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HISTAMINE RELEASE IN RABBIT BLOOD BY DEXTRAN AND DEXTRAN SULPHATE

BY

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Injection of dextran into rats, either intravenously or intraperitoneally, gives rise to reactions having certain similarities to allergic manifestations in man (Morrison, Bloom and Richardson, 1951a). Such reactions are characterized by marked oedema of the feet, jowls and nose, stupor and dyspnoea, followed by scratching (Voorhees, Baker and Pulaski, 1951; Briot and Halpern, 1952a). Urticarial reactions in man following intravenous administration have also been reported (Wilkinson and Storey, 1953). Briot and Halpern (1952b) showed that promethazine and adrenaline both gave some protection against the effects of injected dextran in rats; but Morrison, Richardson and Bloom (1951b), using a variety of antihistamine agents, found only phenindamine ("Thephorin") to be effective. Protection was also obtained by pretreatment with cortisone (Swingle, Fedor, Maxwell, Ben, and Barlow, 1953; Swingle, Fedor, Ben, Maxwell, and Baker, 1953).

Anaphylactoid type reactions in guinea-pigs have been reported following the parenteral administration of certain samples of dextran sulphate with molecular weights above a certain critical level (Walton and Ricketts, 1954). These reactions were not prevented by antihistamine drugs.

In order to determine whether reactions due to dextran or dextran sulphate could be attributed in part to the effects of released histamine, it seemed of interest to determine the effect of incubating them with rabbit blood, the cellular histamine of which is known to be released by a variety of agents capable of causing anaphylactic or anaphylactoid reactions *in vivo* (Code, 1952).

METHODS

Histamine Release in Blood.—All glass apparatus used to handle blood was treated with Silicone D.C. 1107. Pipettes were again treated after they had been used three or four times, but centrifuge tubes in which histamine release had taken place were immersed in a sulphuric acid-chromate cleaning mixture overnight, washed, and then re-treated with silicone before being used again. Rabbit blood was obtained from the incised marginal vein of the ear after the hair surrounding the vein had been clipped short. Those parts of the ear likely to come into contact with blood were carefully smeared with soft paraffin, and the skin at some distance from the incision was gently rubbed with a benzene-impregnated swab to ensure a good flow of blood. Blood was collected in a tube containing ice-cold heparinized saline (NaCl 0.9% w/v, Heparin Evans 3 units/ml.) supported in a beaker containing crushed ice to minimize non-specific histamine release from the cells.

Histamine release reactions were carried out in 50 ml. round-bottomed centrifuge tubes at 37.5° C. The required number of tubes containing equal volumes of the same sample of dilute heparinized blood (usually 5 or 6 ml.), and also those containing solutions of the histamine-releasing agents or inhibitors, were placed in a water bath at 37.5° C. and were left for 15 min. for their contents to reach bath temperature. Histamine-releasing agents or inhibitors were dissolved in 0.9% w/v saline. The volume of such solution added to a tube of dilute blood was always 1 ml., and mixing was accomplished by rapidly swirling the tube. Inhibitors, when used, were always added to blood immediately before the histamine-releasing agents. A control sample of untreated dilute blood was included in each experiment, and equal volumes were maintained in all tubes at each stage by the addition of saline at 37.5° C.

Histamine release was allowed to take place for 30 min. The blood samples were then chilled in ice and centrifuged together at 3,000 rev./min. for 20 min. at room temperature. An aliquot of the supernatant dilute plasma was removed from each tube immediately and the histamine extracted.

Extraction and Assay of Histamine.—4.5 ml. samples of dilute plasma were submitted to the extraction procedure described by McIntire, Roth, and Shaw (1947) within a few minutes of removal from the cells. The final acid eluates were adjusted to *p*H 7.0 with indicator papers reading to 0.3 of a unit and made up to volume just before assay. Histamine present in the eluates was assayed biologically on guinea-pig ileum suspended in Tyrode solution containing atropine sulphate 1 mg./l. Most histamine estimations were carried out with an automatic organ-bath apparatus (Boura, Mongar, and Schild, 1954; Schild,

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1947) by the 2×2 assay technique (Schild, 1942) using 5 groups of 4 responses, though a few solutions were assayed in a hand-operated organ bath by bracketing against the responses from a standard histamine solution.

