STUDIES ON ANTIGENIC COMPETITION*

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At times, the simultaneous presentation of two antigens results in a diminution of the antibody response to one or both antigens. This phenomenon, generally referred to as antigenic competition, has been extensively reviewed by Adler (1, 2). The mechanism of the phenomenon remains unknown.

We have previously studied antigenic competition using two haptenic determinants (3). We found that antibody production to the haptenic groups was depressed when the two antigens were injected simultaneously. The degree of depression of antibody synthesis was the same whether the two haptens were located on the same or on separate carrier molecules. It was also shown that passive antibody to one hapten resulted in suppression of the antibody response to that hapten without affecting the antibody response to the second hapten, provided the two haptenic determinants were located on the same carrier molecule. When the two determinants were located on separate carrier molecules, passive antibody to one resulted in suppression of the antibody response to that determinant and an increase in antibody production to the second determinant.

The experiments to be reported here aim at gaining a greater insight into the mechanism of antigenic competition and its relation to the control of antibody synthesis. Antibody formation against two haptenic determinants located on separate carrier molecules was studied. (*a*) It was found that the extent of competition (the degree of depression of the antibody response) increases as the dose of the competing antigens is increased. (*b*) Antigenic competition is relatively independent of the nature of the carrier molecule. (*c*) Although competition results in a depression of the antibody response to a particular determinant, the affinity of the antibody produced to that determinant is essentially equal to the affinity of the antibody produced by control animals immunized with a single antigen. (*d*) Antigens injected simultaneously, but into separate sites do not compete, suggesting that antigenic competition is not mediated by a circulating factor.

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Materials and Methods

Animals.—New Zealand rabbits weighing 2.0–2.5 kg were used throughout.

Antigens.—Dinitrophenylated (DNP)[1] proteins were prepared according to Eisen et al. (4–6) by reacting the proteins with 2,4-dinitrochlorobenzene at room temperature under alkaline conditions. The product was purified by extensive dialysis.

Arsanilic acid derivatives (R-Azo) of proteins were prepared as follows: For coupling with 1 g of protein, 0.104 g of *p*-arsanilic acid, 0.011 g of KBr, and 0.12 ml of 1 N HCl were dissolved in 13 ml of water and cooled in an ice salt bath. A cold solution of 0.034 g of $NaNO_2$ in 2 ml of water was added to the arsanilic acid solution dropwise during a 2 min period. The resulting solution was added dropwise during a 15 min period to 1 g of protein dissolved in 0.1 M borate buffer at pH 9.5. The reaction was carried out in the cold and the pH was maintained at 9.5 by the addition of NaOH. The product was dialyzed against phosphate-buffered saline (PBS—0.15 M NaCl, 0.1 M potassium phosphate buffer, pH 7.4) and then precipitated by acidification to pH 3.5 with 5 N acetic acid. The precipitate was dissolved and dialyzed extensively against 0.001 M potassium phosphate buffer, pH 7.4.

The concentration of hapten-conjugated proteins was determined by drying known volumes to constant weight at 95–100°C. The dry weights so obtained were corrected for the weight of the buffer present. The degree of DNP substitution was estimated spectrally by assuming that all hapten groups were coupled to ϵ-amino groups of lysine and by using the molar absorbancy of free ϵ-DNP-L-lysine (17,530 at 360 mμ) (7). The degree of substitution of R-Azo derivatives was roughly estimated from their absorbancy in 0.1 N NaOH at 460 mμ, assuming all R-Azo groups were coupled to tyrosine (ϵ = 9,600 at 460 mμ) (8). The values reported for the number of hapten groups per mole of protein are admittedly only rough estimates. However since a single antigen preparation was used for each experiment and, since none of the conclusions are dependent upon an accurate knowledge of the degree of substitution, the use of more critical methods was deemed unnecessary.

