The Production of Lipolytic Activity in Rat Plasma after the Intravenous Injection of Dextran Sulphate

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Samples of plasma obtained from animals which have been injected intravenously with heparin contain an active lipase. This plasma lipase can be conveniently studied *in vitro* by its ability to break down triglyceride which is added to the plasma in the form of a turbid lipid emulsion. The unesterified fatty acids released as lipolysis proceeds go into solution in the plasma in combination with albumin and, in consequence, the turbidity of the system falls (see Robinson & French, 1957). Heparin which is added to normal plasma *in vitro* does not produce an active lipase capable of clearing a triglyceride emulsion and it is presumed that the production of lipolytic activity in plasma after the injection of heparin must require the participation of some component from the tissues.

Other substances behave like heparin in inducing lipolytic activity in the blood on their injection: most of those found to be active have been sulphated polysaccharides of high molecular weight (Constantinides, Cairns & Werner, 1954; Glazko, Strong & Kazenko, 1954; Gillman, Hathorn & Penn, 1956).

In the present study the ability of several dextran sulphate preparations, of different molecular weights and sulphur contents, to induce an active lipolytic system in plasma has been investigated.

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EXPERIMENTAL

Wistar-strain female albino rats with body weight between 180 and 210 g. were used. Heparin and the various dextran sulphates were injected into the femoral vein in 0.9% NaCl soln. The rats were bled under ether anaesthesia, through cannulae inserted into their abdominal aortae, and the blood samples were collected into 3.8% (w/v) trisodium citrate soln. so that the final proportion was 1 ml. of citrate soln. to 9 ml. of blood (French, Robinson & Florey, 1953). Plasma was obtained by centrifuging the blood at 2500 g for 15 min. Plasma from animals injected with heparin will be referred to as heparinized plasma.

Dextran sulphate preparations. The dextran sulphates were prepared and isolated as their sodium salts as described by Ricketts (1952). They can be divided into two groups (see Table 1). Those in group 1 range in S content from 2.3 to 16.2%, but are of the same molecular size. They were prepared from a single dextran sulphate, L3, which showed considerable heterogeneity of S content (average content, 10.9%), by graded precipitation of the cetyl trimethyl-ammonium salt from 2M-NaCl soln. by the addition of water (cf. Scott, Gardell & Nilsson, 1957). The fractions were reconverted into the sodium salts for analysis and for testing.

The preparations in group 2 all have a high S content but their average molecular weights range from 5000 to several million. They were made by sulphating dextran of the stated intrinsic viscosity. The estimate of mol.wt. is based upon the viscosity and takes into account the replacement of H by SO_3Na (Ricketts, 1952).

Some of the properties of similar dextran sulphates have been described by Walton (1952, 1953, 1954). Dextran

Table 1. *Some properties of the dextran sulphate preparations used in this investigation*

L 3/7, 8 and 9 are each relatively homogeneous in S content. B 13 is the type of dextran sulphate used clinically as an anticoagulant. D5 is a relatively homogeneous fraction prepared from plasma-substitute dextran. GA1 is prepared from a dextran of higher mol.wt. than appears in dextran plasma substitute and is fractionally precipitated from ethanol-water to remove the smaller molecules. AL is prepared from undegraded dextran.

Group	Preparation	Intrinsic viscosity of original dextran	S in sodium salt (%)	Approx. estimate of mol.wt.
1	L3/7	—	16.2	} 5×10^3
	L3/8	0.03	13.9	
	L3/9	—	2.3	
2	B13	0.03	18.9	5×10^3
	E2	0.12	12.6	2.5×10^4
	D5	0.32	15.6	2×10^5
	GA1	0.62	16.1	$1-2 \times 10^6$
	AL	1.15	16.9	$> 2 \times 10^6$

sulphates D1 and E1 in his study correspond to the dextran sulphates D5 and E2 used in this investigation. Dextran sulphate B13 is similar to his dextran sulphate I3.

The heparin used in this study was a commercial preparation (Pularin-Evans: 100 units/mg.) and contained 12.1% of S.

Determination of clearing and lipolytic activity. The lipolysis of a turbid triglyceride emulsion by an active plasma is accompanied by a reduction in the turbidity due to the emulsion as well as by a release of unesterified fatty acids and of glycerol.

