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The anticoagulant activity of heparins in dextrose solutions

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Changes with time in the anti-factor Xa activity of several heparins were determined in freshly autoclaved and unautoclaved dextrose solutions. In the former, activity was raised, in the latter a reversible fall occurred at certain heparin concentrations, and the term 'dextrose effect' is applied to the difference in activity in the two types of dextrose solutions. A simple dependence on heparin concentration for the rise in activity in autoclaved dextrose solutions contrasted with a threshold heparin concentration in the unautoclaved dextrose solutions. Above this a fall and below it a rise in activity were demonstrated with one factor Xa method. The pH of the two dextrose solutions differed by one unit but pH was not the principal factor in the effect; salt tended to eliminate the dextrose effect which was found mostly with the high and to some extent also with the low molecular weight fractions of pharmacopoeial heparin. There was no evidence for heparin degradation but activity changes, which differed for different heparins, are thought to be associated with the effects of heparin-dextrose interaction modified in the case of autoclaved dextrose by by-products of autoclaving. Generally, proprietary dextrose solutions gave variable results.

Heparin is a linear polyelectrolyte comprising molecules which range in molecular weight from under 4000 to over 35 000 and which vary in anticoagulant activity. The variation in activity is also associated with species-anatomical source and method of preparation yet the notion still persists that heparin is as a single standardized substance with no more than an inevitable polydispersity of no great clinical significance.

Despite its complexity, heparin activity in neutral aqueous solution has long been known to be stable at temperatures up to those of the sterilizing autoclave (Pritchard 1964), but doubt has arisen about its stability at ambient temperature in dextrose solution in which it may remain up to 24 h while being administered by infusion. Overdosage and underdosage of heparin can have serious consequences and it is therefore essential to resolve doubts about its stability and hence about the quantity of activity actually administered. Jacobs et al (1973) and Okuno & Nelson (1975) claimed that heparin activity in certain infusion fluids either decreased or decreased temporarily; Raper & Johnson (1976) and Goodall et al (1980) found decreases in activity in dextrose solution whilst others (Mitchell et al 1976; Joy et al 1979) found no evidence of instability. Anderson et al (1979), showed increased activity in autoclaved, and decreased activity in unautoclaved, 5% w/v dextrose solutions.

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Relevant systematic clinical studies appear not to have been reported but O'Riordan & McGowan (1970) claimed that heparin therapy is difficult to control when it is administered in 5% dextrose solution, whereas Chessels et al (1972) found similar heparin activity after administration in either sorbitol or dextrose solutions.

Heparins and infusion fluids from different sources and a variety of anticoagulant assays have been used in the investigations so far reported, which make comparisons and, more important, predictions, difficult. In this study three (in one experiment, six) pharmacopoeial heparins, certain chromatographic heparin fractions, freshly made infusion fluids and two anti-factor Xa assays were used in an attempt to control some of the variables which have apparently been present in earlier work.

MATERIALS AND METHODS

Heparins were gifted as follows. Pig mucosal sodium heparin, lot no. 177082, 155 iu mg^{-1}, Leo Laboratories Ltd; beef lung sodium heparin, lot no. 730-EH, 153 iu mg^{-1}, Upjohn Ltd; pig mucosal calcium heparin, batch CH7081, 165 iu mg^{-1}, Laboratoire Choay; pig mucosal calcium heparin, 155 iu mg^{-1}, Glaxo Operations Ltd; pig mucosal sodium heparin, 147 iu mg^{-1}, Riker Laboratories; pig/ox mixed mucosal heparins, 156 iu mg^{-1}, The Boots Company Ltd.

Anhydrous dextrose for parenteral use (B.P.) was given by CPC (U.K.) Ltd. Proprietary infusion

solutions in glass and plastic containers were given by Travenol Laboratories Ltd and The Boots Company Ltd.