The following compounds were prepared (subscripts refer to the estimated number of hapten groups per molecule, assuming a molecular weight of 150,000 for bovine and rabbit gamma globulin and 45,000 for egg albumin): dinitrophenyl bovine gamma globulin (DNP_{57}-BGG), dinitrophenyl rabbit gamma globulin (DNP-RGG), dinitrophenyl egg albumin (DNP_{14}-EA), dinitrophenyl bovine fibrinogen (DNP-BF), and arsanilate-azo-bovine gamma globulin (R-Azo_{27}-BGG), arsanilate-azo-bovine fibrinogen (R-Azo-BF) and arsanilate-azo-rabbit gamma globulin (R-Azo-RGG).

Immunization Procedures.—Rabbits were immunized with a single injection of either 0.5 or 5.0 mg of the indicated antigen or antigens emulsified in complete Freund's adjuvant. The antigens were always administered so that the total volume, generally 2.5 ml, was divided equally among the four footpads and the back of the neck, except as noted in the text. All animals were bled 14 and 21 days after immunization.

Precipitation Reactions.—Antibody measurements were carried out by quantitative precipitin reactions (4, 9) using either R-Azo-BF or DNP-BF as the precipitating antigens. Reaction mixtures were incubated at 37°C for 1 hr and then held at 4°C for 24 hr before assay. Washed specific precipitates were dissolved in 0.02 M sodium dodecyl sulfate (recrystallized

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[1] *Abbreviations used in this paper:* BF, bovine fibrinogen; DNP, dinitrophenylated; DNP-BF, dinitrophenyl bovine fibrinogen; DNP_{57}-BGG, dinitrophenyl bovine gamma globulin; DNP_{14}-EA, dinitrophenyl egg albumin; DNP-RGG, dinitrophenyl rabbit gamma globulin; EA, egg albumin; PBS, phosphate-buffered saline; R-Azo, arsanilic acid derivatives; R-Azo-BF, arsanilate-azo-bovine fibrinogen; R-Azo_{27}-BGG, arsanilate-azo-bovine gamma globulin; R-Azo-RGG, arsanilate-azo-rabbit gamma globulin; RGG, rabbit gamma globulin.

from 95% ethanol). Antibody concentrations were determined from the 278 mμ absorbency of the dissolved specific precipitates after correction for the 278 mμ absorbency contributed by the antigen as calculated from the absorbency at 360 mμ when DNP-BF was used or at 400 mμ when R-Azo-BF was used. $E^{1\%}_{1cm}$ at 278 mμ for rabbit antibody was taken as 14.0.

Antibody Purification.—Anti-DNP antibody was purified from specific precipitates formed at equivalence with DNP-BF by extraction with 2,4-dinitrophenol in the presence of streptomycin sulfate according to the method of Farah et al. (10). Hapten was then removed by chromatography on Dowex I anion exchange resin and the product extensively dialyzed against PBS.

Determination of Antibody Affinity.—Affinity expressed here as the change in standard free energy (ΔF°) for the reaction of antibody with the homologous haptenic determinant N, ε-2,4-dinitrophenyl-L-lysine, was determined by the method of fluorescence-quenching (11) as previously described in detail (12, 13).

Statistics.—The significance of the difference between the means was calculated using a Student's *t*-test adjusted for the appropriate size sample. The significance of the similarity of rank order sets was determined by obtaining a rank correlation coefficient (r') according to the formula $r' = 1 - \frac{6\Sigma\, d^2}{n^3 - n}$ where $\Sigma\, d^2$ is the sum of the squared differences in paired rank order numbers.

Chemicals and Proteins.—Rabbit gamma globulin (RGG), bovine gamma globulin (BGG), bovine fibrinogen (BF) and egg albumin (EA) were obtained from Pentex Inc., Kankakee, Ill. 1-chloro-2,4-dinitrobenzene and *p*-arsanilic acid were obtained from Eastman Organic Chemicals, Rochester, N. Y. 2,4-dinitrophenol and sodium dodecyl sulfate were obtained from Fisher Scientific Co., Fair Lawn, N. J. N,ε-2,4-DNP-L-lysine was obtained from Mann Research Laboratories, New York, N. Y.

