

DESENSITIZATION WITH HAPTENS

Role of Molecular Size in Specific Precipitation, Anaphylaxis, and Desensitization With Dextran Fractions

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SENSITIZED ANIMALS have been made tolerant of multiples of normally fatal doses of allergens in a variety of ways, all of which were transient. Theoretically, lasting desensitization should follow neutralization of sensitizing antibody with specific haptens. This is one of a series of studies of desensitization with haptens,¹⁻⁵ the goal of which is to produce permanent desensitization in actively sensitized animals.

Dextran is an allergen in man.⁶ This limits its usefulness as a plasma expander. It seemed appropriate to study the problem of desensitization of dextran-sensitive animals with a series of haptenic fractions of dextran. Dextran seemed particularly suited to this type of experiment because it has been shown that: (1) it can elicit anaphylaxis in and desensitize passively sensitized guinea pigs specifically⁵; (2) partial hydrolysis results in striking diminution of the capacity of native dextrans to produce wheal and flare reactions in man⁶; (3) dextran fractions with average molecular weights of 3,000 to 4,000 precipitated much less rabbit anti-*Leuconostoc mesenteroides* antibody than did larger dextrans⁷; (4) dextran fractions with average molecular weights of about 10,000 removed only about two fifths as much precipitable antibody as did the native dextran^{8a}; (5) very small dextran fractions (isomaltodextrins) will inhibit precipitation in the human anti-dextran system^{8b}; and (6) low molecular haptens could produce transient desensitization.⁹

Those observations suggested the possibility that dextran fractions would desensitize passively sensitized guinea pigs and that, if this were so, their desensitizing capacities might be related to their molecular weights and to their capacities to precipitate, to inhibit precipitation, and to elicit anaphylaxis. Accordingly native dextran was subjected to a series of fractionation procedures.

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Studies of these fractions showed that progressive molecular cleavage transformed dextran from: (1) an anaphylactogen to anaphylactogenically inactive fractions, (2) an anaphylactogen to specific inhibitors of anaphylaxis, and (3) a specific precipitator to specific inhibitors of precipitation.

MATERIALS AND METHODS

*Antiserum.**—A pneumococcus Type II rabbit serum, which cross precipitated with dextran,^{10, 11} was used (1) to sensitize the guinea pigs used to study the anaphylaxis-provoking and desensitizing effects of low molecular weight dextran fractions; (2) to study specific precipitation by native dextran and its low molecular weight fractions, and (3) to study the ability of low molecular weight dextran to inhibit immune precipitation with native dextran.

Dextran.—Native enzymatically synthesized NRRL B 512-E-SR dextran and clinical dextran, prepared from the same B-512 strain of *L. mesenteroides* by Wyeth Laboratories, were used for the preparation of low molecular dextrans by hydrolytic (acid and enzymatic) degradation and subsequent fractional precipitation. The variations in size of the low molecular fractions were narrowed by precipitation with alcohol and ether, eliminating "head and tail" fractions. Since the individual dextran molecule has only one reducing group, the *Number Average Molecular Weight* (Mn) was determined, as outlined by Isbell and co-workers,¹² using the Folin-Wu method and anhydrous dextrose as reference standard. Technical data on the dextran fractions are summarized in Table I.

Quantitative Immune Precipitation.—Measured quantities of dextran fractions (abscissa, Fig. 1) were dissolved in 1.0 ml. saline, added to 0.5 ml. diluted antiserum (1:3 with saline), and incubated for one hour at 37° C. and then for 24 hours at 0° C. The precipitates were then collected by centrifugation, washed once with chilled saline, and dissolved in 0.1 normal NaOH. The density of the solution was read (Beckman, Model DU with photomultiplier tube, 288 mμ wave length, 0.5 mm. slit width). The values obtained were converted to immune precipitate nitrogen (ordinate, Fig. 1) by means of the extinction coefficient for rabbit antibody E $\frac{1\%}{1 \text{ cm.}}$, which was found to be 16.4 under our conditions.

Inhibition Assays.—Measured quantities of the low molecular dextran fractions (dissolved in 0.5 ml. saline) were added to 0.5 ml. of diluted antiserum (see Fig. 2). After 10 minutes at 37° C., a quantity of native dextran (SR) in 0.5 ml. saline was added to each tube, and also to tubes containing the same amount of antiserum but no low molecular dextran. After further incubation (1 hr. at 37° C., 24 hr. at 0° C.) the immune precipitate was collected and measured as described in the previous paragraph.

