# The Effect of Dextran on the Lysability of Ex Vivo Thrombi

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**The lysability was determined of thrombi formed in Chandler tubes before and after infusion of 500 ml dextran 70 to patients undergoing cholecystectomy. $^{125}$I-labelled fibrinogen was given the day before operation. Following incubation of the thrombi in plasmin, radioactivity remaining in the thrombi and released to the supernatant was determined, as well as fibrinolytic degradation products in the supernatant, using an immunoelectrophoretic method. The dextran infusion was found to increase the radioactivity released from the thrombi to the supernatant from 15.5 ± 7.6% to a maximum of 27.3 ± 8.2% four hours after the infusion ($P<0.001$). A corresponding significant rise of the FDP concentration in the supernatant from 16.8 μg/ml to 44.1 μg/ml was found at the same time. After 24 hours the radioactivity had returned to initial values. The results indicate that dextran infused into patients during surgery increases the lysability of thrombi. It is suggested that this finding at least partly explains the antithrombotic effect of dextran.**

DEXTRAN with an average weight of 75 000 as well as 40 000 has been found to prevent thromboembolism both experimentally[6,13] and postoperatively in humans.[2,5,9,15,18] The antithrombotic effect has partly been ascribed to the decrease of platelet adhesiveness obtained after dextran administration,[4,8,11] partly to the flow improving properties of dextran.[15] Dextran has been shown to initiate fibrin precipitation.[1,19] The morphology of the precipitated fibrin differs from that of normal fibrin by being coarser and more variable in size than normal.[23,29] This has been suggested to make fibrin more easily dissolved by fibrinolytic agents.[23,29] The purpose of this study is to further elucidate the mechanisms of the antithrombotic effect of dextran by studying the lysability of ex vivo thrombi formed in the presence of dextran.

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## Material and Methods

Eighteen patients (14 women and four men) were studied before and after cholecystectomy. The day before operation they received an intravenous injection of fibrinogen labelled with $^{125}$I according to the method of McFarlane.[22] During the operation 500 ml of 6% dextran 70 in normal saline (Macrodex®, Pharmacia, Uppsala, Sweden) was infused in one hour to 12 of the patients. Six patients served as controls and did not receive dextran. Blood samples were collected before, immediately after and at 1, 2, 4 and 24 hours after the dextran infusion. Two ml of blood without anticoagulant was rotated in Chandler tubes[10] at 30 rpm for 1 hour. The thrombi formed were then removed from the loops into plastic tubes, centrifuged for 20 minutes, and after the addition of 2 ml isotonic saline or the same volume of saline containing 4.2 CTA units of plasmin (Novoplasmin®, Novo, Copenhagen), were incubated for 80 minutes at 37 C. After further centrifugation the radioactivity in the thrombus and the supernatant was determined in a γ-spectrometer (Wallac). In the supernatant the concentration of fibrinolytic degradation products (FDP) was also analysed using an immunoelectrophoretic method.[24]

The amount of FDP in simultaneously collected blood samples was determined in 12 patients using the same immunoelectrophoretic method. The factor XIII level in blood was determined in five patients in platelet-poor and platelet-rich plasma, using the method of Lorand et al.[20] modified according to Henriksson et al.[14] The dextran concentration was determined in seven patients with the anthron method.[16] The determinations were kindly performed by AB Pharmacia, Uppsala, Sweden.

To study the effect of dextran in vitro, blood from four

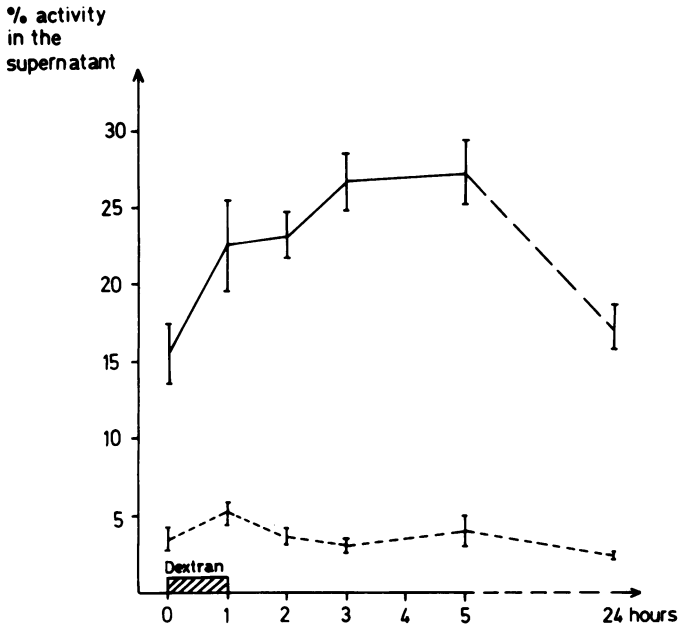


FIG. 1. Radioactivity in the supernatant as % of total activity after incubation with plasmin (——) or NaCl (- - -) before and after dextran infusion. Mean values and SE from 12 patients.

