Particle Size of Antigen in Relation to Antibody-Suppressive Effect of Cortisone. (20885)

ROBERT HANAN* AND JIRO OYAMA. (Introduced by Karl Habel.)

From the U. S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda, Md.

We have previously reported that in rabbits simultaneously immunized with bovine serum albumin (BSA) and either mumps virus(1) or sheep red cell stroma(2) the administration of cortisone uniformly suppresses production of anti-BSA but affects the production of antibody to mumps virus only irregularly and the production of antibody to stroma not at all. These findings suggested that the ability of cortisone to suppress antibody production in the rabbit was influenced by the particle size of the antigen. To determine whether or not this was so the ideal experiment would involve the study of the rabbit's response to the same antigen in 2 widely different sizes, so that factors other than size could be constant. A near approach to this situation was obtained by immunizing rabbits with either BSA or human red cell stroma conjugated with p-bromophenylisocyanate and measuring the antibody thus produced with yet another bromophenylureido-protein so that in each instance antibody only to the phenylureido moiety was measured.

Materials and methods. Preparation of antigens. Three p-bromophenylureido antigens were prepared. Conjugated bovine serum albumin† (PBSA) and egg albumin‡ (PEA) were prepared by the method of Wormall and Hopkins as given by Kabat and Mayer(3). The conjugated red cell stroma (PRC) were prepared in a similar fashion except that to rid the PRC of the bromodiphenylurea reaction product it was necessary to wash with alcohol-ether and ether since solution in alkali and precipitation by acid could not be used to isolate the antigen in this case. The bromine served as a marker to provide some indication of the number of phenylureido linkages on each antigen. Table I lists the Br, N and moisture determinations on these antigens. Since the method used for Br analysis would be influenced by the presence of I, some unconjugated stroma were washed in the same way as the PRC and also analyzed for Br. None was detected. *Antibody N determinations.* When PBSA and PEA were used Antibody (Ab) N was determined by the quantitative precipitin method(4). Antibody to PRC was determined by the quantitative agglutinin method(5).

TABLE I. Analytical Data for Antigens.

	% N	% Br	% moisture
PBSA	13.7	3.2	9.7
PEA	13.4	2.9	10.4
PRC	10.9	5.7	6.0

Experimental. In the first experiment 10 albino rabbits each weighing approximately 2 kg were immunized with 7 intravenous doses of 2 mg each of PBSA given over a period of 2 weeks. From 3 days before until 6 days after the antigen injections 5 of these rabbits were given 4 mg of cortisone§ daily intramuscularly. Six days after the last Ag injection all the rabbits were bled and the serum separated. There was one death in each group (control and cortisone) during immunization.

Results. Quantitative precipitin curves were set up with each of the sera against PBSA and PEA and the Ab N levels thus determined are recorded in Table II. It will be seen that the control group averaged 2.3 (anti-PBSA) or 2.4 (anti-PEA) times as much Ab N as did the cortisone treated group and that moreover there is a distinct separation of the ranges of the 2 groups. Thus the results obtained in rabbits immunized with PBSA are the same as for BSA(1,2). The

* Present address: Leila Y. Post Montgomery Hospital, Battle Creek, Mich.

† Crystallized Bovine Plasma Albumin, Armour and Co., control No. 127-250.

‡ Crystallized Egg Albumin, Armour and Co., lot No. E 90115.

§ Cortone, Merck & Co.

TABLE II. Antibody N Levels in Rabbits Immunized with PBSA and Treated or Not Treated with Cortisone.

Group	Animal #	Anti-PBSA, γAbN/ml	Anti-PEA, γAbN/ml	Anti-PSA / Anti-PEA
Cortisone	145	43	14	3.1
	146	22	5	4.4
	147	70	29	2.4
	148	67	19	3.5
Avg		58	17	3.4
Controls	150	95	29	3.3
	151	180	61	3.0
	152	122	34	3.6
	154	142	40	3.6
Avg		135	41	3.4

homologous Ag, PBSA, precipitated an average of 3.4 times as much Ab N as did a heterologous conjugated Ag, PEA. That this difference was not due to Ab to native BSA was shown by the fact that when quantitative precipitin curves with these sera were set up with BSA very small amounts of precipitate were obtained with sera No. 151, No. 152, and No. 154 and none with the other sera. The difference presumably is due to differences in the portion of the antigen molecules partaking in the phenylureido linkage.

