estrogen dosage was raised. The follicular growth was characterized by granulosal proliferation, resulting in many solid follicles. Apparently, ovarian weight increments resulting from increasing estrogen doses are reflections of this enhanced follicular growth.

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Competition of Antigens as Influenced by Spacing of Heterologous Antigen Injections.* (26806)

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Decreased responses to some antigens following injection of 2 or more antigens have been observed by a number of investigators (1-5). In some cases a crowding out has been shown to occur when an antigen is injected into an animal actively undergoing antibody formation against a previously injected antigen(6-9). Benjamin and Witzinger(7) found that a preliminary injection of horse serum inhibited the formation of hemolysin to sheep cells. Glenny *et al.*(8) found a crowding out of the response to diphtheria toxoid in guinea pigs when the toxoid was injected simultaneously with, or 2, 4, or 6 days after, an injection of normal horse serum. Frolova *et al.*(9) observed a reduction in precipitin response of guinea pigs to ox serum injected 7-14 days after sensitization with horse serum.

In some cases a more rapid response to one antigen in a mixture has been postulated as leading to a crowding out of antibody production against other antigens in the inoculum(1-3). Adler(2) found that simultaneous injection of rabbit serum or hemocyanin with rabbit immune globulin into guinea pigs impeded antibody production against the rabbit immune globulin and that the degree of this suppression was related to the intensity of antibody response to the competing antigens. Abramoff and Wolfe(3), in quantitative studies of the primary response to bovine serum albumin (BSA) and human gamma globulin (HGG), at a 20 mg per kilogram body weight (mg antigen/KBW) dosage level, observed that the chicken system responds earlier to HGG than it does to BSA and the HGG is thus able to crowd out the response to BSA when these antigens are simultaneously injected. However, Abramoff(10) has shown that the selectivity of HGG could be reduced during simultaneous injection of BSA and HGG, at the 40 mg/KBW dosage level, if the animals had been presensitized to BSA. Under these conditions the responses to both of these antigens were significantly reduced.

The present investigation is concerned with further clarification of the mechanisms by which one antigen interferes with antibody production against a second antigen during primary antibody responses. Responses of chickens to single, simultaneous, and spaced injections of BSA and HGG at a dosage level of 40 mg/KBW have been studied.

Materials and methods. Eighty-two New Hampshire Red Chickens (74 males, 8 females) of approximately 34 weeks of age and weighing 2300-4000 g were used in these

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TABLE I. Precipitin Production in Chickens* Injected with 40 mg per kg Body Weight of Bovine Serum Albumin (BSA) and/or Human γ-globulin (HGG).

Days after antigenic stimulus	Group I Single inj. BSA (14)‡	Group II Single inj. HGG (15)	Group III Simultaneous inj. BSA and HGG (15)		Group IV HGG inj. 24 hr after BSA (20)		Group V HGG inj. 48 hr after BSA (19)	
	Antibody (in μg) nitrogen/ml of undiluted serum†							
	BSA	HGG	BSA	HGG	BSA	HGG§	BSA	HGG§
3	0	0	0	0	0	0	0	0
4	0	+	0	+	0	0	0	0
5	+++	133 ± 23	+	171 ± 24	+++	0	42 ± 20	0
6	126 ± 26	280 ± 34	47 ± 18	421 ± 66	82 ± 17	80 ± 10	197 ± 54	0
7	326 ± 52	298 ± 58	328 ± 40	350 ± 60	151 ± 19	200 ± 25	400 ± 55	99 ± 25
8	369 ± 55	245 ± 55	441 ± 59	239 ± 38	172 ± 18	219 ± 22	371 ± 53	291 ± 47
9	314 ± 45	191 ± 50	402 ± 62	190 ± 37	175 ± 16	166 ± 20	281 ± 29	334 ± 45
10	227 ± 36	134 ± 34	358 ± 53	155 ± 36	166 ± 19	110 ± 14	228 ± 21	271 ± 47
12	191 ± 32	78 ± 24	245 ± 46	107 ± 28	113 ± 18	66 ± 10	161 ± 21	210 ± 46
14	138 ± 25	52 ± 19	120 ± 36	68 ± 24	75 ± 14	23 ± 6	94 ± 18	105 ± 23
16	59 ± 12	20 ± 8	75 ± 23	39 ± 18	47 ± 14	12 ± 5	35 ± 13	62 ± 14
18	25 ± 8	12 ± 7	38 ± 13	21 ± 11	11 ± 11	+++	18 ± 8	27 ± 7

* Animals 34 wk of age when inj.
† Mean ± stand. error.
‡ Number in parentheses represents No. of animals in each group.
§ Antibody response to HGG measured from inj. of BSA.

experiments. Antigenic stimulation consisted of intravenous injections of purified soluble BSA‡ and/or HGG§ prepared in 1% saline and standardized to contain 40 mg protein per ml. All injections were given on the basis of 40 mg/KBW.

