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ACTIVATION OF RABBIT SERUM PROTEASE BY DEXTRAN SULPHATE

BY

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Evidence that activation of a proteolytic enzyme may play a part in anaphylactic reactions has been furnished by Rocha e Silva (1950) and Ungar and his co-workers (Ungar, 1947; Ungar and Mist, 1949). Their results are in accord with the hypothesis put forward by Bronfenbrenner (1948), in which histamine release is visualized as taking place during anaphylactic reactions as a consequence of proteolysis.

Among substances which produce anaphylactoid reactions, both peptone (Scroggie, Jaques, and Rocha e Silva, 1947) and trypsin (Kocholaty, Ellis, and Jensen, 1952; Dragstedt and Rocha e Silva, 1941) release cellular histamine and activate serum protease. Various polysaccharides activate protease when incubated with serum or plasma from certain species (Ungar, 1947; Geiger, 1952), and dextran sulphates which give rise to anaphylactoid reactions in guinea-pigs (Walton and Ricketts, 1954) release cellular histamine (Haining, 1955). The present work shows that certain fractions of dextran sulphate are also capable of activating serum protease.

METHODS

Blood was obtained from rabbits as described previously (Haining, 1955). When plasma was required, sodium oxalate at a final concentration of 1 mg./ml. was used instead of heparin. A volume of 1.5 ml. of serum or dilute plasma was incubated with either 0.5 ml. of a solution of 0.9% (w/v) NaCl, or 0.5 ml. of the same solution plus activator, for 3 min. at 37.5° C. and was then diluted with 40 ml. of distilled water. The euglobin fraction was precipitated and the proteolytic activity was estimated by the method described by Geiger (1952). Increases in optical density, due to colour-producing breakdown products of digested bovine fibrinogen, were converted to μg. of L-tyrosine by reference to a standard curve.

The code numbers of the dextran and dextran sulphate fractions used and their physical and biological properties have been described previously (Ricketts, 1952a, b; Ricketts and Walton, 1952; Walton, 1952; Haining, 1955).

RESULTS

Sodium oxalate prevents the *in vitro* release of histamine from rabbit blood cells by dextran sulphate (Haining, 1955). Since this might be due to inhibition of an enzyme, oxalate plasma seemed unsuitable for tests of protease activation. Heparinized plasma was also excluded because low concentrations of heparin activate protease, whereas higher concentrations inhibit activation (Ungar and Mist, 1949). Investigations were therefore begun with fresh rabbit serum.

When rabbit serum was incubated with dextran sulphate D/3 for 3 min. and the precipitated euglobin fraction incubated with denatured bovine fibrinogen, the amounts of colour-forming breakdown products increased with increasing concentrations of dextran sulphate from 4 to 100 μg./ml. When the concentration was increased to 500 μg./ml. there was a large reduction in protease activity (Table I).

TABLE I

ACTIVATION OF PROTEASE IN RABBIT SERUM BY DEXTRAN SULPHATE D/3

Conc. of Dextran Sulphate D/3 (μg./ml.)	Protease Activity (L-Tyrosine Equivalent, μg./ml. of Serum)						P
	Rabbit 27	Rabbit 33	Rabbit 26	Rabbit 32	Rabbit 38	Mean	
Control	14	16	6	28	33	19·4	—
4	30	30	20	18	48	29·2	>0·05
20	39	23	25	51	77	43·0	<0·02
100	63	72	46	74	86	68·2	<0·01
500	1	−6	29	8	0	6·4	>0·05

These results were strikingly similar to the effect of incubating samples of diluted rabbit blood with varying concentrations of dextran sulphate D/3, and to the resulting levels of plasma histamine (Fig. 1). The range of concentrations over which dextran

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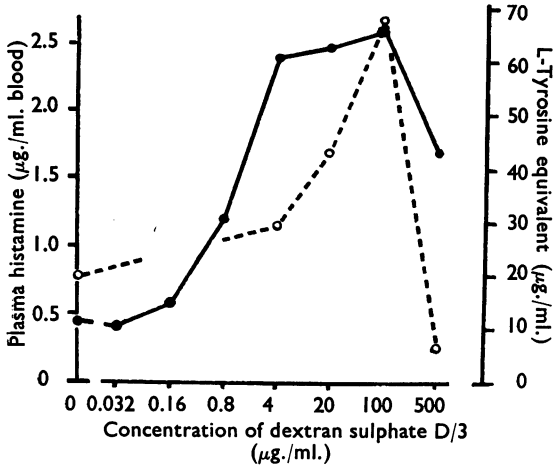

FIG. 1.—The release of histamine from rabbit blood cells and activation of protease in rabbit serum by dextran sulphate D/3. Closed circles, plasma histamine levels after incubating dilute heparinized rabbit blood with dextran sulphate D/3 for 30 min. at 37·5° C. Each point represents the mean value obtained with blood from the same 3 rabbits. Open circles, protease activity in serum after incubation with dextran sulphate D/3 for 3 min. at 37·5° C. Each point represents the mean value obtained with serum from the same 5 rabbits.

sulphate D/3 gave rise to protease activation in serum was within that previously found to release histamine in blood. A concentration of 500 μg./ml. inhibited both histamine release and protease activation.