A suitable technique for the measurement of this reduction in turbidity which uses chyle, collected from the thoracic ducts of rats which have been fed with olive oil as lipid emulsion, has been developed and has already been described (French *et al.* 1953; Robinson & French, 1953). Briefly, clearing of the turbidity is followed in a Hilger Spekker photoelectric absorptiometer modified to carry matched 2 ml. test tubes (internal diam., 0.5 cm.). The test tubes containing the active lipolytic system are incubated at 37° and the appropriate volume of chyle is added with a micropipette. After mixing the contents, serial readings of turbidity are taken at frequent intervals. Initially, clearing of the turbidity occurs at a constant rate and it is this initial rate of clearing which is expressed in the results as a decrease in extinction in a given time.

The active lipolytic system to which the chyle is added usually contains, as well as the active plasma, an aq. 10% (w/v) bovine albumin soln. at pH 8.2: the pH of this albumin solution is adjusted electrometrically with 0.01N-NaOH. No buffer solution is used. The albumin provides excess of acceptor for the unesterified fatty acids which are released as clearing proceeds, and there is only a very slight fall in pH during the course of the enzyme reaction (Robinson & French, 1953; Robinson, Jeffries & French, 1954).

The exact composition of the lipolytic system used varied from experiment to experiment and is given in the legends to the Figures. The total fatty acid content of the chyle samples varied between 20 and 80 mg. of fatty acid/ml.

In some experiments, lipolytic activity was also determined by measuring the glycerol produced by the hydrolysis of the triglyceride of the chylomicra. Glycerol was determined by its conversion into formaldehyde and the measurement of the latter by a colorimetric technique (Korn, 1955). The presence of plasma interfered to a slight extent with the colour development under the conditions used, and a glycerol solution containing the same proportion of plasma as was present in the test samples was used to prepare standard curves.

RESULTS

Effect of the sulphur content of dextran sulphate preparations on the clearing activity induced in plasma by their injection

The three dextran sulphate fractions in group 1 were all of low molecular weight but differed in their sulphur content (see Table 1).

Each fraction was tested for its ability to induce clearing activity by its injection into groups of rats (three animals/group). The rates of clearing of

Fig. 1. The clearing activity induced by dextran sulphate preparations of different S contents. Heparin and dextran sulphate fractions L3/7 (16.2% S), L3/8 (13.9% S) and L3/9 (2.3% S) were injected in 10 μ g. amounts into groups of rats. A group of control rats was injected with 0.9% NaCl soln. The rats were bled 3 min. after their injection and the clearing activity of their plasma was measured by incubating 1 ml. of plasma with 42 μ l. of chyle at 37°. The height of each column represents the decrease in the extinction in 15 min. of individual plasma-chyle systems.

added chyle by the plasma samples obtained from the rats are shown in Fig. 1.

The clearing activity induced by the different preparations fell as their sulphur content was reduced. The most highly sulphated preparation (L3/7) had about 60–70% of the activity of the same amount of heparin and the least highly sulphated preparation (L3/9) had about 10% of the activity of heparin. Fraction L3/9 showed no anticoagulant activity when tested as described by Ricketts & Walton (1953).

The amount of heparin injected was in the range where the clearing activity induced was proportional to the quantity of heparin injected (Robinson, Jeffries & Poole, 1955).

Effect of the molecular weight of dextran sulphate preparations on the clearing activity induced in plasma by their injection

The five dextran sulphate preparations in group 2 were of similar sulphur content but varied widely in their molecular weight (see Table 1). In a preliminary experiment the ability of each fraction to induce clearing activity in rats was assessed. Rats were bled after being injected with varying concentrations of the different dextran sulphates and the rates of clearing of added chyle by the plasma samples obtained were measured *in vitro* (Fig. 2).

There appeared to be marked differences in the clearing activity produced by the various preparations. In general, a rise in the molecular weight of

the dextran sulphate injected was accompanied by a fall in the clearing activity, which could be detected *in vitro*. The activity of the low-molecular-weight preparation B 13, was, except at the highest concentrations tested, 50–60 % of that of heparin. Preparations E 2 and D 5 were less active and, at the highest concentrations tested, there appeared to be a fall in the activity which they induced. When 0.25 mg. of either of these preparations was injected the activity induced was 20–40 % of that induced by the same amount of heparin. Preparations GA 1 and AL produced very little activity on their injection.