Autoclaved and unautoclaved dextrose solutions were prepared with water double-distilled in glass immediately beforehand and contained 5% w/v anhydrous dextrose unless otherwise stated. After autoclaving (121°/55 min) solutions were cooled and used within a few hours, or, where appropriate, after 24 h. The same UGB glass transfusion bottles were used throughout and had been used frequently in the past. Atomic absorption spectroscopy showed that neither water directly from the still, nor dextrose solution sampled (a) from the bottles but unautoclaved, and (b) from the bottles after autoclaving, acquired any of the following metals: Na, K, Ca, Fe, Mg, Al before use.

Heparin stability in dextrose solutions

Heparin (30 iu ml^{-1}) was dissolved in the autoclaved or unautoclaved dextrose solution in glass flasks and maintained at 37 °C. The anticoagulant activity of these stock solutions was determined in duplicate at intervals and changes in activity were related to zero time recovery of added heparin. In one such series, the anti-Xa activities of the stock solutions of three heparins were determined at 0, 1, 2, 3, 4, 5, 24 h on each of five separate days (six in one case); the heparins were chosen on the basis of species (pig mucosal sodium heparin, beef lung sodium heparin) and on the basis of cation (pig mucosal calcium heparin).

Anticoagulant methods

Two anti-factor Xa methods were used. In Yin's method (Yin et al 1973), referred to as method Y, the stock heparin in dextrose solution was diluted by normal human plasma to 5 iu ml^{-1} of which 0·1 ml was used according to method B, standard unit range, as described by the authors. In the method of Denson & Bonnar (1973), referred to as method DB, the stock heparin-dextrose solution was added to the human plasma which had been heated at 56 °C/15 min and centrifuged 2000 rev min^{-1}/10 min, to give a concentration of 0·1 iu ml^{-1} which was then used in the test. Both methods were used as described by Anderson & Harthill (1981), and reagents were obtained in kit form (Sigma; Diagnostic Reagents Ltd for the Y and DB methods respectively).

Chromatographic fractionation of heparin

Pig mucosal heparin (300 mg) was fractionated on a column of Ultrogel AcA-44 (Lane et al 1978) in the manner detailed earlier (Anderson & Harthill 1981) with the exception that three fractions corresponding to 19% (high molecular weight, HMW) 62% (middle molecular weight, MMW) and 19% (low molecular weight, LMW) were isolated by pooling successive 3 ml fractions. Each fraction was concentrated by ultrafiltration (Amicon UM-2 membrane) at 6 °C under nitrogen pressure, desalted on a column of Sephadex G-25 in water and freeze-dried.

Viscosity

Flow times of solutions of pig mucosal sodium heparin (30 iu ml^{-1}) in autoclaved and unautoclaved dextrose solutions were measured according to B.S. 188: 1957 in suspended level short form U-tube viscometers at 37° ± 0·01 °C.

5-hydroxymethylfurfural (5-HMF) was determined by the method of Taylor et al (1971).

RESULTS

Dextrose effect

In freshly autoclaved dextrose solution, heparin (30 iu ml^{-1}) had raised anti-Xa activity, whereas in unautoclaved dextrose solution anti-Xa activity was lowered, a result which generally held, there being only quantitative differences for both anti-Xa methods and all heparins examined. The mean percentage change in activity over 5 h was calculated from the five hourly percentage changes in activity (relative to activity at 0h) for heparin which had been added to the dextrose solutions. The term 'dextrose effect' is applied to the mean difference, Δ, between the changes in heparin activity in autoclaved and unautoclaved dextrose solutions. It is therefore a measure of the effect of the consequences on the anti-Xa activity of the heparin in (unautoclaved) dextrose solution of immediately prior autoclaving of the dextrose solution. A typical example of the effect is given in Fig. 1 for beef lung heparin, which shows altered activity established by 1 h. The other heparins showed similar patterns.

The results of one study of the dextrose effect are in Table 1, where it is clear that activity during the 5 h period was significantly different from activity at zero time. Analysis of variance showed no significant regression of Δ during the 5 h period, but despite that finding, Fig. 1 shows that activity in unautoclaved dextrose solution returned to the original level by 24 h.