EXPERIMENTAL RESULTS

Effects of Antigen Dose on Antigenic Competition.—The immune response to different doses (5.0 and 0.5 mg) of DNP-RGG, R-Azo-RGG and a mixture of DNP-RGG and R-Azo-RGG is shown in Table I. Since the precipitin reactions were carried out with hapten bound to carriers different from those used for immunization, only anti-hapten antibody was measured. It is clear that the degree of antigenic competition decreases if the immunizing dose of the competing antigens is decreased. Simultaneous presentation of 5.0 mg of each of the two antigens results in approximately a 40% depression in the anti-DNP antibody response and an 82% depression in the anti-R-Azo antibody response. In contrast, when animals were immunized with only 0.5 mg of each of the two antigens, the anti-DNP antibody response was not depressed and the anti-R-Azo antibody response was decreased only 57%. In this system it appears that the degree of antigenic competition is greater with higher doses of the immunizing antigens. This is consistent with previously reported results using nonhaptenic systems (2).

Effect of Carrier on Antigenic Competition.—Table II shows the effect of varying the carrier molecule upon antigenic competition between haptenic determinants. Simultaneous immunization with DNP-EA and R-Azo-BGG

TABLE I

*Effect of Antigen Dose on Antigenic Competition**

Antigen	Dose	Rabbit No.	Day 13		Day 20	
			Anti-DNP	Anti-R-Azo	Anti-DNP	Anti-R-Azo
	mg		*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*
DNP-RGG	0.5	5-07	0.05		0.28	
		5-08	0.23		0.56	
		5-10	0.05		0.19	
		5-11	0.21		0.48	
		5-12	0.11		0.52	
		9-60	0.47		0.65	
		9-61	0.89		1.65	
		9-62	0.43		0.83	
Average			0.31		0.65	
R-Azo-RGG	0.5	5-13		0.00		0.26
		5-14		0.03		0.32
		5-15		0.18		0.24
		5-16		0.17		0.18
		5-17		0.07		0.23
		9-63		0.26		0.36
		9-64		0.33		0.20
		9-65		0.36		0.46
Average				0.18		0.28
DNP-RGG	0.5	5-18	0.15	0.13	0.23	0.11
+R-Azo-RGG	0.5	5-19	0.58	0.30	—	—
		5-20	0.16	0.80	—	—
		5-21	0.05	0.21	0.32	0.16
		5-22	0.11	0.13	0.54	0.27
		6-47	0.28	0.01	1.99	0.14
		6-48	0.17	0.01	0.60	0.00
		6-49	0.47	0.04	0.89	0.05
Average			0.25	0.11	0.76	0.12
Depression (average), %			19	50	(−17)	57
DNP-RGG‡	5		0.83 (7)	—	0.97 (21)	—
R-Azo-RGG‡	5		—	0.70 (5)	—	0.90 (5)
DNP-RGG	5		0.31 (5)	0.11 (5)	0.58 (22)	0.16 (22)
+R-Azo-RGG‡	5					
Depression %			63	84	40	82

* Rabbits were immunized with the amount of each antigen (indicated in the table) in complete Freund's adjuvant and bled 13 and 20 days later. The concentration of the antibody was determined by quantitative percipitin reactions with DNP-BF and R-Azo-BF.

‡ The data presented are the mean value for the antibody concentration. The numbers in parenthesis are the number of animals studied. Data on the individual rabbits from which these averages were compiled are presented in Table III and in a previous communication (3).

results in a 61% depression of the anti-DNP antibody response and 88% depression of the anti-R-Azo antibody response on day 14. The degree of depression observed when the two haptens are on different carrier proteins is thus similar to what was observed (Table I) when the two haptens were on identical carrier proteins. It is apparent that the existence of antigenic com-

