·6
34	June 1948	5,000 ,,	14	3	− 0·2
40	July 1948	4,400 ,,	14	2	− 0·8
41	,,	1,300 ,,	14	2	−10·7
42	,,	5,500 ,,	14	2	+ 3·1
44	,,	5,000 ,,	14	2	− 0·7
221	Sept. 1952	5,000 ,,	10	2	+ 7·4
289/500	Nov. 1954	10 ,,	8	3	− 0·7
76	Feb. 1950	5,000 ,,	5	10	− 3·8
72	Dec. 1949	25,000 ,,	4	5	− 0·8
Room temperature (18° C)					
54	Oct. 1948	5,000 u/ml	7	6	+ 3·4
343	May 1956	5,000 ,,	6	4	+ 0·1
69	Sept. 1949	5,000 ,,	6	3	− 3·2
345	June 1956	35,000 ,,	6	3	0·0
360	Nov. 1956	5,000 ,,	5	10	+ 2·3
80	May 1950	5,000 ,,	5	7	− 3·2
376	June 1957	5,000 ,,	5	3	− 0·7
390	Sept. 1957	25,000 ,,	5	–	+ 0·2
393	Oct. 1957	5,000 ,,	4	11	+ 4·9
177	Oct. 1951	5,000 ,,	4	2	− 0·5
429	Oct. 1958	1,000 ,,	3	11	− 0·9
220	Sept. 1952	25,000 ,,	3	3	+ 3·2
Temperature 37° C					
221	Sept. 1952	5,000 u/ml	9	9	−15·9
,,	,,	5,000 ,,	3	4	+ 3·6
282	June 1954	5,000 ,,	8	4	− 0·6
335	Mar. 1956	5,000 ,,	6	8	− 0·2
,,	,,	5,000 ,,	6	3	− 1·5
337	,,	5,000 ,,	6	8	− 0·3
,,	,,	5,000 ,,	6	3	+ 0·9

Fig. 2 shows the effect of heating solutions buffered at pH 2·99, 4·96, 7·5 and 9·96 for a period of 8 hr at 100°. The control material was unheated. This again shows the marked destruction of heparin when heated at an acid pH.

Discussion

The results show that correctly prepared solutions of heparin are stable when stored at 4° for 15 years, at room temperature for over 7 years and at 37° for 6–8 years. Two batches showed a loss in potency, one of 10·7% after 14 years at 4° and the other of 15·9% after 9¾ years at 37°. The remaining batches, with the exception of one showing an apparent 7·4% increase, do not vary widely from expected limits and, since it is unlikely that an increase could occur, it is probable that the original assay was at fault. If we accept these three observations, the percentage change between observations within groups does not differ significantly from the difference between groups at the 3 temperatures tested, suggesting that any change in potency is due to a factor other than storage temperature.

One of the most probable causes of a loss of potency is the incorrect

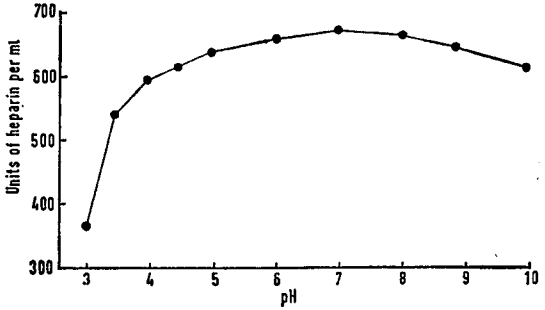

FIG. 1. Potency of heparin solutions after autoclaving at 10 lb./inch² for 10 min at pH values between 3·0 and 10·0.

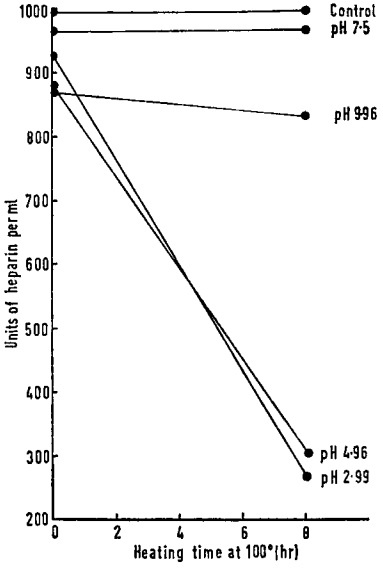

FIG. 2. Potency of heparin solutions, after heating at 100° C for 8 hr at different pH values.

adjustment of the pH of the product. This effect is shown clearly in Figs 1 and 2. The pH is important not only to the stability of the stored product, but also to the stability of mixtures of heparin and dextrose when these are intended as an anticoagulant for blood for heart-lung machines maintaining extra-corporeal circulation in open heart surgery, As injection dextrose B.P. has a pH range of 3·5–6, autoclaving heparin in this solution could destroy 50% of its original potency, and bring about coagulation of the blood.

If heparin is required in a dextrose solution it is best prepared by adding the sterile heparin solution to the bottle containing the sterilised dextrose solution as shortly as possible before use.

Reference

Pritchard, J. (1956). *J. Pharm. Pharmacol.*, **8**, 523–529.