Concentrations of plasma histamine were calculated as μg. of base per ml. of blood, disregarding the volume occupied by the cells.

As a check on the reliability of the techniques used, two samples of rabbit plasma were extracted and assayed for their histamine content several times. The mean estimate of histamine present in the first was 0.22 μg./ml., S.E. 0.02 μg. (7 extractions and assays), and in the second 0.35 μg./ml., S.E. 0.025 μg. (5 extractions and assays). Recovery experiments were also carried out by estimating the histamine content of samples of rabbit plasma with and without added histamine. In 11 determinations the histamine found, calculated as a percentage of the expected value, was 90.0% (S.E. 3%).

Details of the polysaccharides used in these experiments were supplied by Dr. C. R. Ricketts and are given in Table I. Most of the data and the methods used to determine the intrinsic viscosities and molecular weights of the compounds have been published (Ricketts, 1952a, 1952b; Ricketts and Walton, 1952; Walton, 1952).

TABLE I

SUMMARY OF THE POLYSACCHARIDES TESTED EITHER AS HISTAMINE RELEASING AGENTS OR AS INHIBITORS OF HISTAMINE RELEASE

Polysaccharide	Anticoagulant Activity (Units/mg.)*	Approximate Molecular Weight	Intrinsic Viscosity of Parent Dextran
Dextran I		<14,000	0·02
,, E ..		22,000	0·12
,, ...		80,000	0·25
,, D		220,000	0·36
,, ...		1,000,000	0·64
Heparin, Evans ..	93		
Maltotriose sulphate	10 (approx.)		
Dextran sulphate I	15–20	<10,000	0·02
,, ,, E/1	15	40,000	0·12
,, ,, D/3	15	440,000	0·36

* In terms of International Standard Heparin, 130 units/mg.

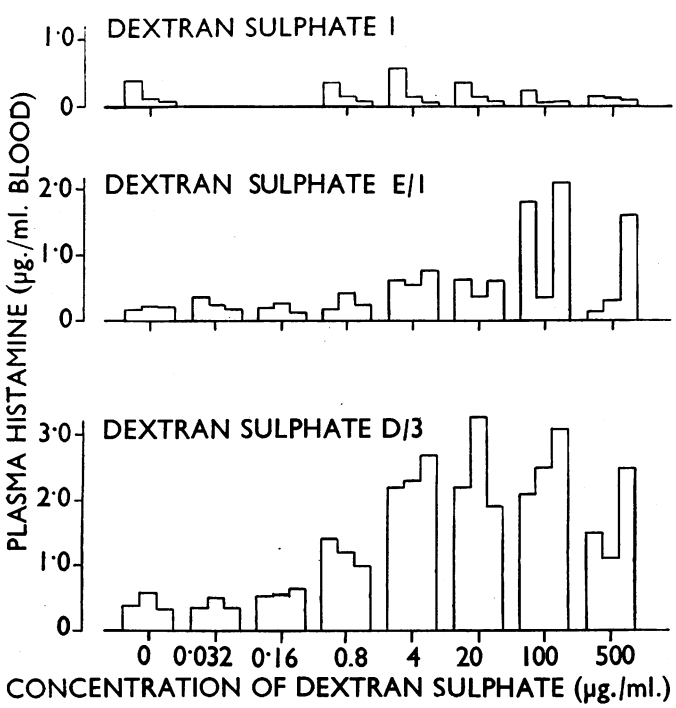

FIG. 1.—Release of histamine from rabbit blood cells by dextran sulphate. Plasma histamine levels after incubating dilute heparinized rabbit blood with varying concentrations of dextran sulphate for 30 min. Samples of dextran sulphate D/3, E/1, and I were each tested on blood obtained from three different rabbits.

RESULTS

Histamine Release by Dextran and Dextran Sulphate

Preliminary experiments showed that incubation of dilute heparinized rabbit blood with dextran sulphate D/3 led to a rise in the level of plasma histamine. Histamine release was initiated by quantities in the region of 0.1 μg./ml. It appeared that 4 μg./ml. was sufficient to release the greater part of the available cellular histamine, since, when the concentration was raised still further to 100 μg./ml., only slight increases in the levels of plasma histamine were obtained.