Fig. 2. The clearing activity induced by dextran sulphate preparations of different molecular weights. Heparin and the dextran sulphates B 13, E 2, D 5, GA 1 and AL were injected in varying amounts into rats. The rats were bled 3 min. after their injection and the clearing activity of their plasma was measured by incubating 1 ml. of plasma with 0.3 ml. of 10% albumin soln. in water (pH 8.2) and 0.1 ml. of chyle at 37°. The height of each column represents the decrease in the extinction in 30 min. of each plasma-albumin-chyle mixture.

The ability of the preparations to induce both clearing and lipolytic activity was studied in more detail by injecting 0.25 mg. of each into groups of rats (three animals/group). This amount of dextran sulphate was chosen since it appeared from the results of the preliminary experiment that it might induce the greatest activity in the plasma.

The rates of clearing and lipolysis of added chyle by the active plasma samples obtained from the rats were measured. The results are shown in Fig. 3. The pattern of both lipolytic and clearing activity is in agreement with that found in the previous experiment. The activity of the preparation B 13 is 60–80 % of that of heparin; the activity of E 2 and D 5 is 20–30 % of that of heparin. The activity of GA 1 and AL is negligible.

Inhibition by dextran sulphates of high molecular weight of the clearing activity induced in plasma by heparin injection

Walton (1952) and Oncley, Walton & Cornwell (1957) have described the interactions of high-molecular-weight dextran sulphates with fibrinogen and with β -lipoproteins. In the experiments which have been described above, though the quantities of dextran sulphate injected were less than those used in Walton's experiments, a similar interaction presumably occurred. It is also known that high concentrations of heparin will inhibit the clearing activity of heparinized plasma *in vitro* (Brown, Boyle & Anfinsen, 1953). That is, heparin itself, in high concentrations, inhibits the lipase which it induces.

In view of these observations the possibility that dextran sulphate preparations of high molecular weight might inhibit the clearing of added chyle by

Fig. 3. The clearing and lipolytic activity induced by dextran sulphate preparations of different molecular weights. Heparin and the dextran sulphates B 13, E 2, D 5, GA 1 and AL were injected in 250 μg. amounts into groups of rats. The rats were bled 3 min. after injection and 0.8 ml. samples of their plasma were incubated with 0.3 ml. of 10% albumin soln. in water (pH 8.2) and 0.1 ml. of chyle at 37°. The decrease in extinction in each plasma-albumin-chyle mixture in 30 min. is represented by the heights of the filled-in columns. Triplicate samples (0.03 ml.) were also removed from the mixtures for the determination of glycerol. The release of glycerol in each system in 30 min. is represented by the heights of the open columns.

heparinized plasma was investigated. This was done in two ways.

First, plasma samples obtained from rats which had been injected with the various dextran sulphates were tested for their ability to reduce the rate of clearing, by a sample of heparinized plasma, of added chyle. It was found that plasma samples from animals injected with the preparations D5, GA1 and AL markedly reduced the ability of heparinized plasma to clear added chyle.

In a second experiment the ability of the various dextran sulphate preparations to inhibit the clearing activity of heparinized plasma was tested directly by adding the dextran sulphates to samples of heparinized plasma and chyle. The results of this experiment are shown in Fig. 4.

Whereas heparin itself and dextran sulphate B13 inhibit the clearing activity of heparinized plasma slightly at the concentrations used, preparation D5 reduces the activity by 50–60% and preparations GA1 and AL reduce the activity by about 80%. The final concentration of heparin and dextran sulphate in this experiment was approximately the same as that reached in the plasma of the rat after the injection of 100 µg. of material/200 g. body wt.

The inhibition of clearing activity by the dextran sulphate preparations of high molecular weight could be due to their interaction with either the lipase or with the lipase substrate, the chylomicron triglyceride, or to their interaction with both the lipase and its substrate. Evidence for a combination of the dextran sulphate with the chylomicron has been obtained as follows.

A sample of chyle (1 ml.) was suspended in 0.9% sodium chloride solution (5.5 ml.) and centrifuged at 114 000 g for 30 min. in the Spinco ultra-centrifuge. The fatty layer at the surface of the centrifuge tube was recovered and redispersed in 1 ml. of 0.9% sodium chloride solution. The infranatant solution and any precipitate of cells were rejected. A 1% solution of the dextran sulphate AL (0.1 ml.) was added to the lipid emulsion and the mixture was incubated at 37° for 30 min. A small sample (A) of the mixture was retained and 5.5 ml. of 0.9% sodium chloride solution was added to the remainder, which was then centrifuged at 114 000 g for 30 min. The lipaemic layer was recovered, redispersed in 0.9% sodium chloride solution and recentrifuged twice more before being finally collected in 1 ml. of 0.9% sodium chloride solution (B). The infranatant solutions from the first (C) and last centrifuging (D) were retained.

