Heparin stability: effects of diluent, heparin activity, container, and pH

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SUMMARY   The effects have been studied of diluent, heparin activity after dilution, container, and pH on the stability of heparin solutions stored under conditions resembling those present during heparin infusion by intravenous drip or syringe pump. Heparin activity was measured by activated partial thromboplastin time and thrombin clotting time (and, in one set of studies, also by factor Xa inhibitor assay and protamine sulphate neutralisation).

Heparin activity was stable for 6 hours regardless of storage conditions. After 24 hours heparin activity was stable when the drug was diluted in 0·9 % saline and stored in plastic, but a small loss of activity was observed in several studies after dilution in 5 % dextrose or storage in glass. A more extensive comparison confirmed a 3-5 % loss in heparin activity over 24 hours after dilution in 5 % dextrose. Changing the pH to 3·5 or 10·0 had little effect on storage stability.

We conclude that heparin activity *in vitro* remains stable during short infusions but recommend dilution in 0·9 % saline and a plastic container when a heparin solution is infused over 24 hours.

Heparin is now commonly given by continuous intravenous infusion rather than by intermittent injection. For this mode of administration heparin is diluted in one of several intravenous infusion fluids and may then remain at room temperature in the infusion bag, bottle, or syringe for 8-24 hours. It is, therefore, important to know if its anticoagulant activity remains stable *in vitro* for this time.

This question has been repeatedly investigated with varying results (Table). Thus, it has been reported that heparin activity is constant for 24 hours regardless of the diluting fluid or its pH over a wide range,[1-4] or that heparin activity is reduced by 40-50% within 1 hour of dilution except in 0·9% sodium chloride,[5] or that heparin activity is reduced by 50-60% after 4-6 hours in all diluents but then increases again with further storage.[6]

Because of these contradictory and sometimes confusing reports about heparin stability *in vitro* we decided to re-investigate the question.

Further, because there is disagreement about the most appropriate test of heparin effect, we decided to measure heparin stability *in vitro* using four tests: the

activated partial thromboplastin time (APTT), the thrombin clotting time (TCT), factor Xa inhibitory activity (Xa time), and protamine sulphate neutralisation.

Methods

STUDY DESIGN

Sodium heparin was added to 500 ml 0·9% saline or 5% dextrose, supplied commercially for intravenous use in plastic bags or glass bottles, to give a heparin activity of 10, 20, or 40 IU/ml, and to 50 ml 0·9% saline or 5% dextrose drawn into a plastic syringe to give a heparin activity of 400 or 1000 IU/ml. These conditions were chosen to mimic those obtaining during heparin infusion by intravenous drip or syringe pump. Heparin activity was assayed immediately and after 3, 6, and 24 hours of room temperature storage. In addition, heparin stability was measured during storage at varying pH.

HEPARIN AND INTRAVENOUS FLUIDS

Mucous heparin BP (1000 or 5000 IU/ml) was obtained from Allen and Hanbury's Division of Glaxo Australia Pty Ltd (Boronia, Victoria). Intra-

Previously reported changes in heparin activity after dilution and storage

Authors	Heparin activity (U/ml)	Storage (hours)	Diluting fluid	pH	Assay	Conclusions
Stock and Warner (1971)[1]	964 200 40	24	5% Dextrose	7·0 6·8 6·6	Not described	No loss of activity
Hodby et al. (1972)[2]	32	24	0·9% Sodium chloride 5% Dextrose Dextrose saline 0·9% Sodium chloride 0·9% Sodium chloride	6·9 7·2 6·9 3·2 6·9	APTT	No loss of activity
Mitchell et al. (1976)[3]	20, 40	48	Normal saline 5% Dextrose	5·2-7·0 5·5-6·8	TCT	No loss of activity
Joy et al. (1979)[4]	20	24	5% Dextrose 0·45% Saline 5% Dextrose	2·0-9·0	TCT	No loss of activity
Jacobs et al. (1973)[5]	20	24	Normal saline 5% Dextrose Saline/Dextrose Ringer lactate Saline lactate	6·8 3·9 3·9 6·3 6·3	Modified TCT	No loss of activity ≃ 50% loss of activity ≃ 40% loss of activity ≃ 50% loss of activity ≃ 50% loss of activity
Okuno and Nelson (1975)[6]	10	24	Normal saline 5% Dextrose 10% Dextrose 2½% Dextrose Hartmann's solution	5·5-6·9	Xa inhibitor TCT	Marked loss of activity within 4 hours, then complete recovery by Xa inhibitor assay but not by TCT

venous fluids were obtained in plastic bags from Travenol Laboratories Pty Ltd (Sydney) and in glass bottles from Abbott Laboratories Pty Ltd (Sydney). Plastic syringes were supplied by Terumo Australia Pty Ltd (Melbourne).