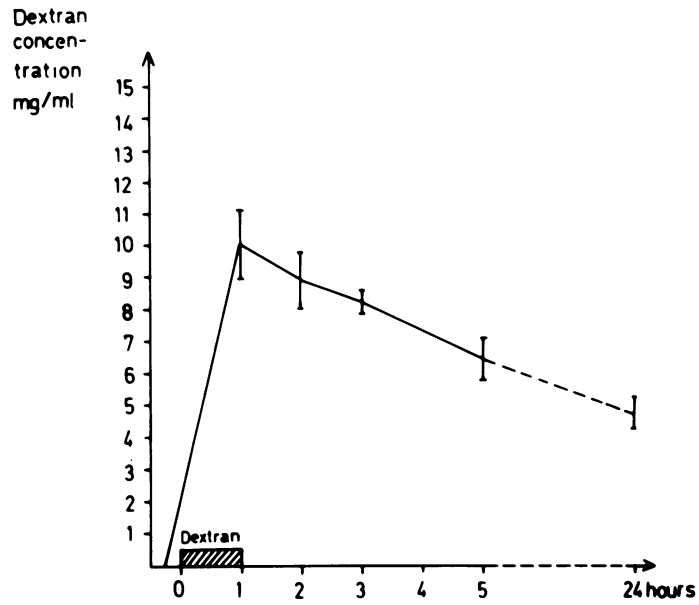


FIG. 3. Dextran concentration in plasma from patients receiving dextran infusion. Mean values and SD from seven patients.

patients labelled with $^{125}$I-fibrinogen was withdrawn. After addition of dextran to a final plasma concentration of 0.5–10%, the blood was rotated in a Chandler loop. The thrombi formed during 1 hour were treated as described above. After the operation all patients were examined for thrombosis using an isotope localisation monitor.[17]

## Results

In the patients receiving dextran the mean radioactivity in the supernatant after incubation with plasmin of the thrombi formed before dextran infusion was 15.5 ± 7.6% of the total activity (Fig. 1). Immediately after the infusion the activity increased to 22.7 ± 9.6% ($P<0.05$) and one hour later to 23.3 ± 5.9% ($P<0.01$). The maximal increase was obtained two hours after the end of the infusion (26.8 ± 7.1%, $P<0.001$). After four hours, the activity remained at the same level (27.3 ± 8.2%) and 24 hours after operation the activity had returned to the initial value (16.3 ± 5.2%). A corresponding rise of the FDP concentration in the supernatant from 16.8 μg/ml to a maximum of 44.1 μg/ml ($P<0.02$) at four hours after the end of the infusion was obtained.

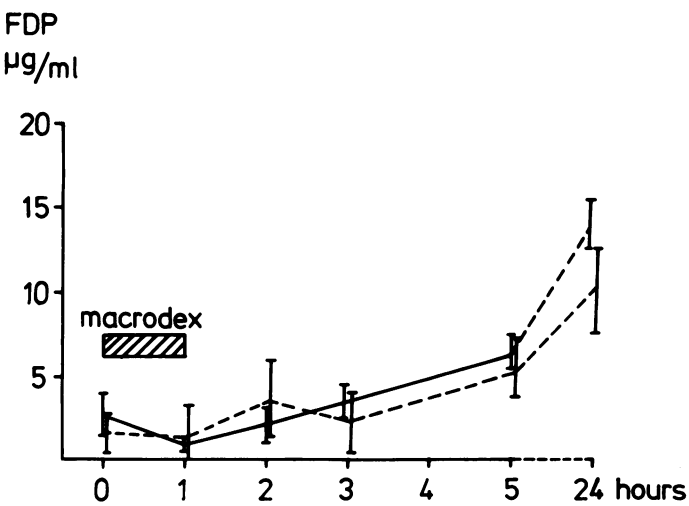


FIG. 2. FDP concentration in the blood from 12 patients receiving dextran (——) and in six control patients (- - -). (Mean ±SE).