*The generous supplies of this serum (Wyeth No. 461) and clinical dextran donated by Wyeth Laboratories in Marietta, Pa., are gratefully acknowledged. The serum was found to contain 3,150 mcg. Ab N per milliliter precipitating with the homologous polysaccharide.

Assay of the Challenging and Desensitizing Effects in Anaphylaxis.—Forty-six guinea pigs, weighing between 180 and 230 grams, were passively sensitized by intraperitoneal injections of 1.0 ml. of diluted antipneumococcal Type II serum (Wyeth No. 461) containing 200 meg. of anti-native dextran-precipitating antibody nitrogen. This also contained 70 to 80 meg. of anti-fraction DF₁ precipitating antibody nitrogen, but no precipitating antibody against fractions HF₁ and HF₅. Forty-eight hours later, known quantities of native dextran and low molecular dextran fractions dissolved in 0.1 ml. saline (see Table II) were injected into a saphenous vein, keeping the injection time as close to 8 to 10 seconds as possible. The challenging effects of native and low molecular dextran fractions were compared. The low molecular fractions were injected in large quantities for better demonstration of any anaphylactogenic action. All animals which had received the low molecular weight fractions were re-injected with native dextran at specified time intervals (see Table III) for evaluation of any desensitizing effects of the low molecular weight dextrans. The degree and duration of desensitization were observed.

TABLE I. TECHNICAL DATA ON PREPARATION AND MOLECULAR WEIGHTS OF THE DEXTRAN FRACTIONS

FRACTION	HYDROLYSIS	ALCOHOL FRACTIONATION	Mn*
B-512-E SR native†	0	insol. 36.6 - 38.5% ethanol	9 Mill.‡
B-512-F DF ₁	10 per cent solution of clinical dextran (Wyeth) in 0.1 N HCl, 99° C. for 60 min. under reflux	insol. 30 - 40% isopropanol	14,000
B-512-F DF ₂		insol. 40 - 50% i.p.a.	8,500
B-512-F DF ₃		insol. 50 - 60% i.p.a.	5,000
B-512-F DF ₄		insol. 60 - 80% i.p.a.	2,200
B-512-F HF ₁	10 per cent solution of clinical dextran (Wyeth) in 0.4 N HCl, 99° C. for 135 min. under reflux	insol. 50 - 60% i.p.a.	1,500
B-512-F HF ₃		insol. 70 - 80% i.p.a.	950
B-512-F HF ₅		insol. 90 - 95% ether (of total vol.)	850
B-512-F EH ₁	24 hr. at 25° C. { 10 per cent solution of clinical dextran (Wyeth) in buffered saline pH5; 1 mg. dextranase (<i>Penicillium funiculosum</i> NRRL 1,768§) per 20 Gm. dextran	insol. 30 - 40% i.p.a.	17,000
B-512-F EH ₂	48 hr. at 25° C. {	insol. 40 - 50% i.p.a.	8,700
B-512-F EH ₃		insol. 50 - 60% i.p.a.	5,400
B-512-F EH ₄		insol. 60 - 80% i.p.a.	2,900

*Number average molecular weight.

†Enzymatically synthesized. Made available by Dr. C. A. Wilham, U.S. Agricultural Research Service, Peoria, Ill.

‡Based on light scattering determination.

§Made available by Dr. H. M. Tsuchiya, U.S. Agricultural Research Service, Peoria, Ill.

RESULTS

Technical details of hydrolysis, isolation, and the molecular weights of the individual dextran fractions are given in Table I. It can be seen that the fractions obtained by mild acid and heat hydrolysis (DF_1 to DF_4) and the fractions obtained by enzymatic hydrolysis (EH_1 to EH_4) have approximately the same molecular size. Intensified acid and heat hydrolysis and subsequent isopropyl alcohol fractionation seemed to result in fractions of somewhat lower molecular size (HF_1 to HF_5). Fraction HF_1 with an average molecular weight of 1,500 may be assumed to consist chiefly of isomaltooctase and an occasional larger polymer. Fractions HF_3 and HF_5 with average molecular weights of 950 and 850, respectively, probably contained isomaltodextrins ranging from isomaltotriose to pentaose, with an occasional larger polymer. Although the number average molecular weight (M_n) does not give the exact degree of polydispersity, it was believed that estimates of the molecular sizes in the individual dextran fractions, based on the technical data in Table I, would be sufficiently accurate for these experiments.