The dextrose effect, Δ (Table 1), for beef lung sodium heparin was greater (P = 0·02–0·05) than

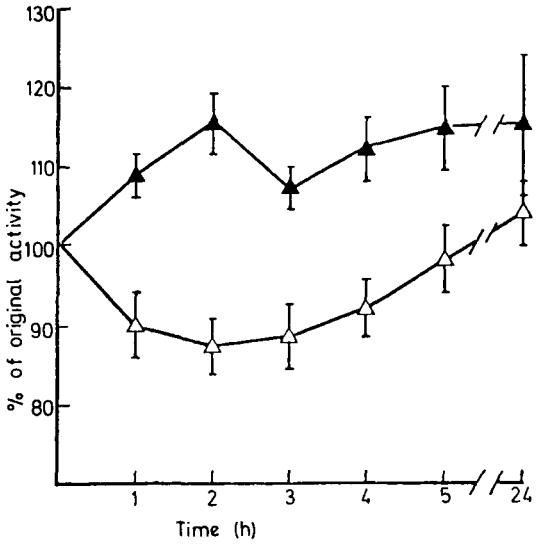

FIG. 1. Changes with time in anti-Xa activity of beef lung heparin in autoclaved, ▲ and unautoclaved, △ dextrose solutions respectively; mean ± s.e.m.; method Y.

that for pig mucus sodium heparin whilst calcium heparin occupied an intermediate position.

When the DB method was used, autoclaved and unautoclaved dextrose solutions were not always examined on the same day and a smaller number of experiments were done, but the dextrose effect (apart from the rise in autoclaved dextrose always disappearing by 24 h) was similar to that seen with method Y and the following results were obtained.

Table 1. Mean percentage change over 5 h, in anti-Xa activity of heparins in autoclaved (A) and unautoclaved (UA) dextrose solutions.

	Mean percentage change in anti-Xa activity over 5 h (±95% confidence limits)			median (confidence limits*)
	A + H	UA + H	Δ	Δ
Pig mucous heparin (Na)	+8·0 ± 2·3	−5·6 ± 2·0	13·7 ± 3·1	12 (8–18)
Pig mucous heparin (Ca)	+9·7 ± 2·5	−7·6 ± 2·0	17·3 ± 2·8	17 (11–23)
Beef lung heparin (Na)	+11·9 ± 2·7	−8·8 ± 3·5	20·7 ± 4·4	21 (15–25)

A + H UA + H: autoclaved, unautoclaved dextrose solutions to which heparin, 30 iu ml^{-1} was added and assayed at hourly intervals using method Y; Δ = A − UA; *, P% for confidence limits for medians are, respectively, 95·68, 95·68, 96·44. Number of duplicate observations, 25 (sodium heparins), 28 (calcium heparin).

Mean percentage change over 5 h (±95% confidence limits) in autoclaved and unautoclaved dextrose solutions respectively: +7·3 ± 2·9; −10·2 ± 3·5; Δ = 17·5 ± 4·8 (pig mucous sodium heparin). For beef lung sodium heparin corresponding results were: +13·0 ± 9·6; −12·1 ± 7·0; Δ = 25·1 ± 8·7.

Metachromatic assay (Lam et al 1976) showed that polyanionic properties of heparin were unchanged during contact with dextrose indicating unimpaired polyanionic activity and absence of hydrolysis during the period when anti-Xa activity was changing.

Heparin concentration in dextrose solutions

At very high heparin concentration (150 iu ml^{-1}) in dextrose solutions the same pattern of raised and lowered activities occurred (Δ = 24, 41, means of pairs of duplicate experiments, method Y, for pig mucous and beef lung sodium heparins, respectively). However, at a heparin concentration of 1 iu ml^{-1} (method Y), the usual fall in activity (obtained with 30 iu ml^{-1}) in unautoclaved dextrose solution did not occur. This was investigated in more detail using the DB method with six pharmacopoeial heparins. Table 2 shows that at 1 and 3 iu ml^{-1} rises in activity were observed in unautoclaved dextrose solution (pH ≈ 6); in autoclaved dextrose solutions at 1, 3, 5 iu ml^{-1} rises were observed as for 30 iu ml^{-1} in the autoclaved solution but were not significant. Hence for freshly autoclaved dextrose solutions the magnitude of rise of activity appears roughly concentration-dependent. But the fall in activity seen in unautoclaved dextrose solution at 30 iu ml^{-1}, and above, was replaced at 5 iu ml^{-1} by no activity changes and by a significant rise at 1 in ml^{-1} (Table 2).