The chickens were treated as follows: Group I: 14 birds received a single injection of BSA;‖ Group II: 15 birds received a single dose of HGG;‖ Group III: 14 animals received successive injections of BSA and HGG within a period of 30 seconds; Group IV: 20 chickens received an HGG injection 24 hours following BSA stimulation; Group V: 19 birds received an HGG inoculation 48 hours after a BSA injection.

Blood was obtained by wing vein or cardiac puncture begining the second or third day after first injection and subsequently on the 4th, 5th, 6th, 7th, 8th, 9th, 10th, 12th, 14th, 16th, and 18th days. The sera were stored at −20°C previous to testing for antibody nitrogen. Individual serum samples were assayed for antibody nitrogen using the Heidelberger quantitative precipitin technic as previously described(10).

Results. Antibody responses to simultaneous injections of BSA and HGG. Earlier studies(3) had shown that antibody responses of animals simultaneously injected with 40 mg/KBW each of BSA and HGG showed equivalent decreases of approximately 50% to both of these antigens as compared with their respective controls. However, these animals were bled only on the 8th day following simultaneous stimulation and it was suspected that peak antibody responses to these 2 antigens were being reached at different times. The present study, in which the complete curve of antibody response was analyzed following simultaneous injection of 40 mg/KBW, showed that antibody responses to BSA and HGG did indeed reach their peaks on different days (Fig. 1B and Table I). It can also readily be seen that antibody responses to BSA and HGG did not differ significantly from their respective controls. These results are in contrast to earlier studies at the 20 mg/KBW dosage level(3). At this dosage level antibody response to BSA was significantly reduced in mean peak titer and occurrence of maximum antibody titer was delayed from the 8th day following a single injection of BSA to the 10th day following simultaneous injection with HGG.

‡ Pentex, Inc., Kankakee, Ill.

§ Generously supplied by J. H. Hink, Cutter Laboratories, Berkeley, Calif.

‖ Injection and bleeding of animals used in this series of experiments were carried out during the same period of time as injection and bleeding of animals used in a study previously reported(10). Therefore, Groups I and II serve as controls for both of these studies.

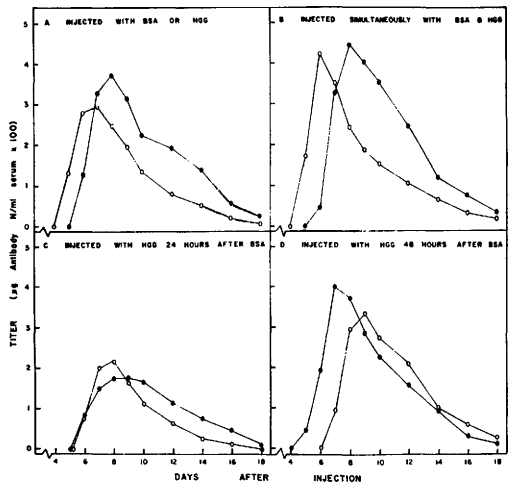

FIG. 1. Precipitin production curves of chickens following single injections (A) of bovine serum albumin (BSA)(●) or human gamma globulin (HGG)(○), or a combination of these 2 antigens inj. simultaneously (B) or at 24 (C) or 48 (D) hr intervals. Curves C and D plotted from day of BSA inj.

The antibody response to HGG was unaffected.

Antibody responses to BSA and HGG following a 24 hour delayed injection of HGG. Earlier studies(3) also had shown a 2-day delay in appearance of the peak response to BSA following simultaneous injection of 20 mg/KBW each of BSA and HGG. Furthermore, it had been demonstrated that at 40 mg/KBW the selective advantage of HGG over BSA could be partially overridden by simultaneous inoculation of BSA and HGG into animals previously sensitized to BSA (10). Consequently, in the present study, injection of HGG was delayed to determine whether this might provide an additional means of influencing the ability of HGG to alter the response to BSA at a dosage level of 40 mg/KBW.