TABLE II

*Effect of Varying the Carrier Molecule on Antigenic Competition**

Immunization	Rabbit No.	Day 14		Day 21	
		Anti-DNP	Anti-R-Azo	Anti-DNP	Anti-R-Azo
		mg/ml	*mg/ml*	*mg/ml*	*mg/ml*
DNP-EA	15-78	1.36		2.66	
	15-79	0.70		1.21	
	15-80	0.40		0.78	
	15-81	0.30		0.37	
Average		0.69		1.26	
R-Azo-BGG	15-84		0.78		1.26
	15-85		0.71		1.89
	15-86		1.12		2.32
	15-88		0.47		1.19
Average			0.77		1.67
DNP-EA	15-89	0.19	0.19	0.17	0.23
+R-Azo-BGG	15-90	0.21	0.04	0.34	0.05
	15-91	0.39	0.06	0.24	0.02
	15-92	0.35	0.06	—	—
	15-93	0.22	0.09	0.27	0.07
Average		0.27	0.09	0.26	0.09
Depression, %		61	88	79	95

* Rabbits were immunized with 5 mg of each antigen in complete Freund's adjuvant and bled 14 and 21 days later. Antibody concentration was determined by quantitative precipitin reactions with DNP-BF and R-Azo-BF.

petition in this system is not dependent upon the use of the same protein as carrier for the two haptens.

Effect of Antigenic Competition on Antibody Affinity.—It might be expected that some of the possible mechanisms for antigenic competition would preferentially affect cells synthesizing either high or low affinity antibody and thus introduce predictable changes in the average affinity of the antibody produced. It has been shown, for example, that induction of partial tolerance affects predominantly high affinity antibody forming cells, leading to a depression in the average affinity of the antibody produced (14). In contrast,

TABLE III

*Effect of Antigenic Competition on the Affinity of Anti-DNP Antibody (Day 20)**

Immunization	Rabbit No.	Anti-DNP	Anti-R-Azo	Anti-DNP ΔF°
		mg/ml	*mg/ml*	*kcal/mole*
DNP-RGG	9-90	1.40 (16)		8.53 (9.5)
	9-91	0.79 (7)		8.71 (13)
	9-92	0.47 (2)		8.33 (6.5)
	9-93	1.25 (14)		9.71 (17)
	9-94	0.52 (3)		7.98 (2)
	9-95	0.44 (1)		8.82 (14)
	4-52	1.34 (15)		9.06 (15)
	4-53	0.78 (6)		8.33 (6.5)
	4-54	0.92 (9.5)		9.40 (16)
	4-55	0.91 (8)		7.92 (1)
	4-56	1.19 (13)		8.53 (9.5)
	7-53	0.70 (5)		8.59 (12)
	7-54	1.11 (12)		8.11 (3)
	7-55	1.06 (11)		8.21 (5)
	7-56	0.92 (9.5)		8.15 (4)
	7-57	0.60 (4)		8.58 (11)
	7-58	2.04 (17)		8.44 (8)
Average		0.97		8.55
DNP-RGG +R-Azo-RGG	9-96	0.35 (5)	0.25 (13)	8.89 (14)
	9-97	0.29 (2)	0.08 (3)	8.52 (7)
	9-98	0.66 (9)	0.13 (6.5)	7.80 (1)
	9-99	0.42 (7)	0.22 (12)	8.29 (5)
	10-00	0.34 (3.5)	0.16 (10)	8.51 (6)
	10-01	0.61 (8)	0.13 (6.5)	8.80 (11)
	4-57	0.34 (3.5)	0.09 (4)	8.79 (10)
	4-58	0.96 (14)	0.36 (17)	9.08 (15)
	4-59	0.76 (13)	0.29 (15)	10.12 (17)
	4-60	0.22 (1)	0.07 (2)	8.66 (8)
	4-61	0.74 (12)	0.34 (16)	9.44 (16)
	7-59	0.67 (11)	0.15 (8.5)	8.21 (4)
	7-60	0.37 (6)	0.09 (5)	8.81 (12)
	7-61	1.37 (17)	0.05 (1)	8.02 (3)
	7-62	1.12 (15)	0.19 (11)	8.87 (13)
	7-63	1.28 (16)	0.28 (14)	8.73 (9)
	7-64	0.67 (10)	0.15 (8.5)	7.99 (2)
Average		0.66	0.18	8.68