Table 2. Mean percentage change over 5 h, in anti-Xa activity of heparins at low concentration in autoclaved (A) and unautoclaved (UA) dextrose solutions.

Mean percentage change in anti-Xa activity over 5 h (±95% confidence limits)					
heparin concn (iu ml^{-1}) in A			heparin concn (iu ml^{-1}) in UA		
1	3	5	1	3	5
+0·9 ± 1·6	+6·2 ± 1·8	+3·7 ± 1·3	+14·4 ± 4·9	+10·7 ± 2·4	−1·9 ± 2·5

Values given are means for six pharmacopoeial heparins: DB method; activities of autoclaved and unautoclaved solutions determined on separate days.
pH ranges: heparins (1–5 iu ml^{-1}) in autoclaved (3·80–4·10) and unautoclaved (5·85–6·50) dextrose solutions.

Throughout this reversal of effect, the pH remained around 6. Hence at low heparin concentration in unautoclaved dextrose solution it is possible, using one factor Xa method (DB), to demonstrate a

rise in anti-Xa activity which is also seen in autoclaved dextrose solution at higher heparin concentration (30 iu ml^{-1}) at acidic pH.

pH and the dextrose effect

The effects of heparin on the pH of freshly-made autoclaved and unautoclaved dextrose solutions are given in Table 3. The change in pH of such solutions with time over 5 h is exemplified by pig mucous sodium heparin in Fig. 2. Over this period, raised anti-Xa activity occurs in the autoclaved acidic solutions whilst temporarily decreased activity occurs in the neutral (sodium heparin) and acidic (calcium heparin) unautoclaved dextrose solution.

Table 3. pH of autoclaved (A) and unautoclaved (UA) dextrose solutions to which 30 iu ml^{-1} heparin has been added.

	Mean pH ± s.e.m.	
Heparin	A	UA
No heparin	4·13 ± 0·04	5·70 ± 0·07
Pig mucous (Na)	5·87 ± 0·18	7·01 ± 0·08
Pig mucous (Ca)	4 82 ± 0·11	6·05 ± 0·02
Beef lung (Na)	5·94 ± 0·10	7·10 ± 0·06

Adjustment of heparin solution in autoclaved dextrose to pH 7·01 with 0·001 M NaOH or by use of the trizmal buffer used in method Y, abolished the rise in activity found in autoclaved dextrose solution and did not cause the fall seen in the (neutral) unautoclaved dextrose solution of heparin. The addition of NaCl also abolished the activity rise.

Increasing concentrations of heparin added to water and dextrose solutions raised pH to a maximum which differed for each solvent. Thus, 30 iu ml^{-1} sodium heparin gave a maximum pH of 7·10 in unautoclaved dextrose but 100 iu ml^{-1} was required to produce a maximum of 7·04 in the acidic autoclaved dextrose solution.

When the pH of autoclaved dextrose solution was raised to the maximum value by higher heparin concentration, a rise in anticoagulant activity was observed over 5 h, as for the lower (30 iu ml^{-1}) concentration which had a lower pH.

The pH maxima produced by calcium heparin in the unautoclaved and autoclaved dextrose solutions were lower than for sodium heparin (~6 and 4·5 respectively).

Salt and the dextrose effect

Addition of 0·9% w/v NaCl to dextrose solution decreased the dextrose effect in both anti-Xa methods, principally due to elimination of the fall in activity in unautoclaved dextrose solution. Also, in the experiments with heparin fractions, the dextrose effect was only observed when desalted fractions were used; undesalted fractions showed no dextrose effect.