If the thrombus was incubated in isotonic saline instead of plasmin, neither radioactivity nor FDP concentration in the supernatant increased (Fig. 1).

The FDP concentration in blood (Fig. 2) did not change during the infusion, but in the following four hours there was a successive increase from 0.8 to 6.3 μg/ml ($P<0.001$). The maximal FDP concentration in blood was found 24 hours after the end of the infusion (mean ± SD = 14.0 ± 5.1 μg/ml, $P<0.001$), followed in the next two days by a gradual decrease.

Factor XIII concentration did not vary significantly during and after the infusion neither in platelet-rich nor in platelet-poor plasma.

The dextran concentrations in plasma are shown in Fig. 3. The mean concentration was maximal at the end of infusion, (10.0 ± 1.0 mg/ml plasma) and then gradually decreased.

In control patients, not receiving dextran, there was no increased lysability after incubation of the thrombi with plasmin as indicated by the radioactivity in the supernatant (Fig. 4). There was no significant rise of the FDP in the supernatant. A moderate rise of FDP in the blood not

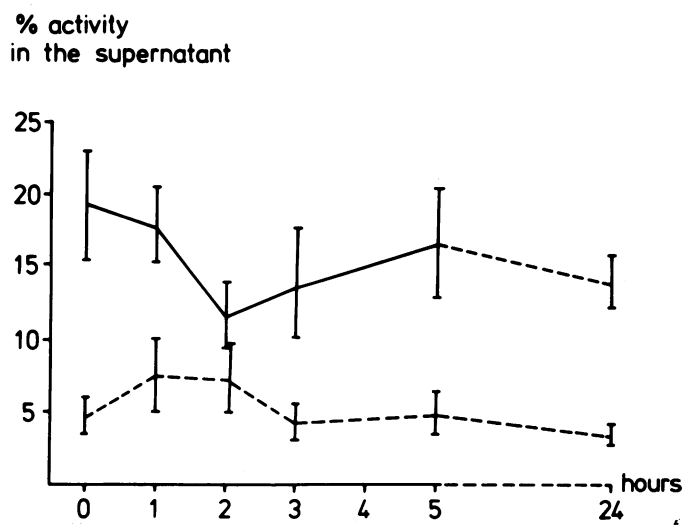


FIG. 4. Radioactivity in the supernatant as % of the total activity after incubation with plasmin (——) or NaCl (----). Mean values and SE from six control patients.

significantly different from that of patients receiving dextran was found (Fig. 2).

After addition of dextran in vitro to blood in increasing amounts to a final concentration of 100 mg/ml plasma, the radioactivity released into the supernatant rose from 12.8% to 46.3% (Fig. 5). When, however, the dextran concentration was 11 mg/ml plasma, roughly corresponding to the highest concentration obtained in vivo, the radioactivity increased only to 20.8% ($P>0.05$).

None of the dextran treated patients, but one of the control patients developed venous thrombosis in the legs as demonstrated by the $^{125}$I-labelled fibrinogen test.

## Discussion

The effect of dextran to decrease the incidence of postoperative thrombo-embolism is well established.[2,5,9,15,18] This effect has been demonstrated most convincingly if the dextran is given during the operation.[15] The antithrombotic effect has at least partly been ascribed to the decrease of platelet adhesiveness.[4,8]

Recently, however, some evidence has appeared indicating that the platelets do not play such a dominating role for development of venous thrombosis.[7,27] Another possible explanation for the antithrombotic properties of dextran may be its effect to improve the blood flow in the muscle veins of the lower legs during the operation.[15]

If the $^{125}$I-fibrinogen test is used to diagnose thrombosis the antithrombotic effect of dextran is less obvious than if phlebography is performed later in the postoperative course.[3] This finding suggests an increased lysability of thrombi formed in the presence of dextran, which thus could contribute to the antithrombotic effect. Muzaffar et al.[23] found that dextran in vitro as well as in vivo changes the structure of the fibrin, resulting in fibrin fibres more variable in size and coarser than normal. In vitro studies have shown that such morphologically changed fibrin clots are more easily dissolved by plasmin.[29]

The increased lysability demonstrated in the present study was apparent as an increased activity of $I^{125}$ in the supernatant. This is due to release of radioactively labelled split products, as previously demonstrated by Nordström and Zetterquist.[25] The amount of FDP in the supernatant after plasmin incubation rose parallel to the radioactivity confirming that the release of radioactivity