The effect of incubating a mixture of euglobin fraction activated by dextran sulphate D/3 with a solution of denatured bovine fibrinogen for varying periods of time is shown in Fig. 2. Heating to 80° C. for 15 min. was sufficient to inactivate the euglobin fraction (Fig. 3). Dextran sulphate D/3 did not cause proteolysis when incubated with denatured bovine fibrinogen for 2 hr. at a concentration of 33 μg./ml., the concentration which would have been present if it were all precipitated with the euglobin fraction in the experiments of Table I.

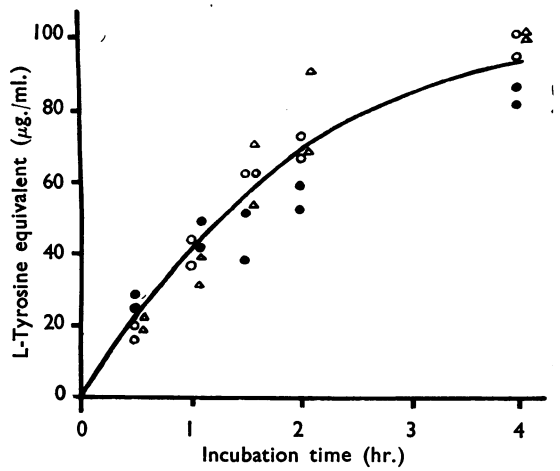

FIG. 2.—Digestion of heat-denatured bovine fibrinogen by the protease of rabbit serum. The protease was activated by incubating serum with dextran sulphate D/3 (100 μg./ml.) for 3 min. at 37·5° C.

The mean molecular weight of a fraction of dextran sulphate has been shown to be a factor in determining its ability to release histamine from rabbit blood cells *in vitro*. If protease activation were concerned in the histamine release reaction, those samples capable of releasing histamine would be expected to activate the protease in rabbit serum also. Three fractions of dextran sulphate were tested, D/3, E/1, and I; the corresponding molecular weights were approximately 440,000, 40,000, and less than 10,000. These were employed at a concentration of 100 μg./ml., since previous experiments had shown that this gave maximum activation in rabbit serum using dextran sulphate D/3. Significant increases in proteolysis were observed with dextran sulphate D/3 or E/1 ($P < 0.05$) but not with fraction I (Table II*a*).

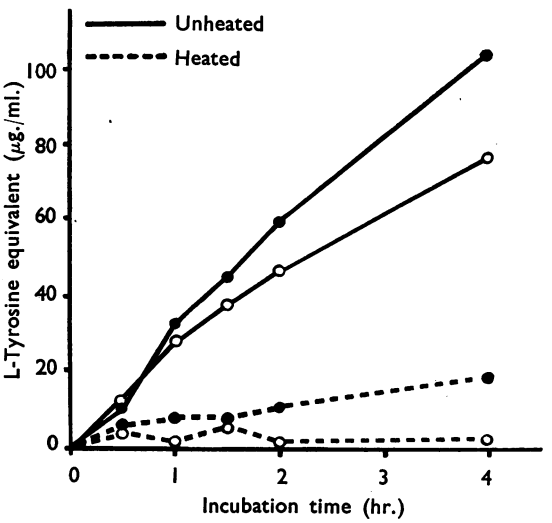

FIG. 3.—Inactivation of rabbit serum protease by heat. Open and closed circles distinguish the 2 different samples of serum used. Continuous lines join values obtained with unheated serum and dotted lines those obtained with serum which had been heated at 80° C. for 15 min. and then rapidly cooled.

When dextran sulphate D/3 was incubated with guinea-pig or pooled rat serum, the results were similar to those obtained with rabbit serum. At a concentration of 100 μg./ml. there was activation of serum protease, but with a higher concentration no

significant increase was obtained. With rat serum 500 μg./ml. gave a value very close to that of the control, and in guinea-pig serum a concentration of 700 μg./ml. had a similar effect (Table II*e* and *g*). Dextran sulphate D/3 also activated protease in oxalated rabbit plasma; concentrations of 4, 20, and 100 μg./ml. of diluted plasma caused graded increases in proteolysis (Table II*c*) comparable with those obtained in rabbit serum (Table I).

