

Anticoagulant activity of heparin in intravenous fluids

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SYNOPSIS The anticoagulant activity of heparin dissolved in intravenous solutions was measured by two different methods of heparin assay. Both procedures showed markedly reduced anticoagulant activity within four hours after the addition of heparin to the solutions. When measured according to the procedure of Yin, heparin in the intravenous solutions fully regained its lost anticoagulant activity after 24 hours at room temperature. When measured by the thrombin time, however, the heparin anticoagulant activity remained reduced. The source of heparin, from either the lung or intestine, does not explain the reduction in anticoagulant activity. Although its cause is unknown, the erratic behaviour of heparin in intravenous solutions stresses the importance of a laboratory monitor of heparin therapy.

While preparing the standard of heparin concentrations, one of us noted that, after a few hours at room temperature, heparin lost some of its anticoagulant activity. Because an increasing number of patients receive heparin in intravenous solutions for various coagulation disorders, we studied the stability of heparin added to various intravenous solutions using a recently introduced heparin assay method.

Material and Methods

Heparin assay was performed according to the procedure of Yin *et al* (1973). Trizmal buffer, cephalin in anticoagulant-free bovine plasma, and activated factor X stabilized in bovine serum were supplied by Sigma Co. (St. Louis, Mo). Heparin assay by thrombin time was done according to the procedure of Quick (1966). Thrombin was obtained from Parke-Davis (Detroit, Mich). Solutions of Ringer's lactate (Hartmann's), normal saline, 5% dextrose, 10% dextrose, and 2½% dextrose in water were obtained from McGraw Laboratories (Mill-edgeville, Ga).

Sodium heparin (Upjohn Co., Kalamazoo, Mich and Medical Chemicals, Melrose Park, Ill) was dissolved in 250 to 500 ml intravenous fluids at a final concentration of 10 u/ml. Sodium heparin from Upjohn is prepared from beef lung and that

from Medical Chemicals is from pig intestine mucosa. Heparin concentrations were measured by both heparin assay procedures at 1 minute, 2 hours, 4 hours, 6 hours, and 24 hours after addition of sodium heparin. As a standard, heparin solution of 10 000 u/ml was diluted with normal saline. To ensure that the heparin assay system were maintained constant throughout the experiment, a small amount of heparin was drawn aseptically from the same vial of the standard heparin solution at each determination. Standardization of heparin was carried out three times to minimize any effects caused by dilution of the standard. To demonstrate further that dilution of the standard solution was kept within a reasonable range, the process was repeated ten times from the same vial of heparin solution. All of the intravenous solutions were stored at room temperature. All the experiments were repeated six times on different days.

Results

The results of heparin assay according to the procedures of Yin are shown in table I, and those by the thrombin time of Quick in table II. To demonstrate that the variation caused by dilution of standard heparin is minimal, the process of dilution was repeated ten times from the same vial of standard heparin. Minimal variation was observed (table V). All the data were a mean of six experiments on different

	Dextrose 2.5%	Dextrose 5%	Dextrose 10%	Normal Saline	Ringer's Lactate
Immediately after heparin	10.01 ± 0.11	10.02 ± 0.10	9.97 ± 0.12	10.03 ± 0.13	10.02 ± 0.10
2 hours	7.46 ± 2.00	7.38 ± 2.27	9.67 ± 2.98	6.62 ± 2.98	6.34 ± 2.44
4 hours	6.66 ± 2.70	4.54 ± 1.11	7.50 ± 3.07	5.84 ± 2.14	5.34 ± 2.43
6 hours	5.20 ± 2.44	4.88 ± 2.98	6.10 ± 4.78	4.55 ± 2.25	4.08 ± 3.46
24 hours	9.46 ± 0.65	10.15 ± 1.12	8.42 ± 1.94	8.75 ± 2.63	7.90 ± 2.59

Table I Anticoagulant activity of heparin (u/ml) according to the procedure of Yin