In some experiments the pH of 0·9% saline and 5% dextrose was adjusted to 3·5 or 10·0 with 0·1 N HCl or 0·1 N NaOH before the addition of heparin.

HEPARIN ACTIVITY MEASUREMENTS

Heparin solutions were subsampled into pooled normal platelet poor plasma for the measurement of heparin activity after each storage interval. After a preliminary dilution, 10 or 20 μl of heparin solution were added to 1·0 ml plasma to yield a calculated final plasma activity of 0·2 IU/ml (assuming no change in activity with storage). All tests were done in duplicate at least five times by two workers using coded samples. Samples were stored on melting ice until tests had been done.

To prepare the normal plasma, blood was collected from volunteer blood donors into 3·8% tri-sodium citrate (9 parts blood:1 volume citrate) and centrifuged at 2000 g for 30 minutes at 4°C. Pooled normal plasma from 20 donors was then stored in small aliquots at −70°C. The same batch of pooled normal plasma was used for each set of studies.

Heparin activity was measured with the APTT and the TCT in all studies, and, in one series of experiments, heparin activity was also measured by a factor Xa inhibitor method (Xa time)[7] and by protamine sulphate neutralisation.[8] All tests were done using a clot-detecting device (Clotek, Hyland), except for the

Xa time, which was measured using a manual (hook) technique.

The observed clotting times for each test were converted to heparin activity (IU/ml) by the use of standard heparin sensitivity curves for each test (see below, and Figs 1-3).

Automated APTT reagent was obtained from General Diagnostics (Morris Plains, New Jersey) and used according to the manufacturer's instructions. The TCT was measured after diluting Topical
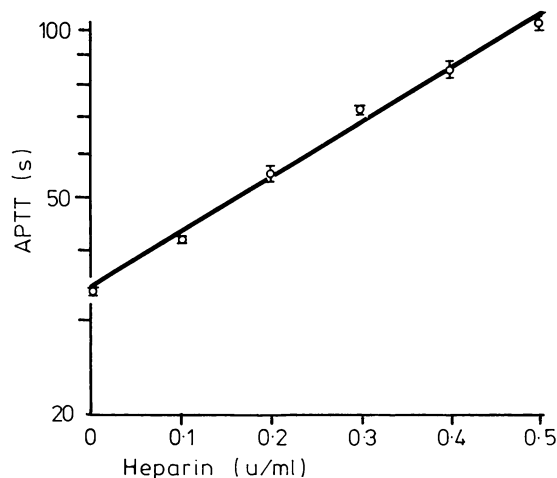

Fig. 1 *Heparin sensitivity of APTT. The mean APTT ± SEM is plotted for each heparin activity tested. The line of best fit was derived by least squares analysis.*
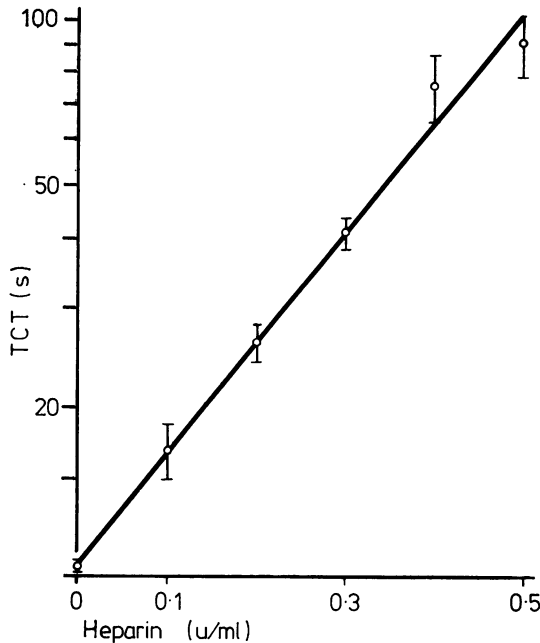
Fig. 2 *Heparin sensitivity of TCT. The mean TCT ±
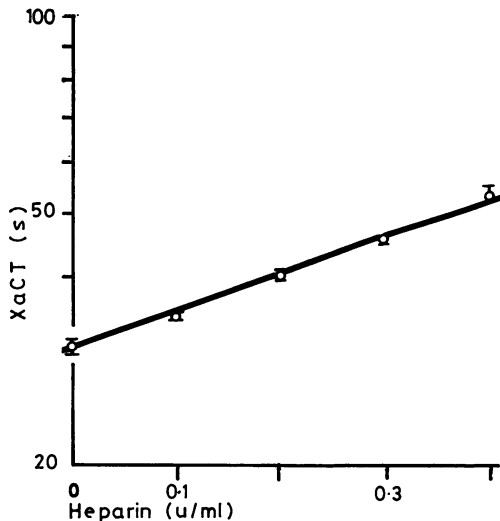SEM is plotted for each activity tested.*