Precipitating Effects.—The precipitation curves for fractions SR, DF_1 – DF_4 , and EF_1 – EF_4 , reacting with the rabbit antipneumococcal Type II serum are given in Fig. 1. Whereas the large molecular dextran SR (molecular weight 9,900,000) precipitated 98 meg. antibody nitrogen at the point of maximum precipitation, fraction DF_1 (M_n 14,000) precipitated only 35 meg.; fraction DF_2 (M_n 8,500) precipitated 19 meg.; fraction DF_3 (M_n 5,000) precipitated 5 meg. antibody nitrogen; fraction DF_4 (M_n 2,200) caused no precipitation. The enzymatically obtained fractions EH_1 – EH_4 showed a similar progressive loss of immune precipitating effect with decreasing molecular weight. This effect seemed slightly better preserved in the EH fractions than in the comparable DF fractions. This may have been due to the fact that the molecular size of the EH fractions was slightly larger than in the corresponding DF fractions. It will be noted also in Fig. 1 that the point of maximum precipitation is reached in the small molecular fractions at lower antigen concentrations than in the larger fractions. This is consistent with the idea that the precipitation was due primarily to the smaller molecules, but does not rule out a significant contribution by larger molecules. It is also consistent with the development of inhibition by small fragments of dextrans. None of the HF fractions had any precipitating effect in this immune system, so they were not included in Fig. 1.

Specific Inhibition of Immune Precipitation.—The small molecular weight dextran fractions were studied for their capacities to inhibit specific precipitation in this pneumococcus Type II antiserum-native (SR) dextran system (Fig. 2). There was no detectable difference in the inhibitory effect of dextran fractions DF_1 to HF_3 , with molecular weights ranging from 14,000 to 950. Therefore, the inhibition of immune precipitation by fraction HF_3 , in the upper part of Fig. 2, exemplifies also the inhibition by the low molecular fractions DF_1 to HF_1 (Table I). Curve SR shows the immune precipitation of the native

dextran. The lower curves in the upper half of Fig. 2 show the amount of precipitation caused by adding 500 or 2,000 mcg. HF_3 to the antiserum before SR was added. Note that 2,000 mcg. of the HF_3 low molecular (Mn 950) dextran inhibited immune precipitation completely. This was roughly twenty times the amount of native dextran which gave maximum precipitation. The