In confirmation of the results of the earlier experiments it was found that, in the presence of a high concentration of the preparation AL, chylomicra could not be cleared by heparinized plasma (Table 2). Furthermore, it was not possible

Fig. 4. The inhibition of the clearing activity of heparinized plasma by various dextran sulphate preparations. The degree of inhibition was determined in systems containing 0.2 ml. of heparinized plasma, 30 µl. of chyle, 0.3 ml. of 10% albumin soln. in water (pH 8.2), 0.9 ml. of normal plasma and 0.1 ml. of either water or a solution in water of either heparin or a dextran sulphate preparation (final concn., 15 µg./ml.). Heparinized plasma was obtained from rats injected with 250 µg. of heparin and bled 3 min. later. The height of each column represents the decrease in the extinction of each system in 30 min. at 37°.

Table 2. Inhibitory effect of the dextran sulphate preparation AL on the clearing of chyle by heparinized plasma

The experiment is described in the text. The fall in extinction at 37° was followed in systems containing, in addition to the lipid mixtures described below, 0.2 ml. of 10% albumin soln. in water (pH 8.2), 0.6 ml. of normal plasma and 0.2 ml. of heparinized plasma obtained from animals bled 3 min. after the injection of 10 units of heparin/kg. body wt.

Lipid mixtures	Fall in extinction in 30 min.
40 µl. of chyle	0.71
40 µl. of A	0
40 µl. of B	0.08
40 µl. of chyle + 0.1 ml. of C	0.09
40 µl. of chyle + 0.1 ml. of D	0.55

to remove the inhibitory effect of the preparation AL by repeated washing of the lipid preparation with 0.9% sodium chloride solution. The infranatant solution from the final centrifuging had very little inhibitory effect on the clearing of chyle by heparinized plasma.

It may be concluded therefore that the dextran sulphate preparation AL interacts with the chylomicron and this may be the explanation of its inhibition of the lipase action: however, it is also possible that, though the dextran sulphate combines with the chylomicron and is carried with it

under the above experimental conditions, its inhibitory effect is nevertheless subsequently exerted on the lipase itself.

Ability of protamine to reverse the inhibition of clearing activity caused by dextran sulphate preparations of high molecular weight

The direct inhibition of clearing activity by the dextran sulphate preparations of high molecular weight suggests that, even if such preparations did induce activity in plasma on their injection, this activity would not be directly measurable *in vitro*.

It is known that protamine will combine with the acid groups of heparin and prevent its action as an anticoagulant (Chargaff & Olson, 1937; Jaques, 1943). Presumably, a similar interaction might occur between protamine and the acid groups of dextran sulphate and this interaction might abolish the inhibitory activity of the high-molecular-weight dextran sulphates. This possibility was investigated by determining the effect of protamine on the clearing and lipolytic activity of plasma samples obtained from rats which had been injected with certain of the dextran sulphate preparations. The results are shown in Table 3.

In the presence of increasing quantities of protamine there is a progressive rise in the clearing and

lipolytic activity of plasma samples obtained from animals injected with the high-molecular-weight dextran sulphate preparations D5, GA1 and AL. The clearing and lipolytic activity of samples of heparinized plasma is relatively unaffected.

It appears therefore that the high-molecular-weight dextran sulphate preparations do induce clearing and lipolytic activity on their injection but that this is demonstrable at a high level in plasma *in vitro* only if the direct inhibition of the activity by the dextran sulphates themselves is prevented.

Production of clearing activity by the dextran sulphate preparations D5 and AL

It was of interest to determine how soon after the injection of the high-molecular-weight dextran sulphate preparations D5 and AL clearing activity could be detected in the plasma. In view of the results already obtained, it is evident that the activity induced could only be determined *in vitro* in these circumstances in the presence of protamine.

Accordingly, groups of rats were bled at different times after the injection of the dextran sulphate preparations. In each case the plasma was separated and added to a tube containing protamine and albumin in solution at 0°. After 10 min. at 0°, chyle was added to each tube and, after a further 10 min. at 0°, clearing of the added chyle was followed at 37°. This order of addition of reactants was followed since, as will be described below, it was found that the activity of the clearing system was rapidly destroyed at 37° in the presence of protamine if chyle, the substrate of the enzyme, was absent.

