

# The Effect of the Successive Administration of Two Antigens to Germfree and Conventional Chickens

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In the germfree chicken, the lymphoid tissue has never been exposed to the multiple stimulating effects of a viable microflora. Less lymphoid tissue is found, which contains fewer plasma cells and secondary nodules (1). Upon stimulation with a single dose of bovine serum albumin (BSA), less precipitating antibody is formed. The data indicate, however, that this antibody formation continues after maximum serum antibody concentrations are reached. In the conventional chicken, on the other hand, precipitating antibody production practically ceases at this point (2). The persistence of antibody formation in the germfree bird was explained by the assumption that in the absence of major antigenic stimulation originating from a viable microflora, the antigenic effect of the BSA on the antibody producing cellular elements persisted. To test this hypothesis, BSA stimulated germfree and conventional chickens were administered a second unrelated protein antigen, human gamma globulin (HGG), just before the anti-BSA antibody concentration reached its maximum value. The results indicate that the administration of this second antigen (HGG) terminated the prolonged production of antibody against the first antigen (BSA). They also show that under these conditions the antibody response to the second antigen is depressed.

## Materials and Methods

Adult 90-110 day old male and female white Leghorn chickens of germfree and conventional status were obtained from the same egg clutches. Germfree birds were housed in plastic isolators with approximately 1100 square cm of floor space per bird. The conventional controls were housed in equivalent floor space but in an open room environment. All birds were fed diet L-289F ad libitum (3).

Germfree and conventional chickens were injected with a single dose of 40 mg of the antigen, either BSA or HGG, via the cubital vein. When two antigens were administered, BSA was given first, followed 7 days later by HGG. The animals were bled on subsequent designated days from the cubital vein to obtain 0.5 ml of chicken plasma. Experimental series I (BSA only) was conducted earlier, series II (BSA, HGG and BSA + HGG) was conducted at a later date.

Precipitating antibody directed against BSA or HGG was determined with a modified Preer technique (2, 4). Half value times (the time needed to reduce a given antibody concentration to half its original value) of anti-BSA and anti-HGG antibody were calculated from the descending limb of the antibody concentration curves, starting at a point where the curve assumed an exponential shape (2).

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### Results and Discussion

A single dose of BSA injected intravenously into the conventional chicken produces precipitating antibody which reaches a maximum concentration approximately 8 days later. Shortly thereafter, this production terminates and the antibody in the circulation is catabolized at the same rate as the overall gamma globulin fraction, which has a half life of 4.2-4.3 days. In the germfree chicken under these conditions less antibody is formed, but its half value time of 8 days indicates that a prolonged antibody production takes place which partially compensates for this gamma globulin catabolism and results in an extended half value time (Fig. 1, series I) (2).

Treatment of the germfree chicken with a second antigen (HGG) just before the anti-BSA antibody concentration reaches a maximum value reduces the half value time for this antibody to 4.5 days (Fig. 1, series II). This indicates that after HGG treatment the anti-BSA antibody concentration now decreases at a rate comparable to the turnover rate of the main body of the gamma globulin fraction and suggests that little or no new formation of anti-BSA precipitin takes place after the tenth day.

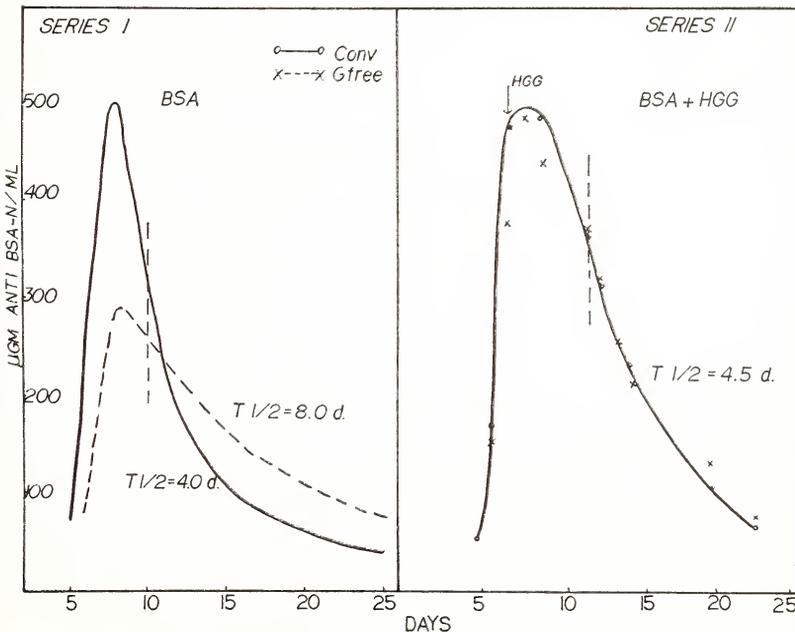


Fig. 1. Anti-BSA precipitins formed in germfree and conventional chickens after a single i. v. dose of BSA (left) and after a dose of BSA, and a dose of HGG administered 7 days later (right).