* Rabbits immunized with 5 mg of each antigen in complete Freund's adjuvant and bled 20 days later. Affinity for DNP-lysine measured by fluorescence-quenching at 20°C in PBS and expressed as the standard free energy change in kcal/mole. Concentration of antibody determined by precipitin reaction with DNP-BF and R-Azo-BF. The numbers which appear in parentheses are rank order numbers (see text).

repeated injections of passive antibody will depress the magnitude of the immune response but will bring about an increase in the average affinity of the antibody present (15). The effect of antigenic competition on antibody affinity was therefore studied.

A comparison of the affinity of anti-DNP antibody produced by animals immunized with only DNP-RGG and by animals immunized simultaneously with both DNP-RGG and R-Azo-RGG is shown in Table III. Affinity is expressed as the change in standard free energy (ΔF°) of binding of the antibody to the homologous haptenic determinant N,ϵ-2,4-dinitrophenyl-L-lysine as measured by fluorescence-quenching techniques. The average affinity of the anti-DNP antibody produced by rabbits immunized with both DNP-RGG and R-Azo-RGG is 0.13 kcal/mole higher than that produced by animals immunized with DNP-RGG alone. Therefore, although antigenic competition results in a diminution of the anti-hapten antibody response, the affinity of the antibody produced is essentially equal to that produced by controls. Similar results were obtained when animals were immunized with 0.5 mg of each antigen.

Each number in Table III is followed by a rank order number in parenthesis. The rank order number denotes the position, in a listing of the particular set of data, where that value would fall if ordered according to its magnitude within the set. For example, rabbit 9-95 produced less anti-DNP antibody than any other animal in this group, therefore the number 1 appears beside the 0.44 mg/ml of anti-DNP antibody it produced. When the various possible rank order lists were compared for likeness, it was found that only the rank order list for the amount of anti-R-Azo antibody produced and the affinity of the anti-DNP antibody produced in the same animal were significantly the same ($P < 0.05$ by rank correlation coefficient). In other words, the animals which produced the highest affinity anti-DNP antibody tended to produce the most anti-R-Azo antibody. No statistically significant correlation was observed between the amount of anti-DNP antibody produced and its affinity.

Relationship between the Site of Antigen Injection and Antigenic Competition.—If antigenic competition is mediated by a humoral (circulating) factor as is implied by observations of Radovich and Talmage (16), then competition would be expected to exist even if the competing antigens were injected so as to drain into different groups of regional lymph nodes. This prediction was tested by immunizing rabbits with DNP-RGG and R-Azo-RGG in the same or in different paws. Details of injection procedure are given in the legend to Table IV. It can be seen (Table IV) that in the animals which received a mixture of both antigens in the same paws, the anti-DNP antibody response was decreased 68% and the anti-R-Azo antibody response was decreased 59% 14 days after immunization. The extent of depression of both the anti-R-Azo and anti-DNP

TABLE IV

*Effect of Site of Antigen Injection on Antigen Competition**

Immunization*	Rabbit No.	Day 13		Day 20	
		Anti DNP	Anti-R-Azo	Anti-DNP	Anti-R-Azo
		mg/ml	*mg/ml*	*mg/ml*	*mg/ml*
1. DNP-RGG (right paws)	9-66	0.70		0.54	
	9-67	0.88		1.00	
	9-68	0.78		0.89	
	9-69	0.58		0.44	
	9-70	0.46		0.36	
	9-71	0.53		0.58	
Average		0.66		0.64	
2. R-Azo-RGG (right paws)	9-72		0.72		0.45
	9-73		0.31		0.27
	9-74		0.39		0.16
	9-75		0.43		0.18
	9-76		0.56		0.71
	9-77		0.67		0.67
Average			0.51		0.41
3. DNP-RGG (right paws)	9-78	0.40	0.25	0.27	0.27
	9-79	0.91	0.60	0.98	0.77
	9-80	0.55	0.84	0.32	0.42
R-Azo-RGG (left paws)	9-81	0.50	0.21	1.00	0.37
	9-82	0.81	1.17	0.65	0.78
	9-83	0.25	0.18	0.06	0.07
Average		0.57	0.54	0.55	0.45
4. DNP-RGG and R-Azo-RGG (right paws)	9-84	0.10	0.23	0.21	0.15
	9-85	0.23	0.15	0.10	0.18
	9-86	0.00	0.16	0.14	0.11
	9-87	0.11	0.29	0.23	0.13
	9-88	0.24	0.08	0.28	0.10
	9-89	0.56	0.37	0.61	0.37
Average		0.21	0.21	0.26	0.17