In the second experiment 12 albino rabbits were immunized with 7 intravenous doses of PRC and 6 of these were treated with cortisone in the same way as the rabbits of the first experiment. All animals were again bled 6 days after the last Ag injection. Quantitative precipitin curves were set up with each of the sera against PBSA and PEA and one quantitative agglutinin absorption was done with PRC. The results are given in Table III. It can be seen that PBSA again precipitated more Ab than did PEA although in this instance they were both heterologous antigens. However the difference is considerably less than in the previous experiment. The control group averaged a slightly higher level of anti-PBSA and anti-PEA (1.3 and 1.1 times respectively) than did the cortisone-treated group but the Ab N ranges of the 2 groups are the same. In the case of the anti-PRC determination, since only one absorption was done and the values obtained represent small differences between relatively large values, little quantitative importance can be ascribed to these data except that they show that there was anti-PRC. There was however no visible agglutination of the PRC when added to these sera, the PRC resuspending quite as easily in these tubes as in the tubes in which saline was present instead of serum. Good agreement was obtained between the N values of the washed sediment of the PRC-saline tubes and the N content of the stock PRC suspension pipetted directly in the digestion flask (139 μg N for the former and 141 μg N for the latter—average of duplicate analyses) indicating that little soluble protein was leached from the PRC by washing.

Discussion. The results provide evidence that the antibody response to antigen of large particle size is less influenced by cortisone treatment than is the case with smaller, soluble antigens. Why this should be is not too clear but it certainly suggests that the effect of cortisone is not anything so fundamental as an interference with gamma globulin synthesis. On the contrary it suggests that cortisone exerts its effect at some point before the antigen reaches the site of antibody synthesis. It is possible that due to their differing physical states the PBSA and PRC are captured by 2 different elements of the reticulo-endothelial system, and that one of these elements is affected by cortisone whereas the other is not.

There is another line of evidence that also indicates that interference with gamma globulin synthesis is not responsible for the cortisone effect. In our work we have found that whether antibody to BSA in the control group is of the order of 0.5-1.0 mg or 0.05-0.1 mg Ab N per ml serum the average Ab levels in these groups were consistently 2 to 3 times those in the corresponding cortisone-treated group. One would expect that if gamma globulin synthesis was involved that less or no relative suppression would occur when the Ab level in the control was low, because then the rate of globulin synthesis should be more nearly equal to the stimulus for antibody production.

Summary. Rabbits were immunized with either p-bromophenylureido-BSA or similarly conjugated human red cell stroma. One-half the rabbits in each group were treated with

TABLE III. Antibody N Levels in Rabbits Immunized with P-HRC and Treated or Not Treated with Cortisone.

Group	Animal #	Anti-PBSA γ Ab N/ml	Anti-PEA γ Ab N/ml	Anti-PRC*	Anti-PBSA / Anti-PEA
Cortisone	167	40	27	18	1.5
	168	42	31	lost	1.4
	169	80	55	31	1.5
	170	16	10	17	1.6
	171	4	0	17	
	172	10	3	20	
	Avg	32	21	21	1.5
Control	173	11	7	14	1.6
	174	12	lost	9	
	175	78	48	27	1.6
	182	38	23	18	1.7
	183	81	44	28	1.8
	184	30	23	12	1.3
	Avg	42	27	18	1.6

* γ Ab N obtained with one absorption of 1 ml of serum.

cortisone. Antibody was measured with a p-bromophenylureido-protein other than the one used for immunization. Cortisone reduced antibody production to the conjugated BSA but affected antibody produced to the conjugated stroma but little, if at all.

1. Overman, J. R., and Hanan, R., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 427.

2. Hanan, R., and Overman, J. R., *ibid.*, 1953, v84, 420.

3. Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry,* Chas. C. Thomas, Springfield, Ill., 1948.

4. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.,* 1935, v62, 697.

5. Heidelberger, M., and Treffers, H. P., *J. Gen. Physiol.,* 1942, v25, 523.

Received January 19, 1954. P.S.E.B.M., 1954, v85.

Stimulatory Effect of BCG on Isoantibody Production in the Rabbit.* (20886)

CARL COHEN. (Introduced by Carl TenBroeck.)

From Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

Although considerable work has been reported on the study of isoantibodies in the rabbit(1-7), there is little reference to the difficulty in inducing isoantibody production. In our study of the mode of inheritance of blood group antigens in the rabbit, the probability of isoantibody being produced in a particular recipient has been very small, even when tests indicate that differences exist between the cellular antigens of the donor and the recipient. Preliminary observations made while using the test of native resistance to tuberculosis devised by Lurie *et al.*(8) suggested that BCG injection might increase the probability of isoantibody production. There is considerable evidence to support the view that in rabbits and guinea pigs infection with tubercle bacilli leads to a nonspecific increase in the ability of these animals to produce antibody. Lewis and Loomis(9) found that hemolytic antibody titre could be increased more than tenfold following the injection of sheep erythrocytes into tuberculous animals. Lurie (10) attributes this increased productivity to the generally increased activity of the reticulo-

* This study was supported in part by grants from The American Trudeau Society of the National Tuberculosis Association and the National Microbiological Institute of the National Institutes of Health.