	Dextrose 2.5%	Dextrose 5%	Dextrose 10%	Normal Saline	Ringer's Lactate
Before heparin	9.57 ± 0.06	9.55 ± 0.06	9.58 ± 0.06	9.58 ± 0.06	9.50 ± 0.06
Immediately after	22.10 ± 1.21	22.10 ± 0.54	23.30 ± 0.46	22.00 ± 1.44	22.08 ± 1.43
2 hours	18.45 ± 0.34	22.20 ± 1.47	20.50 ± 2.16	18.10 ± 0.44	17.05 ± 0.12
6 hours	11.08 ± 0.12	15.58 ± 0.18	15.28 ± 0.06	16.10 ± 0.14	10.54 ± 0.18
24 hours	10.58 ± 0.06	16.40 ± 0.13	18.05 ± 0.27	17.18 ± 0.13	10.55 ± 0.06

Table II Anticoagulant activity of heparin (seconds) by the thrombin time of Quick

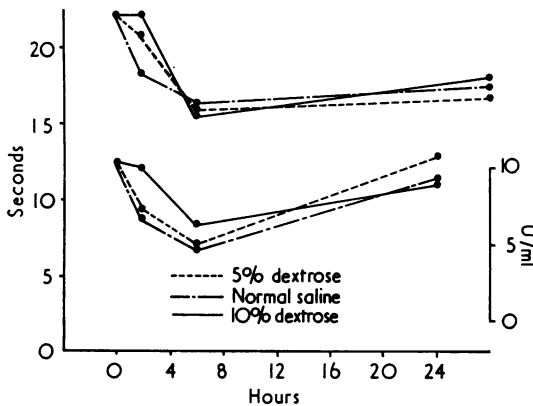


Figure Anticoagulant activity of heparin in intravenous solutions measured by the thrombin time (upper trace) and by the procedure of Yin (lower trace).

days and their standard deviations. Heparin quickly lost its anticoagulant activity within two hours in dextrose, normal saline, and Ringer's lactate solutions. The loss of heparin activity was demonstrated

	Heparin (u/ml)	Thrombin Time (sec)
Without heparin	0	5.9 ± 0.8
1 min	10.0 ± 0.0	26.5 ± 0.6
2 hours	7.8 ± 0.7	26.8 ± 1.0
4 hours	8.5 ± 0.9	26.0 ± 1.2
6 hours	8.7 ± 0.8	26.8 ± 0.7
24 hours	10.0 ± 0.2	26.8 ± 1.4

Table III Heparin assay and thrombin time in 500 ml distilled water

in assays by both procedures. The activity of heparin reached its nadir at six hours. The pH of the solutions remained stable throughout the experiment, ranging from 5.5 to 6.9. The results of assay of anticoagulant activity of heparin in 500 ml distilled water are shown in table III. A loss of anticoagulant activity is observed with the procedure of Yin, while the thrombin time remains unchanged. Table IV shows the anticoagulant activities of heparin from two different sources measured by the thrombin time in normal saline solution. No difference between the two preparations is demonstrated. Similar results are obtained in 2½%, 5%, and 10% dextrose and Ringer's lactate solution.

	A	B
Before heparin	10.03 ± 0.00	9.90 ± 0.10
Immediately after	21.67 ± 1.70	22.33 ± 1.70
2 hours	18.67 ± 0.48	17.67 ± 0.62
6 hours	16.27 ± 0.20	16.07 ± 0.24
24 hours	17.40 ± 0.28	17.20 ± 0.00

Table IV Anticoagulant activity of heparin from lung preparation (A) and intestine preparation (B) measured by the thrombin time of Quick (seconds)

	Seconds	Units/ml
Mean	57.06	9.71
Range	54.0-58.8	9.2-10.0
Standard deviation	2.08	0.36
Coefficient variation (%)	3.65	3.70

Table V Variation of heparin standard according to the process of dilution (n = 10)

Discussion

Heparin is often added to intravenous fluids and thus is continuously infused for as long as 24 hours. This method of administration is generally regarded as obtaining a stable anticoagulant activity throughout, once the infusion rate has been adjusted (Genton, 1974). It is known that the dose of heparin needed is related to body weight and is influenced by the condition of the patient, such as shock, fever, hepatic function, and the presence of active thrombosis. However, little is known about the effect of intravenous fluids on the anticoagulant activity of heparin. The literature is not only scarce but is conflicting (Stock and Warner, 1971; Jacobs *et al*, 1973).