Results obtained with three different fractions of dextran sulphate, and also the parent dextrans, are shown in Figs. 1 and 2. Dextran sulphate I (mol. wt.<10,000) did not release histamine at concentrations between 0.8 μg. and 500 μg./ml. The E/1 fraction of higher molecular weight (40,000) behaved qualitatively like the D/3 fraction (mol. wt. 440,000), but its concentration threshold for histamine release was higher, and the mean values of plasma histamine obtained at each concentration were lower, than with the latter. Results obtained with dextran showed that the molecular weight of a particular fraction was again a factor in determining its ability to initiate histamine release. The D fraction (mol. wt. 220,000) showed marked activity although its threshold concentration was higher than that of

the corresponding dextran sulphate. The E fraction (mol. wt. 22,000) showed relatively little, if any, activity, and the I fraction (mol. wt.<14,000) none at all, at concentrations between 0.8 μg. and 500 μg./ml.

When samples of blood from the same rabbit were incubated with either dextran D or dextran sulphate D/3, increasing levels of dextran D from 0.02 to 2.0 mg./ml. gave rise only to a slow increase in the level of plasma histamine, whereas dextran sulphate D/3 over the same range of concentrations caused a gradual reduction (Fig. 3).

The Effect of Molecular Size of Dextran on Histamine Release

Six dextran fractions were tested for their abilities to release histamine from rabbit blood cells *in vitro*. The intrinsic viscosities of the samples used ranged from 0.02 to 0.64 and represented a range of molecular weights from less than 14,000 to more than 250,000. Each fraction was incubated with 6 ml. of diluted heparinized blood from the same rabbit for 30 min. at a concentration of 100 μg./ml.

The dextran of intrinsic viscosity 0.02 did not cause a significant rise in the plasma histamine levels above those of the control samples, but increases obtained with those fractions having intrinsic viscosities ranging from 0.12 to 0.64 were significant at the P. 0.05 level (Table II).

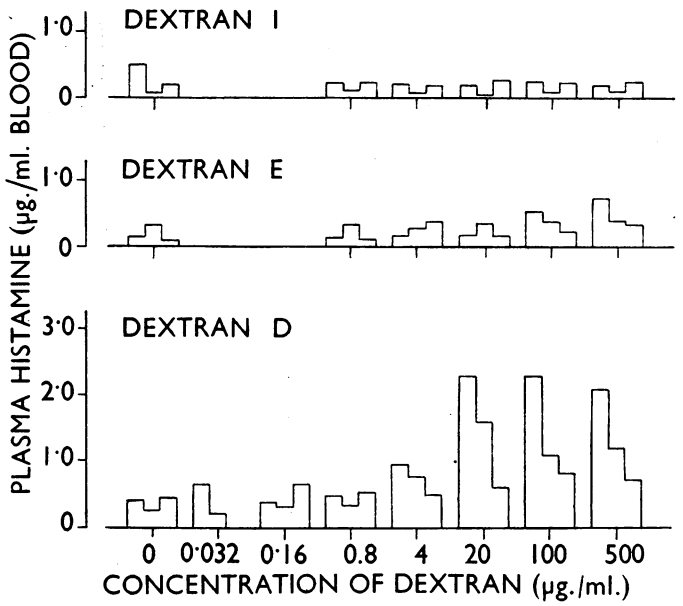

FIG. 2.—Release of histamine from rabbit blood cells by dextran. Plasma histamine levels after incubating dilute heparinized rabbit blood with varying concentrations of dextran for 30 min. Samples of dextran D, E, and I were each tested on blood obtained from three different rabbits.

TABLE II

THE *IN VITRO* RELEASE OF HISTAMINE FROM RABBIT BLOOD CELLS BY DEXTRANS OF VARYING INTRINSIC VISCOSITIES

Diluted rabbit blood was incubated with dextran 100 μg./ml. for 30 min. at 37·5° C.