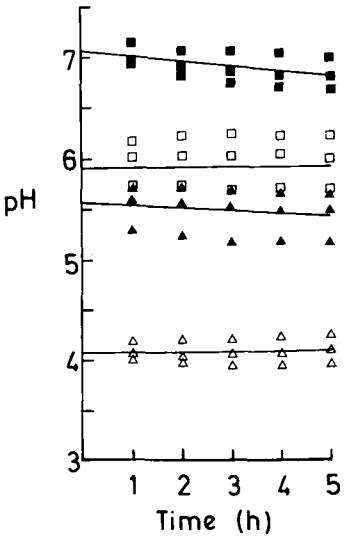

FIG. 2. Changes in pH, with time, of pig mucous sodium heparin in autoclaved and unautoclaved dextrose solutions. ▲, ■ heparin (30 iu ml^{-1}) in autoclaved and unautoclaved dextrose solutions, respectively; △, □ autoclaved and unautoclaved dextrose solutions, respectively.

Proprietary dextrose solution and container effects

Proprietary dextrose infusions obtained from different sources and in containers of different materials (glass and plastic) were a source of variable results from which a consistent pattern of changes in activity did not emerge. Processing information was not available but presumably the infusions had been sterilized by autoclaving; presumably also the adsorption properties of the containers differed as would the nature of any substances leached from them. The possible influence of such effects on the anticoagulant test cannot be overlooked although at present our knowledge of them is inadequate.

Viscosity

The flow times for autoclaved and unautoclaved dextrose solutions differed by 0·1 s over 0–5 h and by 0·2 s at 24 h. The flow times for these solutions containing heparin (pig mucous sodium; 30 iu ml^{-1}) differed by 1·3, 1·2 s at 0 h and 5 h respectively and by 1·6 s at 24 h. Viscometer flow times were around 230 s. These differences suggest that the autoclaved

and unautoclaved dextrose solutions behave as different solvents for the heparin in which different interactions are occurring. The small changes in flow times over 0–24 h are deemed non-significant.

Molecular weight of heparin and the dextrose effect

The three pig mucosal sodium heparin fractions were known, from earlier data (Anderson & Harthill 1981), to have the following molecular weight ranges: HMW, 16 000–25 000; MMW 10 400–16 000; LMW, 5450–10 400. The dextrose effect is found (Table 4) predominantly with the high and low molecular weight fractions which together comprise about 38% of the unfractionated heparin.

Table 4. Mean percentage change, over 5 h, in anti-Xa activity of heparin fractions in autoclaved and unautoclaved dextrose solutions.

Heparin fraction	Mean percentage change in anti-Xa activity over 5 h ±95% confidence limits		
	A + H	UA + H	Δ
HMW	+15·5 ± 9·1	−15·1 ± 7·1	30·6 ± 12·3
MMW	+8·8 ± 6·9	+1·8 ± 4·6	7·0 ± 4·6
LMW	+10·0 ± 6·7	−9·0 ± 5·8	19·0 ± 3·3
Unfractionated	+8·0 ± 2·3	−5·6 ± 2·0	13·7 ± 3·3

A + H, UA + H, Δ as in Table 1: 10% w/v dextrose solutions; significance of differences of Δ: HMW–MMW, $P < 0{\cdot}01$; MMW–LMW, $P < 0{\cdot}01$; HMW–LMW, $P = 0{\cdot}05$–$0{\cdot}1$; numbers of determinations, 10, 8, 4 for HMW, MMW, LMW respectively.

The dextrose effect for HMW appears larger than that for LMW even though significance of the difference is barely established for the number of results available for LMW. The dextrose effect for the unfractionated heparin differed significantly from that of HMW ($P < 0{\cdot}001$) and MMW ($P = 0{\cdot}02$–$0{\cdot}05$) but not from that of LMW ($P > 0{\cdot}1$).

5-HMF

5-HMF is one of the principal by-products of dextrose heat-degradation in the autoclave. Freshly autoclaved 5% w/v dextrose solutions contained $0{\cdot}89 \pm 0{\cdot}16 \cdot 10^{-4}$ M 5-HMF whereas freshly autoclaved 10% w/v dextrose solutions contained $2{\cdot}16 \cdot 10^{-4}$ M 5-HMF (mean of two determinations) and the rises in anti-Xa activity in autoclaved 5 and 10% w/v dextrose solutions were not significantly different.