The conflicting reports on the stability of heparin in intravenous fluids appear, in part, to result from the rather insensitive and inaccurate heparin assay methods that the studies were based upon; all these studies used the thrombin time or its modification. This method lacks sensitivity and specificity, especially at low heparin levels (Yin *et al*, 1973). The anticoagulant activity of heparin is complex. It is generally thought that heparin enhances an alpha-2 globulin molecule in plasma, which is also known as the plasma inhibitor. The plasma inhibitor interferes with thrombin and therefore blocks the conversion of fibrinogen to fibrin (Genton, 1974). Recently the inhibitor has been reported to serve as an inhibitor of activated factor X and thereby to interfere with the conversion of prothrombin to thrombin (Yin *et al*, 1973). Based on the latter mechanism, a new heparin assay method was introduced by Yin *et al*. This measures submicrograms of heparin with a high reproducibility.

Using this procedure, our study showed an initial loss of heparin activity in intravenous fluids; activity was fully recovered after 24 hours. This pattern of loss of anticoagulant activity was seen in all the fluids tested—2½% dextrose, 5% dextrose, 10% dextrose, normal saline, and Ringer's lactate solutions. This is in sharp contrast to the study of Jacobs *et al* who, using a modified thrombin time, found that heparin in normal saline did not lose its anticoagulant activity for 24 hours. Although they observed a substantial loss of heparin activity in other intravenous fluids (4.3% dextrose with 0.18% sodium chloride, lactate, and 5% dextrose), they failed to detect the rebound of anticoagulant activity after 24 hours. Our studies, using the thrombin time, also failed to show a full recovery of heparin anticoagulant activity in 24 hours. The heparin assay used in their study is rather insensitive. The moderate insensitivity of heparin assay based

on the thrombin time is also demonstrated in table III. In this table an experiment was done using 500 ml distilled water added to heparin. While the procedure of Yin detected a moderate loss of anticoagulant activity, the thrombin time remained unchanged throughout the experiment. Chessells *et al* (1972) using a within-patient cross-over trial to compare 5% dextrose and 5% sorbitol as diluents for heparin, demonstrated no detectable loss of heparin activity in either solution. The *in vivo* study of Chessells *et al* may not prove or disprove the results of the *in vitro* studies, because of accumulative anticoagulant activity in the body, which may obviate the *in vitro* determination, and because of its *in vivo* metabolism which is relatively unknown (Genton, 1974). Furthermore, the partial thromboplastin time used in their study may not accurately reflect the anticoagulant activity produced by heparin. The inaccuracy of partial thromboplastin time for heparin control has been reported recently (Congdon *et al*, 1973); (Pitney *et al*, 1970). A low pH was also stated to influence the anticoagulant activity of heparin (Stock and Warner, 1971). Our study, however, showed a fairly stable pH of the intravenous fluids throughout the experiments and none of the fluids was below pH 3, a critical level below which the anticoagulant activity of heparin is reportedly inhibited.

The reason for a temporary loss of heparin activity is not known and further study is needed. Several reasons are conceivable: heparin transiently alters its molecule in fluids; heparin is insufficiently dissolved in solutions; the fluids interfere with the plasma inhibitor or they influence the conversion of various coagulation stages. Whatever the cause, such capricious behaviour of heparin in fluids stresses the importance of a regular heparin monitor in patients undergoing heparin therapy, in contrast to the opinion of those who prefer a selected schedule of heparin administration which requires no laboratory control (Bauer, 1964).

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