Results of the experiment in which the injection of HGG was withheld until 24 hours after that of BSA indicate that the response to BSA was significantly reduced (Table I, Fig. 1A and 1C). Thus, although the response to HGG was itself somewhat reduced, these results indicate that, at this dosage level, such a delay of HGG injection enables HGG to regain the selective advantage over BSA previously observed at the 20 mg/KBW dosage level.

Antibody responses to BSA and HGG following a 48 hour delayed injection of HGG. Results of the preceding experiment raise the question as to whether a lapse of 48 hours between injection of BSA and HGG would produce an even greater suppression of the response to BSA. Whereas antibody response to BSA was reduced following a 24-hour delayed injection of HGG, it can readily be seen that antibody responses to BSA and HGG in animals in which the injection of HGG was delayed for 48 hours approximate the responses of their respective controls (Table I, Fig. 1A and 1D). Statistical analyses in which responses of Groups II and V to HGG were treated as if superimposed in time, revealed no significant differences between them. Similarly, the amount of antibody produced against BSA in this experiment was not significantly different from that of its control group.

Discussion. It has been shown previously that both bovine serum albumin and human gamma globulin are "good" antigens in the chicken system(3,10). However, HGG has been shown to possess certain selective advantages over BSA(3). At the 20 mg/KBW level chickens which readily produced satisfactory amounts of antibody in response to single injections of BSA produced significantly less antibody against this antigen when concurrently injected with HGG. Furthermore, the peak response to BSA was delayed 2 days, whereas the response to HGG remained unchanged. In the present studies the selectivity of HGG was altered under circumstances of simultaneous injection of BSA and HGG at the 40 mg/KBW level insofar as antibody response to HGG failed to suppress the response to BSA. These data suggest that specific optimal dosage levels exist for each antigen which permit expression of its maximal antibody response. In this respect, some correlation with the secondary response studies of Barr and Llewellyn-Jones (4) is possible. They found that a preliminary injection of *H. pertussis* vaccine brought about interference with the diphtheria response following subsequent immunization

with a combined alum-precipitated diphtheria-pertussis prophylactic. Interference did not occur, however, when the dosage of both antigens in the secondary injection was increased considerably.

It is also evident that the ability of one antigen to interfere with the antibody response to another is dependent upon the prior immune state of the host(1,4,10,11). Abramoff(10) has shown that the selectivity of HGG can be reduced during simultaneous injection of BSA and HGG, at the 40 mg/KBW level, if the animals are presensitized to BSA. The present studies, at the same antigenic concentration, indicate that the selectivity of HGG over BSA may also be reduced by delaying injection of HGG for 48 hours.

The mechanism by which spacing of injections influences the ability of one antigen to alter the antibody response to another antigen appears to be associated with altered induction periods. A competition occurred between BSA and HGG under those experimental conditions in which induction periods were altered so as to be of the same duration for both antibody responses; *i.e.*, simultaneous injection at 20 mg/KBW of each antigen (3), simultaneous injection of 40 mg/KBW of each antigen following previous sensitization with BSA(10), and HGG injection 24 hours following BSA stimulation (present study). Thus, it would appear that under conditions in which these 2 antigens are predisposed to stimulate the antibody-forming mechanism at approximately the same time, a competition of antigens occurs, the extent of which then appears to be conditioned by dosage(3 and present study), spacing of injections, and previous immune state of the animals(10).

Summary. 1) Antibody responses of chickens to single, simultaneous, and spaced injections of bovine serum albumin and human gamma globulin at a dosage level of 40 mg per kilo of body weight are described. 2) Under conditions of simultaneous injection of BSA and HGG antibody responses to both BSA and HGG approximate those of their respective controls. 3) Results of the experiment in which injection of HGG was withheld until 24 hours after that of BSA indicate that antibody response to BSA was significantly reduced. 4) Antibody responses to BSA and HGG in animals in which injection of HGG was delayed for 48 hours approximate the responses of their respective controls. 5) It appears that the character of the antibody response to simultaneous injections of BSA and HGG is regulated by the parameters of dosage level and timing of injections.

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