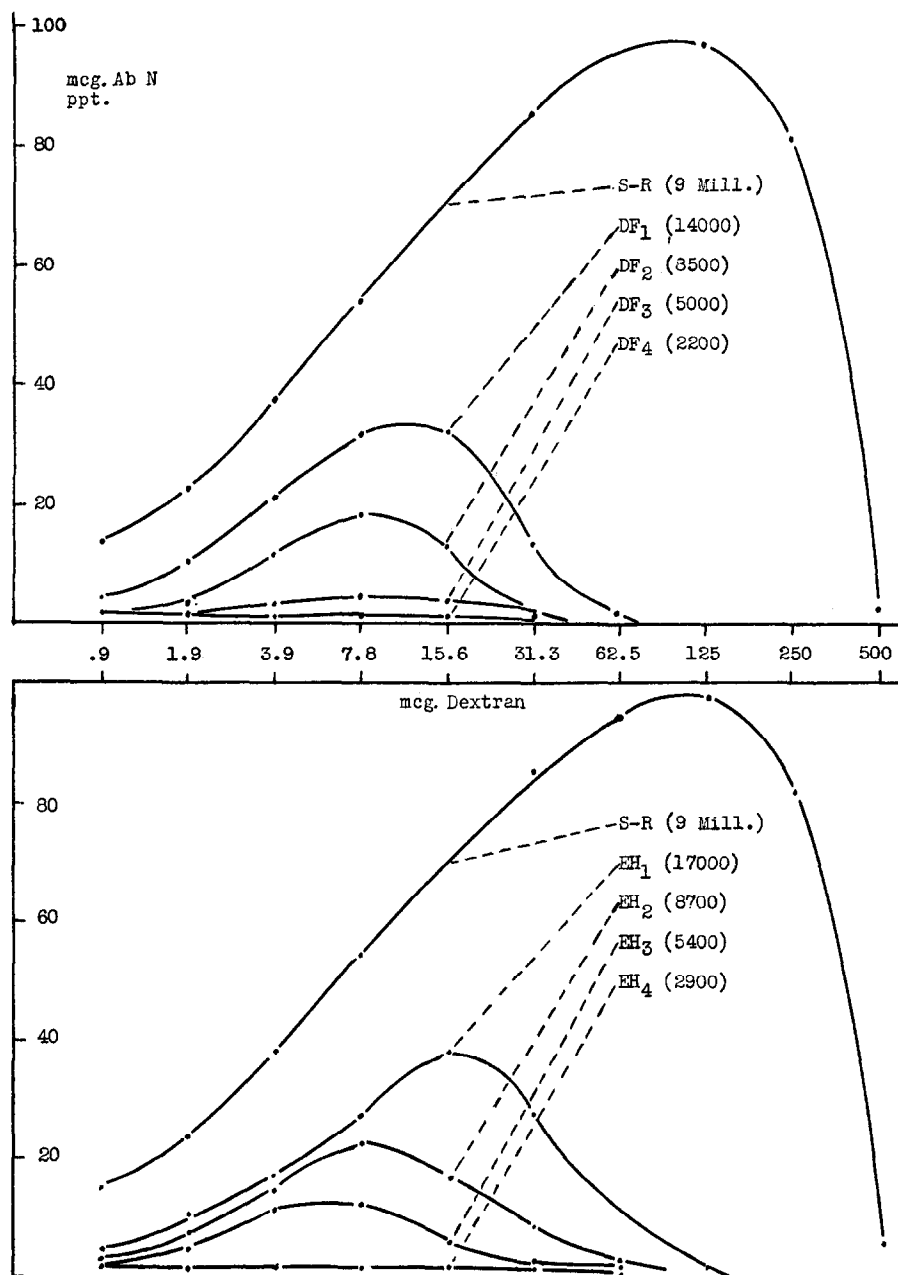


Fig. 1.—Immune precipitation of dextran fractions, effect of decreasing molecular weight (see text).

lower half of the figure demonstrates that fraction HF_5 had much less inhibitory activity than fraction HF_3 . But the addition of even 8,000 mcg. of HF_5 to the antiserum did not abolish precipitation entirely.