Fig. 3 *Heparin sensitivity of factor Xa inhibitor assay
(XaCT). The mean XaCT ± SEM is plotted for each
activity tested.*

Thrombin (Parke Davis, Detroit, Michigan) to an
activity of 5 units/ml in 0·1 M calcium chloride and
adding 0·1 ml of this reagent to 0·2 ml platelet poor
plasma.

The Xa time was measured using the Deci Unit
range of method A of Yin *et al.*[7] Reagents for this
test were obtained from Sigma Chemicals Co (St
Louis, Mo).

Protamine sulphate neutralisation was done as
described by Refn and Vestergaard[8] using dilutions
of protamine sulphate (10 mg/ml) supplied by Boots
Co Ltd (Nottingham, England).

HEPARIN SENSITIVITY CURVES AND HEPARIN RECOVERY FROM PLASMA

The sensitivities of the APTT, TCT, and Xa CT to
heparin are shown in Figures 1-3. These curves were
derived by adding appropriate dilutions of the
heparin batch used in the stability studies to pooled
normal platelet poor plasma and measuring clotting
times in duplicate on five separate occasions.

To check heparin recovery from the pooled
normal plasma used in these studies, protamine
sulphate was used to neutralise a crystalline porcine
sodium mucous heparin (169 IU/mg) obtained by
courtesy of Dr Peter Forsell, Allen and Hanbury's,
Boronia, Victoria. 100 IU of this heparin was
neutralised by 1·045 mg of protamine sulphate. When
0·2 IU/ml of this standard heparin was added to
pooled normal plasma, 0·19 IU/ml could be
measured by protamine sulphate neutralisation, in-
dicating substantially complete recovery.

STATISTICAL METHODS

Lines of best fit for heparin activity-test response
curves were derived by the method of least squares.
Change of heparin activity with time was tested by
paired *t* test and analysis of variance. All results in
text and figures are expressed as mean ± standard
error of mean (SEM).

Results

HEPARIN RECOVERY

At zero time, all heparin solutions showed the pre-
dicted heparin activity with all test systems used, so
that subsequent results were expressed as a percent
of the initial measured activity.

PLASTIC CONTAINERS

There was no significant loss of activity when
heparin was diluted in 0·9% saline and stored for 24
hours in plastic containers (bag or syringe) (Figs 4,
5, 7). This occurred regardless of the heparin dilu-
tion, the storage pH, and whether heparin activity
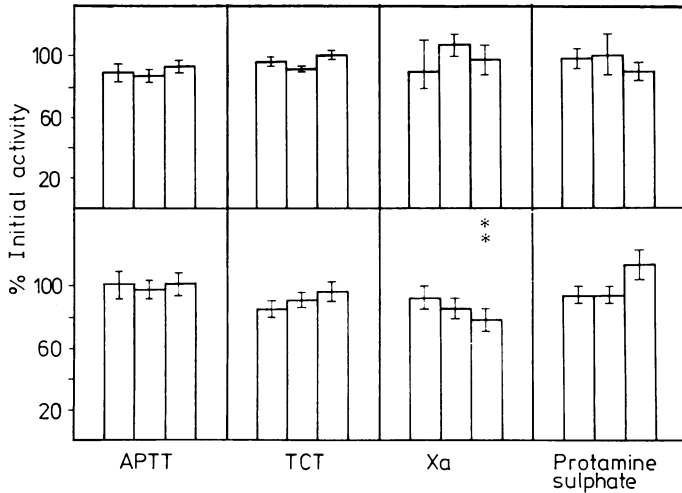was measured by APTT, TCT, or (with the 10 IU/ml