* The four groups of rabbits received antigen emulsified in complete Freund's adjuvant as follows: (1) Received 5 mg of DNP-RGG in 1 ml of emulsion divided between the right front and right hind paws; (2) Received 5 mg of R-Azo-RGG in 1 ml of emulsion divided between the right front and right hind paws; (3) Received 5 mg of DNP-RGG in 1 ml of emulsion divided between the right front and right hind paws and 5 mg of R-Azo-RGG in 1 ml of emulsion divided between the left front and left hind paws; (4) Received 5 mg of DNP-RGG and 5 mg of R-Azo-RGG (mixed prior to emulsifying) in 1 ml of emulsion divided between the right front and right hind paws. Animals were bled at 13 and 20 days after immunization. Anti-DNP and Anti-R-Azo concentrations were determined by quantitative percipitin reactions using DNP-BF and R-Azo-BF.

response was significant at $P < 0.05$ (Student's t-test). In contrast, when animals received both antigens in separate paws (DNP-RGG in the right front and right hind paws and R-Azo-RGG in the left front and left hind paws) there was no depression of the antibody response. Thus, it appears that antigenic competition only occurs when both antigens are injected so as to drain into the same regional lymph nodes.

DISCUSSION

It has been shown that antigenic competition is greater when larger doses of antigen are employed. Furthermore, competition between haptenic determinants exists to a comparable degree whether the haptens are bound to identical or different carrier proteins. Although antigenic competition is manifested as a decrease in the magnitude of the antibody response, the affinity of antibody produced is not decreased. In addition, it was shown that in the system studied, antigenic competition occurs only when both antigens are injected so as to drain into the same regional lymph nodes.

Current theorization in immunology appears to favor a selectional hypothesis (17) to explain the basic characteristics of the immune response (18). One presumes that the individual lymphoid cell possesses a limited potential with regard to antibody synthesis and that this limited potential is determined prior to contact with antigen. Regardless of the detailed somatic mechanism for generating the diversity of the adult lymphoid cell population, antigen functions in such a theory merely to select cells capable of synthesizing antibody of appropriate specificity. A variety of evidence (19–22) suggests that antigen must undergo some ill defined macrophage-associated "processing" or "localizing" step prior to contact with lymphoid cells in order to initiate specific cell proliferation and antibody synthesis. Therefore, according to a selectional theory, antigenic competition cannot exist at the level of the antibody forming cell. Antigenic competition could theoretically be due to one or more of the following: (*a*) a competition for some aspect of the processing system; (*b*) the production of a nonspecific inhibitor of antibody synthesis; (*c*) an alteration of local lymph node architecture in response to one antigen which temporarily and nonspecifically depresses the response to a second antigen; (*d*) an alteration of the localization or the distribution of one antigen as a result of a concomitant immune response to a second antigen; (*e*) the induction of partial tolerance to one or both of the competing antigens; (*f*) suppression of antibody synthesis to one antigen by cross-reacting antibody formed to the second antigen.

A variety of evidence suggests that suppression by cross-reacting antibody is not involved. (*a*) Competition has been reported in the past using non–cross-reacting antigens (1, 2). (*b*) We have previously shown that competition and suppression by passive antibody occur at different points in the sequence of

events leading to antibody formation (3). (*c*) It has been shown here that antigenic competition between two non–cross-reacting haptens occurs to essentially equal degrees whether they are coupled to the same or different carrier proteins.