Addition of $0{\cdot}79$–$7{\cdot}9 \cdot 10^{-4}$ M 5-HMF to unautoclaved dextrose solution did not produce the raised anti-Xa activity which was consistently found in autoclaved dextrose solutions, and is unlikely to be the cause of the rise in activity.

Concentrations of 5-HMF and the by-product named P_1 by Taylor et al (1971) in proprietary dextrose solutions varied, particularly as between glass and plastic containers and showed no correlation with mean percentage change in anti-Xa activity over 5 h observed when heparin was added to these solutions.

DISCUSSION

Variables contributing to conflicting comment on heparin stability have included the source and concentration of heparins used, the chosen anticoagulant test, pH, ionic strength, the source and container of the dextrose solution and the duration of the stability study (Jacobs et al 1973; Okuno & Nelson 1975; Mitchell et al 1976; Raper & Johnson 1976; Joy et al 1979; Anderson et al 1977, 1979; Goodall et al 1980). There is no evidence for the degradation of heparin, in sterile solutions of dextrose at pH 5–7, maintained at up to 37 °C over 48 h, but explanation of 'instability', or rather ex vivo anticoagulant activity which fluctuates in response to such variables, is necessary.

Reversible decrease of activity of heparin (Okuno & Nelson 1975; Anderson et al 1977, 1979) in dextrose solution could correspond to reversible conformational changes in the interacting polyelectrolyte molecules caused by heparin-dextrose association in the anticoagulant tests which are effectively protease-modifying assays with a plasma clotting end point. Investigations of such systems must consider the factors that affect the behaviour of polyelectrolytes of which heparins are complex examples. For example, heparin-dextrose solutions are diluted, probably by various solutions (including plasma prepared in a variety of ways) in order to use the different clotting tests which are essentially complex polyelectrolyte systems in which interactions are pH-, concentration-, and salt-dependent.

The dextrose effect. Dextrose solution for infusion is autoclaved and hence contains small quantities of 5-HMF and certain acids. After autoclaving, the composition continues to change but the process is incompletely understood (Taylor et al 1971; Sturgeon et al 1980). Freshly autoclaved dextrose solutions were used to achieve constancy of composition; unautoclaved dextrose solutions were also used because at least 95% unchanged dextrose is present in autoclaved dextrose solution in addition to the by-products and it is desirable to discriminate

between the influence on heparin activity of dextrose and its by-products.

Reversible reduction of heparin activity in unautoclaved dextrose solution (Fig. 1) rules out destruction of heparin. Reversible conformational change in heparin molecules brought about by association with dextrose is a possible explanation. The rise in activity in the acidic autoclaved dextrose solution (Fig. 1) is associated with the autoclaving by-products of dextrose in presence of dextrose. The difference between the lowered and raised activities is called the dextrose effect on the basis that the effect is initiated by dextrose and modified in the autoclaved preparation by the by-products produced by the treatment.

pH and salt. Sodium heparin (30 iu ml^{-1}) raises the pH of autoclaved dextrose solution from about 4 to 6 and that of unautoclaved dextrose solution from about 6 to 7. Calcium heparin also raises the pH in both types of dextrose solution but to one unit less than the sodium heparins. Thus calcium heparin in unautoclaved dextrose solution has approximately the same acidic pH as sodium heparin in autoclaved dextrose solution (Table 3), but the calcium heparin preparation shows a fall in activity, and the sodium heparin preparation a rise. Further, by increasing the sodium heparin concentration to 100 iu ml^{-1} in autoclaved dextrose solution, a pH of 7·04 is attained but there is still a rise in activity. Hence pH by itself cannot be the determining factor in change of activity.