Fig. 4 *Heparin stability tested by APTT, TCT, Xa time (Xa), and protamine sulphate neutralisation after heparin was diluted to 10 IU/ml and stored in commercial solutions of 0·9% saline (above) or 5% dextrose (below) sold for intravenous use in plastic bags. Bars represent percent of initial activity ± SEM after 3, 6, and 24 hours' storage. **P <0·01 when activity after storage was compared with initial activity.*
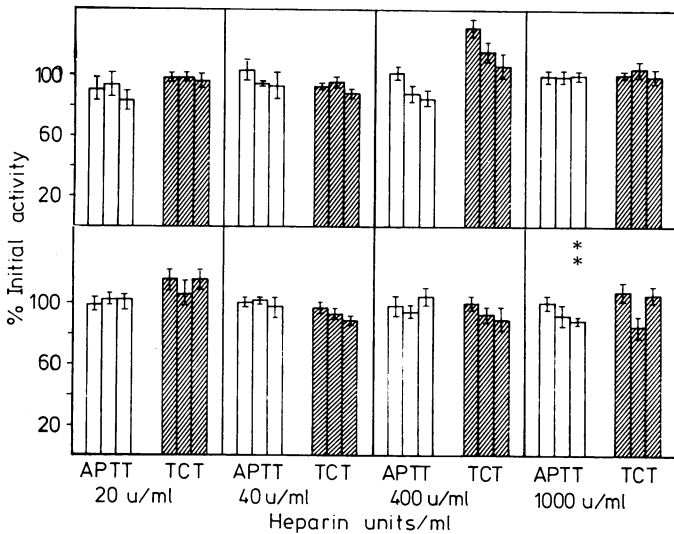

Fig. 5 *Heparin stability tested by APTT (clear bars) and TCT (shaded bars) after dilution to 20 IU/ml, 40 IU/ml, 400 IU/ml, and 1000 IU/ml in 0·9% saline (above) or 5% dextrose (below), and storage for 3, 6, and 24 hours in plastic bags (20 IU/ml and 40 IU/ml) or plastic syringes. Bars represent percent of initial activity ± SEM. *P <0·05 **P <0·01 (compared with initial activity).*

solution) by Xa time or protamine sulphate neutralisation. Similarly, there was at most a small loss of activity when heparin was diluted in 5% dextrose solution and kept in plastic containers. There was a statistically significant decrease in activity after 24 hours when the 10 IU/ml solution was tested by Xa time (Fig. 4), the 40 IU/ml solution was tested by TCT (Fig. 5), the 1000 IU/ml solution was tested by APTT (Fig. 5), and the pH 10, 20 IU/ml solution was tested by APTT (Fig. 7), but the apparent loss of activity was small: 9-11% in all cases except for a 23% loss of activity measured by Xa time.

GLASS BOTTLES
In several experiments a statistically significant 10-14% loss of activity was recorded after 24 hours' storage in glass bottles. This was seen when activity was measured by APTT (10 IU/ml, 0·9% saline) or TCT (40 IU/ml, 0·9% saline; 20 IU/ml, 40 IU/ml, 5% dextrose) (Fig. 6).