Rabbit	Plasma Histamine (μg./ml. Blood)						
	Control	Intrinsic Viscosity of Dextran Fraction					
		0·02	0·12	0·25	0·36	0·44	0·64
38	0·25	0·32	0·37		2·9		0·81
39	0·58	0·31	1·4	3·2	2·7	3·1	2·2
42	0·69	0·80	1·4	2·0	1·8	2·6	2·1
44	0·25	0·46	0·67	2·3	2·5	1·9	1·2
47	0·17	0·98	0·64	0·92	2·0	1·7	0·64
49	0·43	0·41	0·76	1·2	2·7	3·0	0·62
Mean values	0·40	0·55	0·87	1·9	2·4	2·5	1·3

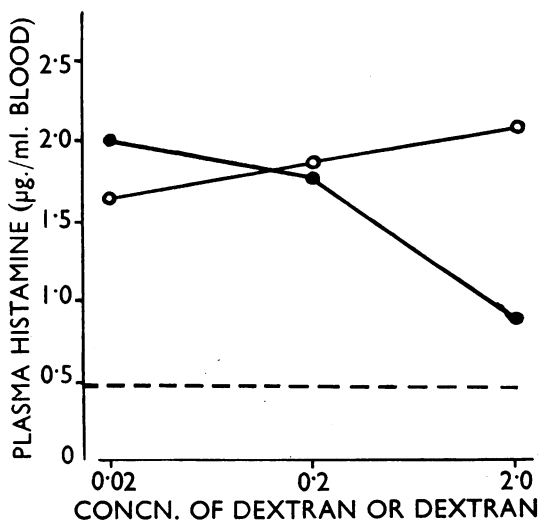

FIG. 3.—Release of histamine from rabbit blood cells. The effects of higher concentrations of dextran and dextran sulphate. Plasma histamine levels after incubating dilute heparinized rabbit blood with varying concentrations of dextran D and dextran sulphate D/3 for 30 min. Each point on the graph represents the mean of three determinations on different samples of blood. Broken line=control. Closed circles=dextran sulphate D/3. Open circles=dextran D.

Evidence that the additional histamine found in the plasma during these experiments was derived from the cellular fraction of blood, and not from the plasma or the polysaccharides themselves, was obtained by incubating dextran sulphate D/3 or dextran D with dilute rabbit plasma for 30 min. at concentrations which had previously been found effective in causing histamine release in blood. No increase in plasma histamine was obtained as a result of this procedure (Table III).

TABLE III

THE FAILURE OF DEXTRAN D OR DEXTRAN SULPHATE D/3 TO INCREASE THE LEVEL OF HISTAMINE AFTER INCUBATION WITH DILUTE RABBIT PLASMA FOR 30 MIN. AT 37·5° C. IN DUPLICATE EXPERIMENTS

Experiment	Treatment of Dilute Rabbit Plasma During Incubation Period	Histamine Present in Dilute Rabbit Plasma μg./ml.	
1	Control (incubation only)	0·15	0·13
	Incubation with dextran sulphate D/3 0·02 mg./ml.	0·11	0·16
	Incubation with dextran sulphate D/3 0·5 mg./ml.	0·12	0·15
2	Control (incubation only)	0·14	0·08
	Incubation with dextran D 0·13 mg./ml.	0·09	0·08
	Incubation with dextran sulphate D/3 0·02 mg./ml.	0 06	0·12
	Incubation with dextran sulphate D/3 0·5 mg./ml.	0·16	0·09

Identification of the active plasma constituent was carried out by comparing the responses of guinea-pig ileum to alternate equiactive doses of histamine and of plasma extract after the addition of mepyramine maleate (0.002 or 0.003 μg./ml.) to the bath. The same degrees of inhibition and rates of recovery were obtained with histamine and with plasma extracts from blood which had been incubated with either dextran D or dextran sulphate D/3 (Figs. 4 and 5). Since mepyramine is a highly specific inhibitor of histamine (Schild, 1947), these results make it seem likely that the active substance present in the extracts was either histamine or some closely related substance.