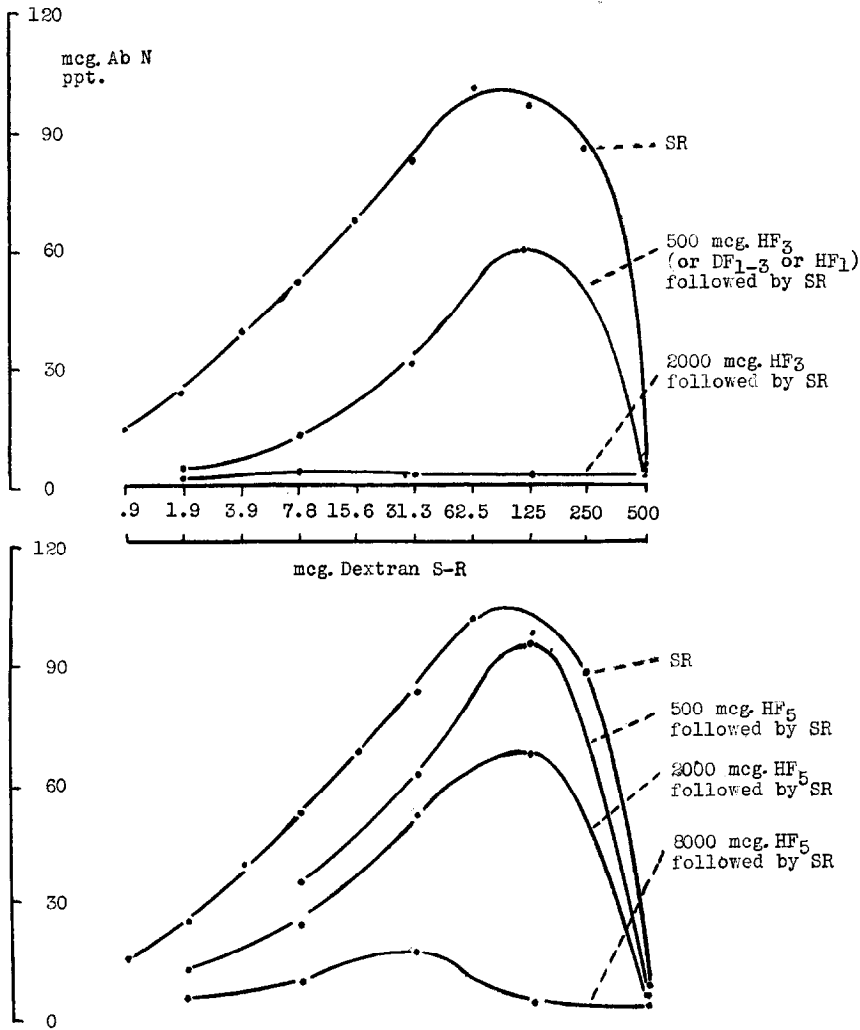


Fig. 2.—Inhibition of immune precipitation of dextran by low molecular fractions.

Challenging and Desensitizing Effects of the Fractions in Passive Anaphylaxis.—Guinea pigs passively sensitized by intraperitoneal injections of rabbit antipneumococcal Type II serum were challenged by intravenous injections of dextran fractions 48 or 72 hours later. As seen in Table II, 1.66 mg. of native SR dextran produced fatal anaphylaxis in 3 sensitized guinea pigs; 0.55 mg. killed 11 of 12 animals, while one exhibited a severe anaphylactic shock. When challenged with 0.18 mg. of native dextran, one animal died, one had a moderate reaction, and 2 exhibited no anaphylactic symptoms. It was concluded that a challenging dose of 0.55 mg. native dextran could be expected to elicit fatal anaphylaxis in the large majority of animals and could be considered to be approximately LD_{50} .

TABLE II. ANAPHYLACTOGENIC EFFECTS OF DEXTRAN FRACTIONS*

NO. OF GUINEA PIGS	INTERVAL AFTER SENSITIZATION (HR.)	FRACTION USED (MOL. WT.)	CHALLENGE DOSE I.V. (MG.)	ANAPHYLACTIC REACTION			
				AD†	SEVERE	MODERATE	NONE
3	48	Native SR (9 Mill.)	1.66	3	1		
8	48		0.55	7			
4	72		0.55	4			
4	48		0.18	1		1	2
10	48	DF ₁ (14,000)	50.0			1	9
8	48	HF ₁ (1,500)	50.0				8
9	48	HF ₅ (850)	50.0				9

*Passive sensitization: 1.0 ml. diluted rabbit antipneumococcal Type II serum intraperitoneally, containing 200 mcg. ab N precipitating with native SR dextran or 70-80 mcg. Ab N precipitating with fraction DF₁.

†Anaphylactic death.

The effects of Wyeth clinical dextran in anaphylaxis and precipitation (not shown in Fig. 1 and Table II) were found to approximate those of native dextran very closely.

Neither of the low molecular fractions (HF₁ and HF₅) produced anaphylactic reactions in the passively sensitized guinea pigs even when 100 times the LD₅₀ of native dextran (Table II) was given. Fraction DF₁ elicited a moderate anaphylactic reaction in 1 of 10 animals.

The desensitizing effect of the low molecular dextrans was then tested by challenging, with native SR dextran, those guinea pigs which had been given the large doses of fractions DF₁, HF₁, HF₅ (Table III). The time interval between the injection of the low molecular fractions and the native SR dextran varied between 14 min. and 24 hr. The challenging doses of native SR dextran were 1 or 3 LD₅₀ (0.55 mg. and 1.66 mg.). It may be seen that native dextran elicited no anaphylactic reaction in the group which had been given the DF₁ (Mn 14,000) fraction previously. Apparently the large dose of the low molecular DF₁ fraction had desensitized them for at least 24 hours. In the