Results presented in this paper are also inconsistent with the idea that competition is due to the induction of tolerance to one or both of the immunizing antigens. It has previously been shown that partial tolerance is characterized by the production of low affinity antibody (14). We have shown that although antigenic competition results in a decrease in antibody production, the affinity of the antibody produced is equal to or slightly greater than that produced by control animals immunized with only a single antigen. Thus, antigenic competition does not appear to be due to the same mechanism as immunological tolerance.

In the simplest hypothesis implementing a "competition" for some aspect of the processing system as an explanation for antigenic competition, one might assume that antigen processing was the rate-limiting step in the sequence of events leading to antibody synthesis. From a simplistic point of view it would follow that the total amount of antibody produced (to all immunizing antigens) will remain constant as long as these antigens utilize identical pathways in the processing system. It is interesting to note that the sum of the amounts of antibody produced to both antigens by animals immunized with two antigens simultaneously is approximately equal at the two immunizing doses studied. Also, the sum of the anti-DNP and anti-R-Azo antibody responses of animals immunized simultaneously with 0.5 mg of both antigens is approximately equal to the antibody response of control animals immunized with only 0.5 mg of DNP-RGG. In contradiction to this simple view, the amount of anti-DNP or anti-R-Azo antibody produced by animals immunized with 5 mg of a single antigen is significantly greater than the sum of anti-DNP and anti-R-Azo responses of animals immunized with both antigens simultaneously. This contradiction, however, might be due to an amplification system resulting from cell division initiated by processed antigen.

Radovich and Talmage (16) and Adler (2) have shown that antigenic competition was more marked if a delay of several days was imposed between the injection of the two antigens. In addition, Radovich and Talmage (16) noted that in cell transfer studies with irradiated recipients, greater competition was observed if a larger number of sensitized cells were administered. They concluded that competition is mediated through the production of a nonspecific inhibitor of antibody synthesis. If such an inhibitor exists, it might be either localized to the site of antigenic stimulation (regional lymph nodes) and only exert its effect locally, or it might circulate freely and exert its effect systemically. Since, as reported here, antigenic competition is not observed when two antigens are injected so as to drain into different groups of regional lymph nodes, an inhibitor, if it exists, must be localized to the site of antigenic

stimulation. It seems unlikely in terms of our observations that a systemic inhibitor mediates antigenic competition. It should be noted that these results are superficially in conflict with a recent report by Eidinger et al. (23). A variety of differences in experimental procedure may account for the apparent discrepancy. Eidinger et al. (23) employed intravenous administration of a particulate antigen and allowed a time interval of 7 days before footpad injection of the second antigen. In contrast, the work reported here involves the simultaneous footpad injection of soluble antigens emulsified in complete Freund's adjuvant.

Thus antigenic competition appears to occur at some step in the sequence of events leading to antibody synthesis which is independent of specific lymphoid cells. The detailed mechanism remains unknown. The data presented suggests that competition is not mediated by suppression due to cross-reacting antibody synthesis, by tolerance induction or by a systemic inhibitor of antibody synthesis. Possibilities of a local inhibitor of antibody synthesis, an alteration in antigen localization, distribution, or "processing", or an alteration of lymph node architecture are all consistent with available data.

SUMMARY

Antigenic competition was studied in a haptenic system. It was found that: (*a*) The extent of competition is greater when larger doses of antigen are employed. (*b*) Antigenic competition appears to be independent of the carrier molecule. (*c*) The affinity of the antibody produced in antigenic competition is approximately equal to the affinity of antibody formed by animals immunized with only one antigen. (*d*) Antigenic competition only occurs when both antigens are injected so as to drain into the same regional lymph nodes.

The results suggest that antigenic competition occurs locally at the site of antigen stimulation and is not mediated by a circulating factor, by tolerance induction, or by suppression due to synthesis of cross-reacting antibodies.

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