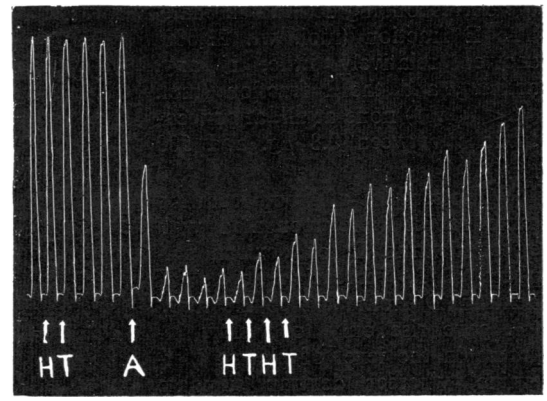

FIG. 4.—Regular contractions of isolated guinea-pig ileum suspended in Tyrode solution from alternate approximately equiactive concentrations of histamine (as acid phosphate) 0.03 μg./ml. (H), and diluted plasma extract from rabbit blood after incubation with dextran D (T). At A, mepyramine maleate 0.002 μg./ml. was allowed to remain in contact with the preparation for one minute.

Inhibitors of Histamine Release

When carrying out procedures involving histamine release in whole blood it was desirable to have some means of producing complete inhibition of the reaction. Sodium oxalate was known to prevent the *in vitro* release of histamine from the blood cells of sensitized rabbits by antigen (McIntire, Roth, and Richards, 1949). Its effect on the release reaction to dextran sulphate D/3 in normal rabbit blood was therefore determined. Levels of plasma histamine were compared after incubating dilute heparinized rabbit blood with dextran sulphate D/3 100 μg./ml. (*a*) alone, (*b*)

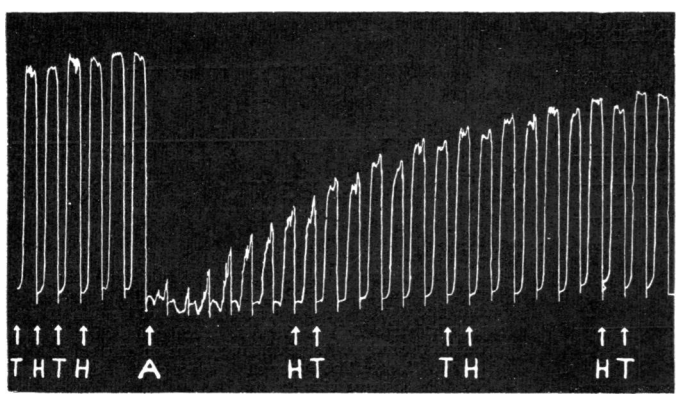

FIG. 5.—Regular contractions of isolated guinea-pig ileum suspended in Tyrode solution from alternate approximately equiactive concentrations of histamine (as acid phosphate) 0.03 μg./ml. (H), and diluted plasma extract from rabbit blood after incubation with dextran sulphate D/3 (T). At A, mepyramine maleate 0·003 μg./ml. was allowed to remain in contact with the preparation for one minute.

after the addition of sufficient sodium oxalate to give a concentration of 0.021 M during the incubation period, and (*c*) with the same quantity of sodium oxalate added at the end of the incubation period. The results are shown in Table IV. They indicate that histamine release was prevented completely by the sodium oxalate when added before the dextran sulphate D/3, but that the same quantity added after histamine release had taken place

TABLE IV

INHIBITORY EFFECT OF SODIUM OXALATE 0·021M ON THE RELEASE OF HISTAMINE FROM RABBIT BLOOD CELLS INCUBATED WITH DEXTRAN SULPHATE D/3 100 μG./ML.