Table 3. *Effect of protamine on the lipolytic and clearing activity induced in plasma by the injection of heparin and various dextran sulphates*

The fall in extinction at 37° was followed in systems containing 0.2 ml. of 10% (w/v) albumin soln. in water (pH 8.2), 0.02 ml. of chyle, 0.2 ml. of protamine sulphate solution and 0.5 ml. of plasma from rats bled 3 min. after the injection of 250 µg. of either heparin or the dextran sulphate preparation D5, GA1 or AL. Triplicate samples (0.06 ml.) were also removed from each mixture after 0 and 30 min. for the determination of glycerol.

Material injected	Final protamine concn. (µg./ml.)	Clearing activity (decrease in extinction in 30 min.)	Lipolytic activity (µg. of glycerol released/30 min.)
D5	0	0.13	6.3
	10	0.18	7.9
	60	0.35	25
	200	0.38	30
GA1	0	0.06	0
	10	0.08	6.7
	60	0.40	23.5
	200	0.38	31.5
AL	0	0.08	0
	10	0.21	10
	60	0.26	20.5
	200	0.26	19
Heparin	0	0.78	57
	10	0.92	69
	60	0.83	48
	200	0.76	40

Fig. 5. The clearing activity induced by the injection of 250 µg. of the dextran sulphate preparations D5 and AL. Groups of rats were bled 30, 90 or 180 sec. after their injection. Each plasma (0.4 ml.) was mixed at 0° with 0.2 ml. of 0.05% protamine soln. and 0.3 ml. of 10% albumin soln. in water (pH 8.2). After 10 min. 20 µl. of chyle was added and, after a further 5 min. at 0°, the decrease in the extinction of each system was followed at 37°. The height of each column represents the decrease in the extinction in 30 min. of an individual plasma system.

The results in Fig. 5 show that clearing activity is present in the blood within 30 sec. of the injection of the dextran sulphate preparations D5 and AL.

Effect of protamine on the stability of the enzyme system induced in plasma by heparin and dextran sulphate

In a previous study (Robinson, 1956) it was shown that, when heparin was removed from heparinized plasma on a column of an anionic-exchange resin, the clearing activity of the eluate from the column was lost very rapidly at 37°. Activity was maintained at 37° if heparin was added to the eluate. It was concluded that the stability of the enzyme system in heparinized plasma was due to the presence of heparin.

Since protamine was presumably combining with heparin and dextran sulphate in the plasma in the experiments which have already been described, it seemed possible that the stability of the enzyme system might also be reduced in these circumstances. The following experiment suggested that this was so.

Samples of heparinized plasma were incubated for a short time at 37° with varying concentrations of protamine. After cooling to 0°, chyle was added to each plasma sample and the rate of clearing of the chyle was measured at 37°. In a control series the plasma samples were first incubated alone at 37° before the protamine solutions and chyle were added at 0°, and the clearing of the chyle was followed at 37°.

Table 4. *Effect of protamine on the stability of the enzyme system induced in plasma by the injection of heparin*

Rats were injected with 0.25 ml. of heparin soln. (100 µg./ml.) and bled 3 min. later. In the test systems 0.5 ml. of plasma was incubated for 10 min. at 37° with 0.3 ml. of 10% albumin soln. in water (pH 8.2) and 0.2 ml. of protamine sulphate soln. Then, at 0°, 0.03 ml. of chyle was added and the clearing of the added chyle was followed at 37°. In the control systems 0.5 ml. of plasma was incubated for 10 min. at 37° with 0.3 ml. of the albumin soln. and then mixed, at 0°, with 0.2 ml. of the protamine sulphate soln. After 10 min. 0.03 ml. of chyle was added and, after a further 5 min. at 0°, clearing of the added chyle was followed at 37°.

Final protamine concn. (mg./ml.)	Decrease in extinction in 15 min.	
	Test	Control
0.4	0	0.19
0.2	0	0.18
0.15	0	0.18
0.1	0.04	0.23
0.05	0.24	0.26
0.02	0.25	—
0	0.24	0.20

The results in Table 4 show that the clearing activity of heparinized plasma was destroyed in 10 min. at 37° in the presence of a sufficiently high concentration of protamine. In this experiment, as the final protamine concentration was raised from 0.05 to 0.2 mg./ml., the clearing activity of the plasma after incubation at 37° fell to zero. In the control series clearing activity could be demonstrated at all the concentrations of protamine tested.