CONFIRMATORY STUDIES
In a second series of studies, 10 IU/ml heparin was stored for 24 hours in 20 500-ml plastic bags of 0·9% saline and 20 500-ml bags of 5% dextrose, and
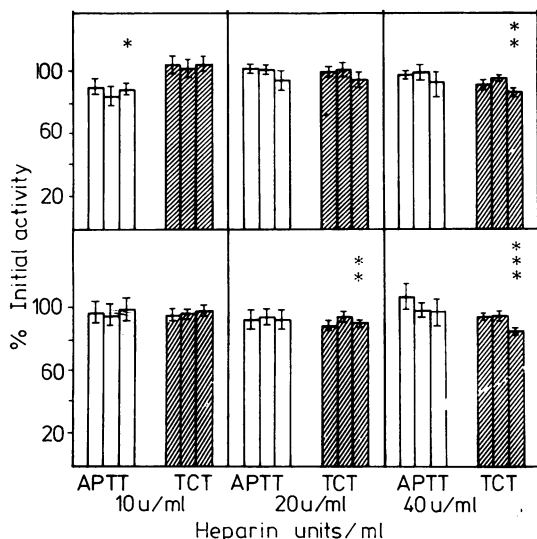
Fig. 6 *Heparin stability tested by APTT* (clear bars) *and TCT* (shaded bars) *after dilution to 10 IU/ml, 20 IU/ml, and 40 IU/ml in commercial solutions of 0·9% saline* (above) *or 5% dextrose* (below) *sold for intravenous use in glass bottles. Bars represent percent of initial activity ± SEM after 3, 6, and 24 hours' storage.*
*P <0·05 **P <0·01 ***P <0·001 (compared with initial activity).

Fig. 7 *Effect of pH 3·5 and pH 10 on heparin stability tested by APTT* (clear bars) *and TCT* (shaded bars) *6 and 24 hours after dilution of heparin to 20 IU/ml in 0·9% saline* (above) *or 5% dextrose* (below) *sold for intravenous use in plastic bags. Bars represent percent of initial activity ± SEM *P <0·05.*

heparin activity was measured by APTT and TCT at zero time and after 24 hours.

There was no change of heparin activity in 0·9% saline (24-hour APTT = 99·9 ± 0·6%; 24-hour TCT = 100·3 ± 0·6% of initial activity), but a very small though statistically significant decrease of heparin activity in 5% dextrose (24-hour APTT = 97·3 ± 0·9%, 24-hour TCT = 95·1 ± 1·3% of initial activity; P <0·01 for both observations).

Discussion

Our results suggest that there is no loss of heparin activity when heparin is diluted in 0·9% saline and stored in plastic for 24 hours at room temperature. Heparin also appears to be stable for 6 hours under other storage conditions, but a small loss was observed in some studies after 24 hours when the drug was diluted in 5% dextrose or stored in glass; this loss was no greater than 10-15% of initial activity. Further studies comparing heparin stability in 0·9% saline and 5% dextrose in plastic bags, using a larger sample of 20, confirmed that heparin is stable for 24 hours in 0·9% saline, but showed a 3-5% loss of activity in 5% dextrose. Any loss of activity associated with storage at the pH extremes
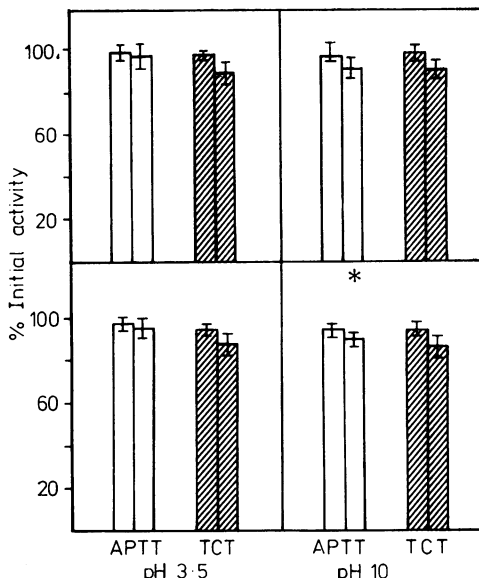
of 3·5 and 10·0 was also small.

These conclusions are similar to those of most previous reports[1-4] and are consistent with the stable anticoagulant response to a 12-hour infusion of heparin diluted in 5% dextrose observed by Chessels *et al.*[9]

Our results do not confirm the marked loss of heparin activity reported after dilution in 5% dextrose by Jacobs *et al.*,[5] and after dilution in 5% dextrose or 0·9% saline by Okuno and Nelson[6] (Table), and we are unable to explain these discrepancies. Our methods of measuring heparin activity differ from those used by Jacobs *et al.*[5] but are similar to those of Okuno and Nelson.[6]

We conclude that heparin activity *in vitro* remains stable during a short infusion, but, because of the small loss in activity after 24 hours when heparin is diluted in 5% dextrose or stored in glass, we recommend dilution in 0·9% saline and storage in plastic containers when a heparin solution is to be infused over 24 hours.

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