However, the anti-HGG precipitin response to this second injection again demonstrates the difference in half value time between germfree and conventional birds that was observed in animals which had received a single injection of BSA or HGG (Fig. 2 and Table I). In con-

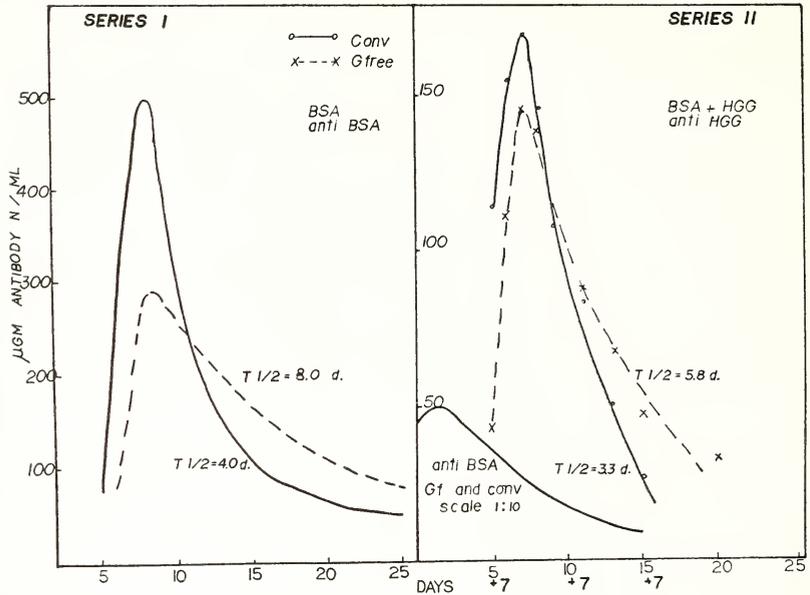


Fig. 2 Precipitin response after a single dose of BSA (left) and after a dose of BSA, and a dose of HGG administered 7 days later (right). Time scale anti-HGG response starts at day of injection of HGG (see text).

ventional chickens the anti-HGG antibody shows a half value time slightly less than the approximate 4 day value established for the half life of chicken gamma globulin. The half value time in germfree birds is 50% higher than the gamma globulin half life and almost twice the half value time found in conventional animals, again indicating a prolonged antibody formation in the absence of other major antigenic stimuli. The data also demonstrates the difference between the anti-BSA and anti-HGG response in general, which seems to be caused by an earlier termination of the major phase of anti-HGG antibody production, resulting in lower maximum concentrations which are reached a day earlier than in the case of the anti-BSA response.

The quantitative aspects of these studies have been brought together in Table I. These data show, that the subsequent administration of the second antigen (HGG) suppresses the extended anti-BSA antibody response in the germfree bird but apparently increases the magnitude of this response. These effects are not seen in the conventional animal. On the other hand, the response to HGG, the second antigen, is definitely depressed in both groups as a result of the earlier administration of BSA.

The data consistently show a difference in half value time between anti-HGG and anti-BSA antibody produced in conventional chickens. Assuming that during the period in which these values were determined no appreciable new formation of precipitating antibody occurs, then this suggests that the half life of anti-HGG antibody is shorter than



that of anti-BSA antibody, and also somewhat shorter than the half life of the total ammonium sulfate precipitated gamma globulin fraction. With the controversy about the nature of the antibody resulting from a primary stimulation in the chicken not yet resolved (5, 6), it is difficult to speculate about the meaning of these differences.

The picture which emerges from these studies is that when major antigenic stimuli are absent, as in the germfree chicken, stimulation with BSA results in a response which continues beyond the eighth day, when maximum titers are reached. The administration of a second unrelated antigen, e.g. HGG, produces an instantaneous increase in the amount of anti-BSA antibody released to the circulation, but the protracted response is suppressed. The second antigen, on the other hand, seemingly finds only part of the potential antibody producing system available. The resulting anti-HGG response in both groups is depressed in comparison to that of animals receiving only a single injection of this antigen. However, the germfree animal demonstrates a prolonged response to this second antigen that is similar to that found after the administration of a single antigen.

#### Summary

Germfree and conventional chickens were administered two unrelated antigens, BSA and HGG. The second antigen, HGG was given one day before anti-BSA precipitins reached a maximum value. This resulted in a suppression of the extended anti-BSA precipitin formation otherwise observed in germfree chickens. Formation of anti-HGG precipitin was depressed in both experimental groups, but the germfree birds demonstrated a prolonged response to HGG similar to that seen after the administration of a single antigen.

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