It was found, however, that in the control series in the presence of high concentrations of protamine, the rate of clearing of chyle by the heparinized plasma fell off rapidly as the reaction proceeded. Thus when the final protamine concentration was 0.2 mg./ml., the fall in extinction initially was 0.12/10 min., and this had declined, after clearing for 30 min., to 0.04/10 min. In the absence of protamine, on the other hand, the fall in extinction initially was 0.15/10 min., and, after 30 min., was 0.13/10 min.

This suggests that the instability of the enzyme system in heparinized plasma in the presence of a sufficiently high concentration of protamine persists in the presence of chyle. This is in contrast with the protective effect of chyle on the clearing activity of heparinized plasma from which heparin had been previously removed by treatment with an anionic-exchange resin (Robinson, 1956).

DISCUSSION

From the results of this study the level of clearing activity induced in plasma by the injection of dextran sulphate appears to be directly related to the degree of sulphation of the dextran sulphate molecule. However, it appears that factors other than the degree of sulphation of the molecule may also be important, since none of the dextran sulphate preparations, with a sulphur content equal to or greater than that of heparin, was as efficient as heparin in inducing clearing activity in plasma.

It is not possible to determine the effect of the molecular weight of the injected dextran sulphate on the level of clearing or lipolytic activity induced in the plasma since the preparations of high molecular weight markedly inhibit the enzyme system which they induce. It is at least partly for this reason that the level of activity which can be directly demonstrated *in vitro* falls with an increase in the molecular weight of the preparation injected. In the presence of protamine, which presumably combines with the dextran sulphate and abolishes its inhibitory effect, the clearing activity produced by the dextran sulphates GA 1 and AL, each of which has an average molecular weight of over a million, is raised from a negligible

value to a level which approaches that produced by the preparations of much lower molecular weight. The possibility that in the preparations GA1 and AL there are a few dextran sulphate molecules of a low molecular weight, which are really responsible for the clearing activity produced under these conditions, cannot be excluded, though preparation GA1 was fractionated to remove smaller molecules and preparation AL was prepared from undegraded dextran.

The observation that the stability of the enzyme system in heparinized plasma is markedly reduced in the presence of protamine suggests that the apparent inhibition of the clearing of chyle by heparinized plasma in the presence of protamine which was previously reported (Robinson *et al.* 1954) may, in fact, have been due to the rapid destruction of the enzyme in these circumstances. It is also possible that the ability of injected protamine to increase alimentary lipaemia and to delay the removal of ingested or injected fat from the blood may be partly explained by a reduction of the stability of the same lipolytic enzyme system (Brown, 1952; Spitzer, 1953; Bragdon & Havel, 1954; French & Morris, 1957).

It is possible that some component necessary for the activity of the lipolytic system induced in blood by the injection of heparin or dextran sulphate is normally located on the inner surface of the vascular wall and is released from there into the bloodstream (see Robinson & French, 1957). Such a component could be either the lipase itself or some substance capable of initiating lipolytic and clearing activity in the plasma. The observation that the dextran sulphate preparations, D5 and AL, with average molecular weights of 200 000 and more than a million respectively, induce clearing activity in the plasma within 30 sec. of their injection is in accordance with this view.

SUMMARY

1. Plasma samples obtained from rats which have been injected with dextran sulphate contain an active lipase. The activity of this enzyme can be measured by the ability of an active plasma sample to clear the turbidity of chyle added to it *in vitro*.

2. The level of activity induced by the injection of dextran sulphate is directly related to the degree of sulphation of the dextran sulphate molecule.

3. With an increase in the molecular weight of the dextran sulphate injected the level of clearing activity demonstrable *in vitro* falls. This is at least partly due to the inhibition of the clearing activity by the high-molecular-weight dextran sulphates themselves. An increase in the activity in the

plasma, after the injection of such preparations, can be demonstrated in the presence of protamine: it is presumed that the protamine combines with the dextran sulphate and so abolishes its inhibitory effect.

4. The inhibition of clearing activity by the high-molecular-weight dextran sulphates may be due to their interaction with the chyle.

5. The dextran sulphate preparations D5 and AL, with average molecular weights of 200 000 and 1–2 million respectively, produce clearing activity in rat plasma within 30 sec. of their injection.

6. The observation that the clearing activity of a plasma sample is rapidly destroyed at 37° *in vitro* in the presence of protamine supports the view that the sulphated molecules which induce this activity may also stabilize the enzyme system.

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