Rabbit	Plasma Histamine (μg./ml. Blood)			
	Control Incubation with Normal Saline	Incubation with Dextran Sulphate D/3 100 μg./ml.	Sodium Oxalate 0·021M Added Before Incubation with Dextran Sulphate D/3 100 μg./ml.	Sodium Oxalate 0·021M Added After Incubation with Dextran Sulphate D/3 100 μg./ml.
35	0·29	2·4	0 38	2·3
39	0·64	3·0	0·13	3·1
33	0·55	1·5	<0·06	1·2
40	0·57	1·6	0·22	1·6
32	0·44	3·7	0·14	3·7
Mean values	0·50	2·44	<0·19	2·38

did not interfere with the amount of histamine found after extraction and assay. Since the values for plasma histamine obtained when sodium oxalate was added to blood before the dextran sulphate D/3 were lower than those obtained with untreated blood, it seems likely that the sodium oxalate in addition to inhibiting the histamine release due to dextran sulphate D/3 also prevented a non-specific release of histamine from the cells during the incubation period. On the basis of these results, sodium oxalate was used in further experiments to stop the release of histamine as required.

Dextran sulphate of high molecular weight was found to possess a dual action, behaving as an activator of the histamine-release mechanism in low concentrations and as an inhibitor at higher concentrations. This fact indicated that fractions of low molecular weight that were incapable of releasing histamine might behave as inhibitors. This hypothesis was tested by incubating samples of dilute heparinized blood containing either dextran I or dextran sulphate I with dextran sulphate D/3 100 μg./ml. Dextran I at concentrations of 100 μg./ml. and 500 μg./ml. failed to cause any reduction in the amount of histamine released, but dextran sulphate I gave mean reductions of 21% and 60% of plasma histamine respectively when the same concentrations were used (Table V).

TABLE V

EFFECTS OF DEXTRAN SULPHATE I AND DEXTRAN I ON THE RELEASE OF HISTAMINE FROM RABBIT BLOOD CELLS INCUBATED WITH DEXTRAN SULPHATE D/3 100 μG./ML.

Rabbit	Plasma Histamine (μg./ml. Blood)					
	Control	Dextran Sulphate D/3 100 μg./ml.	Dextran Sulphate D/3 100 μg./ml. Plus			
			Dextran I		Dextran Sulphate I	
			100 μg./ml.	500 μg./ml.	100 μg./ml.	500 μg./ml.
31	0·63	1·4			0·69	0·30
31	0·27	1·2	1·3	1·2	0·62	0·22
26	0·57	3·7	3·2	3·4	2·8	1·2
38	1·25	3·0	3·4	3·5	3·5	2·1
39	0·56	2·9	3·8	3·8	2·9	1·7
Mean percentage reduction of plasma histamine ..			−10	−10	21	60

These results suggested that sulphation of the dextran was necessary in order to obtain compounds capable of inhibiting the histamine release reaction, and it seemed possible that sulphation of other polysaccharides would give rise to the same properties. Recent work (Ricketts, 1952a) showed that sulphuric esters of polysaccharides of low molecular weight possessed anticoagulant activity *in vitro*. The effect of one of these, maltotriose sulphate, was compared with that of heparin in its ability to inhibit the histamine-releasing action of dextran sulphate D/3 in rabbit blood. The results are shown in Table VI. On a weight for weight basis, maltotriose sulphate was more effective as an inhibitor of histamine release than heparin; the mean reduction in plasma histamine after heparin 500 μg./ml. was 34%, and that after maltotriose sulphate 500 μg./ml. was 77%.

TABLE VI

INHIBITORY EFFECTS OF HEPARIN 500 μG./ML. AND MALTOTRIOSE SULPHATE 500 μG./ML. ON THE RELEASE OF HISTAMINE FROM RABBIT BLOOD CELLS INCUBATED WITH DEXTRAN SULPHATE D/3 100 μG./ML.

Rabbit	Plasma Histamine, μg./ml. of Blood			
	Control	Dextran Sulphate D/3 100 μg./ml.	Dextran Sulphate D/3 100 μg./ml. Preceded by Heparin 500 μg./ml.	Dextran Sulphate D/3 100 μg./ml. Preceded by Maltotriose Sulphate 500 μg./ml.
14	0·12	1·9	1·4	0·28
43	<0·14	0·78	0·17	0·12
2a	<0·09	1·6	1·2	0·37
4a	<0·09	1·0	1·0	0·40
30	0·16	1·8	1·1	
31	0·18	0·97	0·67	
Mean percentage reduction of plasma histamine ..			34	77