TABLE III. INHIBITION OF ANAPHYLAXIS BY LOW MOLECULAR DEXTRAN FRACTIONS (DESENSITIZATION)*

GUINEA NO. OF PIGS	LOW MOLEC- ULAR FRAC- TION (DOSE)	RE-CHALLENGE		ANAPHYLACTIC REACTION				DESENSITI- ZATION
		TIME AFTER 1ST CHAL- LENCE	DOSE OF SR (MG.)	AD†	SEVERE	MODERATE	NONE	
3	DF ₁ 50 mg.	20-30 min.	0.55				3	Effective and lasting > 24 hr.
3	DF ₁ 50 mg.	20-30 min.	1.66				3	
4	DF ₁ 50 mg.	24 hr.	1.66				4	
2	HF ₁ 50 mg.	15-20 min.	0.55				2	Effective but transient; > 20 min., < 3 hr.
3	HF ₁ 50 mg.	15-20 min.	1.66				3	
2	HF ₁ 50 mg.	3 hr.	1.66	2				
1	HF ₁ 50 mg.	3 hr.	0.55	1				
1	HF ₅ 50 mg.	14 min.	0.55				1	Weak and transient; > 14 min., < 40 min.
3	HF ₅ 50 mg.	10-20 min.	1.66		3			
5	HF ₅ 50 mg.	20-40 min.	1.66	4		1		

*The passively sensitized guinea pigs, after injection with the low molecular dextrans, received 1 or 3 LD₅₀ of native SR dextran.

†Anaphylactic death.

group given fraction HF₁ (Mn 1,500) the desensitization was transient. All of those animals died when rechallenged with the native dextran 3 hours after they had received the HF₁ dextran. The desensitization, if any, afforded by the HF₅ (Mn 850) fraction was weak and was almost gone when the pigs were rechallenged 20 to 40 min. later.

DISCUSSION

The decrease in precipitating activity with decreasing molecular size of B-512 dextran fractions is illustrated in Fig. 1. No significant difference was observed when the precipitation curves obtained with low molecular weight dextrans produced by acid and heat hydrolysis were compared with the curves of the enzymatically hydrolyzed dextrans of corresponding molecular size. Fractions DF₁ and EH₁, with average molecular weights of 14,000 and 17,000, respectively, precipitated less than 50 per cent as much antibody (35 meg.) as was precipitated by native SR dextran (98 meg.). Some precipitating activity remained in fractions DF₃ and EH₃ with average molecular weights of 5,000 to 5,400. But at the average molecular weights of 2,200 to 2,900, the fractions ceased to produce precipitates. These observations are similar to those of Glynn and associates⁷ that dextran of an average molecular weight of 3,000 to 4,000 was markedly less active in precipitating rabbit dextran antibody than was dextran of an average molecular weight of 25,000. Our results are also in agreement with those obtained by Goodman and Kabat.^{8a} Their fraction 2 (average molecular weight 35,000) precipitated 42 per cent of the antipneumococcal antibody nitrogen precipitable by native dextran B-512. Their fraction 1 (average molecular weight 10,000) precipitated 41 per cent of the antibody nitrogen precipitable by native B-512 dextran. Our DF₁ fraction (Mn 14,000) precipitated 36 per cent of the pneumococcal antibody nitrogen precipitable by native B-512 dextran. The small difference may be accounted for, in part, by our shorter incubation and aging period (24 hours at 0° C. instead of their 7 days) prior to the collection of the immune precipitate.

The effect of our low molecular weight dextran fractions in inhibiting immune precipitation of native SR dextran was about the same for the range of 14,000 to 950 Mn. The marked decrease of the inhibitory effect of HF₅ (Mn 850) suggests that this fraction consisted of isomaltodextrins, the majority of which were probably smaller than isomalto-pentaose, which was shown by Goodman and Kabat^{8c} to be the most effective inhibitor in this cross-precipitating system.

One purpose of this investigation was to determine if there was any correlation between the progressively reduced molecular weights of the dextran fractions and the loss of their precipitating, anaphylactogenic, and desensitizing powers. This was planned in the hope that such a correlation would lead to easy and reliable predictions of the desensitizing capacity of a variety of haptens.