Regarding salt, several facts may be considered. First, NaCl tends to eliminate the dextrose effect, possibly by preventing dextrose-heparin association or any resulting conformational changes. Second, neutralization of autoclaved dextrose solution of heparin abolished the rise in activity and, in view of the evidence against a primary role for pH by itself, this abolition was probably due to salt formed during neutralization. Third, chromatographic fractions of heparin which were not desalted after fractionation failed to show the dextrose effect. These facts can be related to theory. In the absence of added electrolyte, heparin chains will tend to adopt extended forms, a tendency which could be accentuated by a non-ionic substance like dextrose. This could in turn decrease the chance of specific interaction of heparin with the active site in antithrombin III on which heparin anticoagulant activity depends, resulting in decreased activity. Freshly autoclaved dextrose solutions contain mainly dextrose but also some acidic by-products which compete for available heparin counterions. Another type of heparin conformational change, possibly folding, could follow this ionic disturbance resulting in increased availability of the relatively short dodecasaccharide chain segments with which the anticoagulant activity of heparin is associated (Laurent et al 1978). Electrostatic interaction between heparin and antithrombin III will occur so long as the prevailing pH promotes ionization of the groups involved, and within certain limits of salt concentration.

Hence the role of pH in the dextrose effect is generally permissive whilst dextrose, added salt, and acidic autoclaving dextrose by-products have more active roles possibly through influence on heparin conformation.

The viscosity results support the suggestion of differences in heparin interaction in the autoclaved and unautoclaved dextrose solutions.

Anticoagulant tests and concentration. Two anticoagulant tests based on a common clotting factor, Xa, have been shown (Anderson & Harthill 1981) to give different results for a single heparin and either of these tests, amongst others, has in the past been selected arbitrarily to investigate heparin stability in dextrose solution. It was considered desirable to limit the investigation to two factor Xa tests since factor Xa is a principal location of action of heparin in the clotting sequence and since they differ in a known and limited way thus allowing a certain degree of comparison of the results.

The three heparins at the higher concentration of 30 iu ml^{-1} in dextrose solutions showed dextrose effects by both methods. However, only the DB method revealed (Table 2) increased activity at low concentration (<5 iu ml^{-1}) in unautoclaved dextrose solution in which decreased activity was found at the higher concentration by both methods. One important conclusion is that arbitrary choices of method (even of similar type like factor Xa methods), can lead to apparently conflicting results. The Y and DB methods differ in concentration of components, particularly calcium ion, known to behave as a condensed counterion for heparin (Braud et al 1980) with special affinity for the L-iduronic acid carboxyls and hence of importance where heparin-protein interaction underlies the effect being measured. Another conclusion is that since an identical effect (rise in activity) may be accompanied by either neutral or acidic pH, which is determined by heparin concentration, concentration is a dominant factor in the dextrose effect. Hitherto, choice of concentration appears to have been arbitrary.

Preliminary studies with the thrombin time test (Michalski et al 1978) and the peptide substrate

method of Teien et al (1976) gave variable results for heparin activity in dextrose solutions and detailed studies would be required to characterize activity changes using these methods.

Heparin fractions. Pharmacopoeial heparins are mixtures of related molecules which vary with source and preparation method and amongst which anticoagulant activity is not necessarily uniformly distributed hence the observed difference in dextrose effect in molecular weight fractions (Table 4) is important and could have contributed to reported discrepancies in heparin stability studies in which heparins from different sources have been used. The dextrose effect is given principally by the tails of the molecular weight distribution, the parts most likely to vary between different heparin preparations. The HMW fraction gives the most pronounced effect and the larger molecules would be the most likely to undergo conformational changes which could be the basis of the dextrose effect.

Proprietary dextrose solutions. Dextrose solutions for infusion have been autoclaved and are often of proprietary origin. The plastic or glass container may leach substances into, or adsorb autoclaving by-products from, the solution, which could influence heparin interaction in the anticoagulant test system. Our variable results with such solutions, considered with the complex effects now demonstrated using freshly made solutions not accompanied by container effects, clearly suggest that uniform results are not likely to be obtained when proprietary dextrose solutions are used and continued study was not undertaken.

Conclusion. The present work shows that the 'stability' of heparin in dextrose solutions essentially reflects its behaviour as a polyelectrolyte, and when introduced into the blood circulation, more powerful interactions will replace the dextrose association which gives rise to the ex vivo result. Hence difference in clinical effect between infusions of heparin in different fluids could only be determined in clinical study.

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