In order to confirm that dextran sulphate I and heparin were not interfering with the extraction or assay of histamine, four samples of dilute heparinized blood from the same rabbit were incubated with: (*a*), sodium oxalate to prevent histamine release, followed by dextran sulphate D/3; (*b*), dextran sulphate D/3 followed after incubation by sodium oxalate; (*c*), dextran sulphate D/3 followed after incubation by sodium oxalate, and then by dextran sulphate I; and (*d*), dextran sulphate D/3 followed after incubation by sodium oxalate and then by heparin. In each sample the final molarity of sodium oxalate was 0.019, and dextran sulphate D/3 100 μg./ml. was present during the incubation period. The concentrations of dextran sulphate I and heparin were similar to those used in previous experiments summarized in Tables V and VI. The results are presented in Table VII. Comparison of the plasma histamine

TABLE VII

FAILURE OF HEPARIN AND DEXTRAN SULPHATE I TO INTERFERE WITH THE EXTRACTION AND ASSAY OF HISTAMINE RELEASED FROM RABBIT BLOOD BY INCUBATION WITH DEXTRAN SULPHATE D/3

Rabbit	Plasma Histamine (μg./ml. Blood)			
	Blood Plus Sodium Oxalate, then Dextran Sulphate D/3 (100 μg./ml.) Before Incubation A	Blood Plus Dextran Sulphate D/3 (100 μg./ml.) Before Incubation, then Sodium Oxalate After Incubation B	Blood Plus Dextran Sulphate D/3 (100 μg./ml.) Before Incubation, then Sodium Oxalate and Dextran Sulphate I After Incubation C	Blood Plus Dextran Sulphate D/3 (100 μg./ml.) Before Incubation, then Sodium Oxalate and Heparin After Incubation D
35	0·38	2·3	1·9	2·5
39	0·13	3·1	3·0	2·9
33	0·06	1·2	1·2	1·2
40	0·22	1·6	1·5	1·5
32	0·14	3·7	3·7	3·5
Mean values	0·19	2·38	2·26	2·32

values given in columns B, C, and D makes it clear that neither dextran sulphate I nor heparin caused any reduction in the level of plasma histamine, so that the inhibitory effects on histamine release previously obtained could not be attributed to interference with the extraction or assay of plasma histamine.

DISCUSSION

These results show that certain samples of both dextran and dextran sulphate will release histamine, or some closely related substance, from the cells of rabbit blood *in vitro*. Of interest is the observation that dextran and dextran sulphate show differences in their behaviour as releasing agents at high concentrations. The quantity of histamine released by a particular fraction of either dextran or dextran sulphate depends upon its concentration. With dextran, the molecular weight of the fraction used is important in determining its activity.

The influence of concentration and molecular weight of dextran sulphate on its behaviour in biological reactions has been previously described by Walton (1952), who stressed the points of similarity between non-specific precipitation of fibrinogen by dextran sulphate at physiological *p*H and the precipitin reaction between antigen and antibody. It is interesting to note that the behaviour of dextran sulphate as a histamine-releasing agent and as a precipitant of fibrinogen has the following features in common. (1) Release of histamine and precipitation of fibrinogen occur only with samples of dextran sulphate above a certain molecular weight. (2) Optimum concentration of dextran sulphate is necessary for maximum histamine release or fibrinogen precipitation, since an excess inhibits either reaction. (3) Precipitation of fibrinogen and release of histamine are inhibited by heparin or by dextran sulphate of low molecular weight. Whilst these similarities may be incidental they would not be entirely unexpected if interaction between dextran sulphate and a protein constituent of the plasma was a prerequisite for the initiation of histamine release.

Since dextrans are polymers consisting of glucose molecules joined through 1:6 α glucoside linkages as branched chains (Ricketts and Walton, 1952), the intrinsic viscosity of any particular sample gives an indication of its molecular weight and the size of its constituent molecules. The dextrans used in these experiments were polydisperse, and therefore only the average molecular weights of the different fractions were known. Equal concentrations of dextrans having intrinsic viscosities from 0.12 to 0.44 gave rise to graded increases in the quantities of histamine released under the same conditions, in spite of the fact that increasing values of intrinsic viscosity were accompanied by parallel reductions of molarity.