No activation of protease was observed in rat serum after incubation with New Zealand agar at a concentration of 830 μg./ml. for 3 min. The same sample of agar was tested for its ability to release histamine from rabbit blood cells *in vitro* by the method previously described (Haining, 1955). In one experiment, incubation of rabbit blood with agar (100 μg./ml.) for 30 min. gave a plasma histamine level of 1.2 μg./ml. compared with 0.14 μg./ml. of blood for the control sample, whereas a concentration of 710 μg./ml. in a different sample of blood increased the level from 0.25 μg./ml. to 2.2 μg./ml.

Dextran D caused insignificant increases in protease activity in rabbit serum, rat serum or oxalated rabbit plasma even with concentrations as high as 50 mg./ml. (Table II).

TABLE II

PROTEASE ACTIVATION IN SERUM AND PLASMA

System	Conc. of Polysaccharide (μg./ml.)	L-Tyrosine Equivalent (μg./ml. Serum or Dilute Plasma)	No. of Observations	*P*
a Rabbit serum	Control	17·0		
	Dextran sulphate I 100	30·4	5	>0·05
	,, ,, E/1 100	47·4	5	<0·05
	,, ,, D/3 100	55·8	5	<0·01
b Rabbit serum	Control	16·8		
	Dextran D 2,000	16·4	5	
	,, 10,000	18·3	6	
	,, 50,000	21·7	6	>0·05
c Oxalated rabbit plasma	Control	6·6		
	Dextran sulphate D/3 4	15·4	5	<0·02
	,, ,, 20	24·1	5	<0·01
	,, ,, 100	30·9	5	<0·05
d Oxalated rabbit plasma	Control	6·6		
	Dextran D 2,000	6·3	5	
	,, 10,000	9·5	5	>0·05
	,, 50,000	9·9	4	>0·05
e Pooled rat serum	Control	4·0		
	Dextran sulphate D/3 20	9·9	5	>0·05
	,, ,, 100	19·0	4	<0·01
	,, ,, 500	5·2	5	>0·05
f Pooled rat serum	Control	2·4		
	Dextran D 2,000	0·7	3	
	,, 10,000	5·3	4	>0·05
	,, 50,000	13·3	4	>0·05
g Guinea-pig serum	Control	96		
	Dextran sulphate D/3 100	172	10	<0·05
	,, ,, ,, 700	80	7	>0·05

DISCUSSION

There is considerable indirect evidence to suggest that histamine release may in some circumstances depend upon a proteolytic mechanism (Ungar, 1953; Rocha e Silva, 1950). A close correlation exists between histamine release and protein breakdown both during antigen-antibody reactions *in vitro*, and during incubation of normal tissue with Tween 20, octadecylamine, morphine sulphate, and compound 48/80. Furthermore, these agents and also peptone and a variety of polysaccharides have been shown to activate the proteolytic enzyme present in guinea-pig serum (Ungar, Damgaard, and Hummel, 1953; Ungar and Damgaard, 1955). In the present experiments, the parallelism between histamine release and protease activation by dextran sulphate is sufficiently close to suggest a relation between the two phenomena.

It is difficult to explain the inactivity of dextran D as a protease activator. Since dextran and dextran sulphate are so closely related structurally, it is unlikely that the mechanisms by which they release histamine differ to any great extent. Failure to activate protease by a dextran known to be capable of releasing histamine may therefore indicate that the activation of protease by dextran sulphate is unrelated to histamine release. However, it is significant that agar—which like dextran D also released histamine when incubated with rabbit blood and yet failed to activate rabbit or rat serum protease—does activate protease in guinea-pig serum (Ungar and Mist, 1949). Humphrey and Jaques (1955) found that rabbit plasma incubated with agar developed proteolytic activity towards denatured haemoglobin. The possibility therefore remains that failure to demonstrate activation of protease in the present experiments can be attributed to failure to employ optimum conditions.

The significance of the inhibitory effect of high concentrations of dextran sulphate on histamine release and protease activation is not clear. Similar effects have been observed in other *in vitro* anaphylactic and anaphylactoid reactions (Ungar *et al.*, 1953), in the precipitin reaction between antigen and antibody, and in the precipitation which occurs when increasing concentrations of dextran sulphate of high molecular weight are added to a solution of fibrinogen (Walton, 1952). Furthermore, when excess antigen is incubated with tissue from a sensitized animal, the amount of protease activation is diminished (Ungar *et al.*, 1953; Geiger, 1952).

Summary

1. Certain fractions of dextran sulphate activated protease when incubated with oxalated rabbit plasma or the sera of rabbits, rats and guinea-pigs. No protease activation was observed with dextran (approximate mol. wt. 220,000).

2. The samples of dextran sulphate which activated protease were the same as those which released histamine from rabbit blood cells; they had molecular weights of approximately 40,000 and 440,000. A sample with a molecular weight of 14,000 was inactive.

3. When dextran sulphate (approximate mol. wt. 440,000) was incubated with rabbit serum there was an optimal concentration for maximum activation of protease. The range of concentrations over which protease activation occurred was within that previously found to cause histamine release in rabbit blood.

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