The interesting observation was made that, with decreasing molecular size, the anaphylactogenic effect of the dextran fractions was apparently lost sooner

than the precipitating effect. This is shown by the fact that fraction DF₁ (Mn 14,000) produced (nonfatal) anaphylaxis in only one of 10 passively sensitized guinea pigs, although it precipitated 36 per cent as much antibody as was precipitated by the native dextran. Those pigs were sensitized with 200 meg. of precipitating anti-native dextran antibody nitrogen, or about 75 meg. of antibody nitrogen precipitable by fraction DF₁. It had been shown previously that 55 meg. anti-dextran antibody nitrogen from this same Type II antiserum would sensitize guinea pigs regularly against native dextran.⁵ Fatal anaphylactic sensitization has been induced in guinea pigs by Kabat and Benacerraf¹³ with as little as 30 meg. of antibody nitrogen. It is difficult to explain the failure of 75 meg. of precipitating anti-DF₁ antibody nitrogen (plus an unmeasured amount of univalent anti-DF₁ antibody) to sensitize pigs effectively against large amounts of DF₁ (Mn 14,000), even when allowances are made for the differences in sensitizing capacity of various antisera. Ovary and Karush¹⁴ reported a somewhat similar inability to provoke passive cutaneous anaphylaxis with monovalent haptens (not dextrans).

There was no apparent correlation between the capacities of the low molecular dextran fractions to inhibit immune precipitation and to desensitize guinea pigs. For example, fraction HF₁ (Mn 1,500) inhibited immune precipitation as well as did fraction DF₁ with a molecular weight of 14,000. But DF₁ (Mn 14,000) maintained desensitization much more effectively than fraction HF₁ (Mn 1,500). By the same token, large amounts of fraction HF₅ (Mn 850) still inhibited immune precipitation, whereas the desensitizing effect of HF₅ was negligible. The short duration of specific desensitization with low molecular haptens is in accord with the early observations of Tillet, Avery, and Goebel.⁹ Ovary and Karush,¹⁴ who studied the role of other haptens in passive cutaneous anaphylaxis, thought that "the transitory protection by the hapten is due to its ready dissociation from antibody (and its rapid removal from the site of reaction) . . . which is thus rendered available for further reaction with the complete antigen."

These experiments indicate that lasting desensitization with related polysaccharides should be sought in haptens with molecular weights in excess of 14,000.

The Mn 14,000 dextran fraction was weakly anaphylactogenic, but the Mn 1,500 fraction was not. Both of these fractions produced desensitization. Dextran is an allergen in man. This limits its usefulness as a plasma expander. These findings suggest that reactions to infusions of clinical dextran could be prevented by prior desensitizing infusions of dextran fractions, with molecular weights larger than 1,500 and smaller than 14,000.

SUMMARY

Clinical dextran was subjected to varying degrees of acid and enzymatic hydrolysis and fractionation with alcohol and ether. The number average molecular weights (Mn) of the different fractions varied from 17,000 to 850.

Cross-precipitation with Type II antipneumococcal rabbit serum decreased with the molecular size of the fractions. Precipitation occurred with the Mn 5,000 but not with the Mn 2,200 fraction.

Precipitation was inhibited about equally by fractions Mn 14,000 to 950. There was much less inhibition by a smaller fraction (Mn 850.)

Native and clinical dextrans elicited fatal anaphylactic shock regularly in guinea pigs which had been passively sensitized with Type II antipneumococcal rabbit serum. The Mn 14,000 dextran fraction elicited a moderate reaction in only one of 10 similarly sensitized animals. Smaller fractions, even in large doses, did not provoke anaphylaxis at all. Varying degrees of desensitization against native dextran followed large challenging doses of several of the fractions. Desensitization was complete for 24 hours with the Mn 14,000 fraction, complete but transient (less than 3 hours) with the Mn 1,500, and negligible with the Mn 850 fraction.

Studies of these fractions showed that progressive molecular cleavage transformed dextran from (1) an anaphylactogen to anaphylactogenically inactive fractions, (2) an anaphylactogen to specific inhibitors of anaphylaxis, and (3) a specific precipitator to specific inhibitors of precipitation. But there was no parallelism between the progressive reduction in molecular weights of the fractions and the loss of precipitating, anaphylactogenic inhibitory and desensitizing powers.

Dextran is an allergen in man. This limits its usefulness as a plasma expander. These findings suggest the need to investigate the possibility that reactions to infusions of clinical dextran could be prevented by prior desensitizing infusions of dextran fractions, with molecular weights larger than 1,500 and smaller than 14,000.

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