In what way molecular size influences the histamine-release mechanism is not clear. The actual shape of the molecule may be of importance, and activation of the release mechanism may be directly related to molecular size, or may depend simply upon the presence of molecules above a certain critical size. In this respect, the smaller release of histamine obtained when using

dextran of intrinsic viscosity 0.64, as compared with those with values of 0.36 or 0.44, may have been owing to the smaller number of molecules present.

It seems probable that molecular weight is also a factor in determining whether a fraction of dextran sulphate will cause histamine release. No attempts were made to confirm this, since by analogy with the anticoagulant action of dextran sulphate (Ricketts, 1952b) it was thought that the degree to which a particular fraction had been sulphated might also prove to be an additional factor in modifying activity. Those samples which were available at the time had not been sulphated to the same extent.

Apart from confirming that the histamine released into rabbit plasma was derived from the cells, and not from the releasing agents themselves, or as a result of some action on the plasma constituents, no attempts were made to determine its origin. It seems likely, however, that it was derived from the platelets, which are considered to be the main carriers of histamine in rabbit blood (Code, 1952).

Those dextran sulphates effective in causing histamine release have also been reported to cause clumping of platelets *in vitro* and *in vivo* (Walton, 1954). The possibility exists, therefore, that consequent damage to platelets could be sufficient to cause liberation of their histamine. According to Rocha e Silva (1950), platelets form aggregates, and later disintegrate, during anaphylactic shock in the rabbit; this breakdown of platelets can be correlated with release of histamine. This is in contrast to the stable aggregations of platelets which form after the intravenous injections of various polysaccharides.

In the present experiments the fact that, above a certain level, increasing concentrations of dextran sulphate D/3 gave rise to decreasing levels of released histamine points to the presence in plasma or cells of some mechanism leading to histamine release which can be either reversed or blocked by the dextran sulphate. Such mechanism could be entirely independent of any direct damaging effect which a dextran or dextran sulphate may have on platelets.

It is rather striking that substances which inhibit the histamine release in rabbit blood by one particular agent are frequently effective against a variety of others. Sodium oxalate, in addition to preventing histamine release by dextran sulphate, has also been used to inhibit that due to a variety of primary amines, quaternary pyridinium esters, morphine (McIntire, Roth, and Sproull, 1951), to antigen-antibody reactions in normal rabbit blood (McIntire, Roth, and Sproull, 1950), and to addition of the specific antigen to blood from a sensitized rabbit—a property shared by heparin (Dragstedt, Wells, and Rocha e Silva, 1942; McIntire, Roth, and Richards, 1949). Heparin has also been shown to inhibit histamine release in normal rabbit blood by proteose and trypsin (Dragstedt, Wells, and Rocha e Silva, 1942). There were also indications that it would reduce the quantity of histamine released from the cells of normal rabbit blood incubated with washed antigen-antibody complex—a property shared by dextran sulphate I (Haining, 1953).

That the same inhibitors are effective in influencing the release of histamine due to such a variety of apparently dissimilar agents suggests that the number of pathways leading to histamine release in rabbit blood may be very restricted.

Summary

1. Certain fractions of dextran and of dextran sulphate were found to cause the release of histamine when incubated with rabbit blood.

2. With dextran, histamine release was dependent upon concentration and molecular weight. Samples having molecular weights between 22,000 and 1,000,000 were effective, but not those below 14,000.

3. Samples of dextran sulphate with molecular weights below 10,000 did not release histamine, but those with values from 40,000 to 440,000 showed a graded ability to do so. Dextran sulphate differed from dextran in that an optimum concentration was necessary in order to obtain maximum histamine release.

4. The release of histamine in rabbit blood by dextran sulphate D/3 was inhibited by sodium oxalate, dextran sulphate, heparin, and maltotriose sulphate. The importance of the ester sulphate groupings in this respect was shown by the fact that dextran (m.w.$<$14,000) was ineffective.

I wish to express my thanks to Professor A. C. Frazer for his continued interest in this work and for provision of the necessary facilities, and to Dr. P. B. Marshall for his sympathetic direction and advice.

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