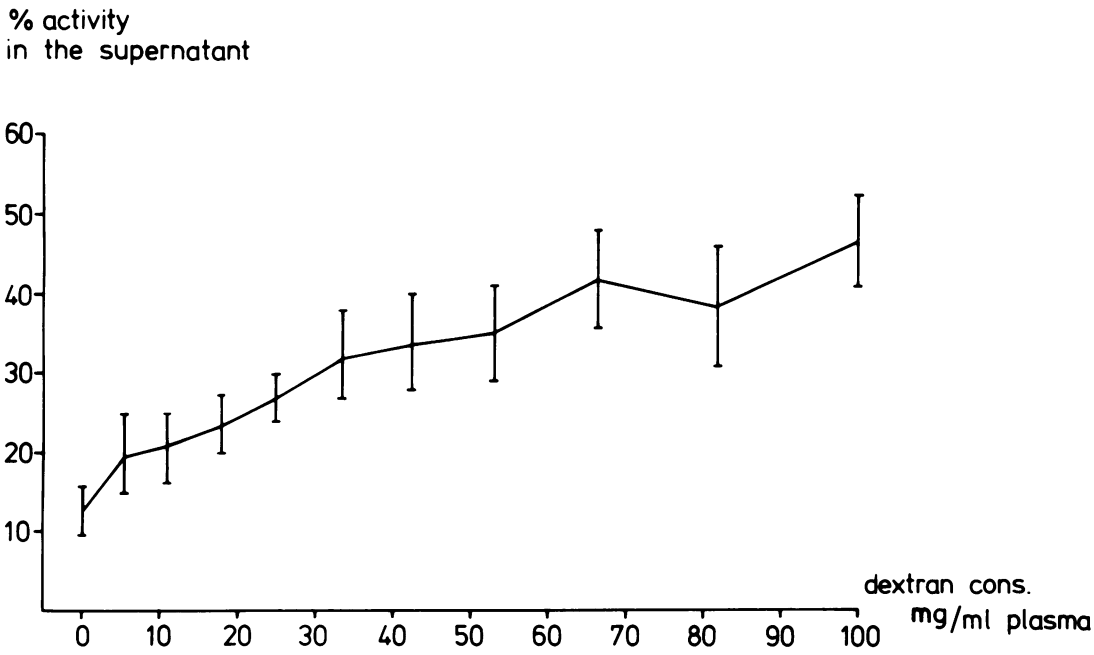


FIG. 5 Radioactivity in the supernatant as % of total activity after incubation of thrombi with plasmin. Dextran added in vitro. Mean values and SE from four patients.

from the thrombi really corresponds to a plasmin-digestion of the fibrin in the thrombi. The increased lysability was apparent already at the end of the dextran infusion, but reached a maximum about four hours after the end of the infusion. Thus the curve for increased lysability did not parallel the curve for dextran concentration. This finding agrees with the decrease of platelet adhesiveness after dextran infusion, which also reaches a maximum after four hours.[8,11] Some relationship between the effect of dextran on fibrin lysability and platelet function is therefore suggested. It has been shown by Paterson and Dhall[28] that infusion of dextran is followed by a transient platelet aggregation with release of ADP. Although the ADP content of the platelets is normalised within one hour after the end of the dextran infusion, releasable amount of ADP was decreased for many hours. When platelets aggregate, other substances such as antiplasmins, inhibitors of the plasminogen activation and factor XIII have been found to be released.[12,21,26,30] Factor XIII is known to initiate the cross-linking of fibrin fibers, thereby making the thrombi more stabile. Lack of factor XIII increases the lysability of thrombi. In the present study no evidence was found to indicate a decreased activity of factor XIII after dextran infusion.

When dextran was added in vitro the increased lysability was small as long as the dextran concentration was below 11 mg/ml plasma, which is the highest concentration obtained in vivo. The lysability increased with increasing dextran concentration, which is in agreement with the in vitro findings by Tangen et al.[29] When dextran was given to patients the maximal increase of lysability was found not at maximal dextran concentration but when this concentration had decreased to 6.4 mg/ml. Thus the increased lysability observed in the present ex vivo study is not related to the concentration of dextran, and probably due to some interaction between dextran and blood occuring in vivo, possibly via the platelets.

Dextran does not initiate general fibrinolysis.[11] The FDP found in the blood of the patients in this study was not caused by the dextran infusion, but by the operative procedure, as apparent from the finding of FDP also in the control patients.

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