Duration of Antibody Response to Soluble Antigen

ANTIBODY production after immunization with particulate antigens is known to persist over long periods of time. By contrast, immunization with soluble antigens in the absence of adjuvants is generally thought to give responses of relatively brief duration. Recently, it has been reported that antibody production continued for several hundred days in rabbits given one or several intravenous injections of a number of soluble antigens[1]. Antibody produced late in the course of the response gave negative ring tests, failed to sensitize the guinea pig intestine, and could be detected only by a passive hæmagglutination technique. This communication is concerned with similar observations on the response of chickens to intravenous injections of bovine serum albumin (BSA). One major finding of this study is the persistence of precipitating antibody up to 313 days after injection.

Antisera were obtained at various times after two spaced (50 days) intravenous injections of BSA (40 mg/kg body-weight). Antibody activity was measured both by precipitin tests and by Farr's technique[2], which depends on the differential solubility of free ^{131}I-BSA and ^{131}I-BSA bound to antibody in half-saturated ammonium sulphate. The dilution effect, 'avidity', was determined by comparison of the antigen-binding capacity of serum at two concentrations of BSA. The antigen-binding capacity was determined as described by Farr: sera were diluted until 33 ± 10 per cent of a constant amount of antigen was precipitated after addition of ammonium sulphate. Correction factors were applied to normalize all values to 33 per cent binding. The following formula was used to calculate the antigen-binding capacity: (μg BSA nitrogen added/ml.) × (0·33) × (reciprocal of dilution binding 0·33 of added antigen).

The antigen-binding capacities of the three animals tested were remarkably constant over the 226-day period considered (Table 1), the only difference noted being an increase in 'avidity' between 87 and 241 days after immunization. Because all samples were tested at the same time, this avidity difference may represent an effect of storage. According to Farr[2], complexes formed at the 0·33 reference point with high avidity sera have an overall molar ratio of one antibody molecule to two antigen molecules. The weight ratio of BSA (mol. wt. 70,000) to antibody (160,000) in such complexes would be essentially one. Thus, the antigen-binding capacity of high-avidity sera approximates the actual antibody content, provided one is testing bivalent antibody. The precipitating antibody concentrations expected in the sera under investigation then would be 4–10 μg nitrogen/ml., clearly less than the resolving power of most quantitative precipitin methods.

Table 1. ANTIGEN-BINDING CAPACITY (ABC)* AT VARIOUS TIMES AFTER IMMUNIZATION

Bird No.	Days after second injection			
	87	241	297	313
28	0·93/1·6 (58)	1·4/1·8 (78)	1·5/2·0 (75)	1·2/1·7 (71)
29	1·4/2·4 (58)	1·2/1·7 (71)	0·90/1·3 (69)	1·2/1·6 (75)
30	0·54/1·1 (49)	0·72/1·1 (65)	0·93/1·4 (66)	0·74/1·1 (67)

* The figures given are: ABC at 0·16 μg BSA nitrogen per ml./ABC at 1·6 μg BSA per ml. (percentage 'avidity'). ABC values calculated for 0·25 ml. serum.

Because of the contention[1] that late antibody is non-precipitating, experiments were carried out to test rigorously for the presence of precipitating antibody. Mixtures of serum and ^{131}I-BSA of varying concentrations were made in both 0·9 and 8 per cent sodium chloride. The mixtures were incubated at 37° C for 6 h and then at 5° C for several days. The radioactivity of each tube was determined in a well-type scintillation counter both before and after centrifugation and removal of the supernatant fluid. In this manner, it was possible to detect significant precipitation of antigen in the absence of any readily visible precipitate. Control tubes containing antibody to a non-related protein retained less than 2 per cent of the added antigen, even in the absence of washing.

In all samples tested, complete antigen precipitation occurred over a wide range of concentrations (Fig. 1) and, significantly, at high enough concentrations to account completely for the antigen-binding capacity data. Because this method allows fairly accurate delineation of the region of slight antigen excess, it is also possible to estimate antibody content based on known equivalence ratios of antibody to antigen. Such estimates of antibody content were generally several times higher than the estimates based on the antigen-binding capacity. This is not entirely unexpected because the avidities of the sera were less than 100 per cent, that is, higher antigen-binding capacity values would be expected if higher concentrations of antigen were used. These results show that the late antibody response of chickens consists mainly of precipitating antibody. Because of evidence[3] indicating that non-precipitating antibody is not responsible for passive hæmagglutination reactions, it is expected that the use of more sensitive methods also would reveal the presence of precipitins in the late response rabbit antisera[1].

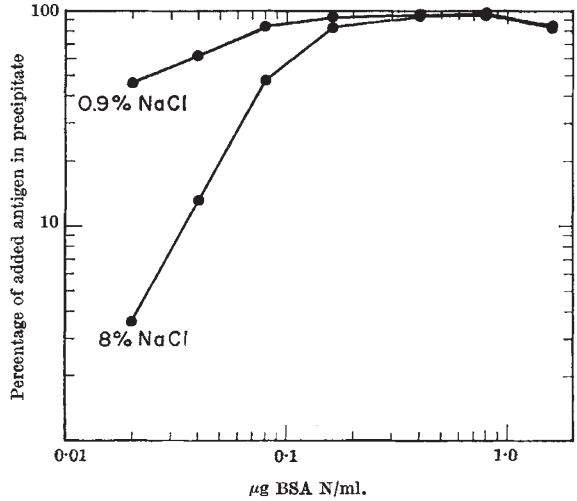

Fig. 1. Bird 29, 297 days after injection. Approximate equivalence concentration, 0·8 μg nitrogen/ml. Note marked inhibition by 8 per cent sodium chloride in antibody excess region

Although not directly related to the main point of this communication, the occurrence of a marked inhibition zone in extreme antibody excess deserves comment. As shown in Fig. 1, inhibition of antigen precipitation was greatest in the presence of 8 per cent sodium chloride. This is surprising, because it is well established that maximum precipitation occurs at this salt concentration with avian antisera. By adding ammonium sulphate to one-half saturation, all the antigen could be precipitated from the supernatants in this region. Therefore, the primary interaction between antigen and antibody was not affected.

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[1] Richter, M., and Haurowitz, F., *J. Immunol.*, **84**, 420 (1960). Blumer, H., Richter, M., Cua-Lim, F., and Rose, B., *J. Immunol.*, **88**, 669 (1962).
[2] Farr, R., *J. Infect. Dis.*, **103**, 239 (1958).
[3] Stavitsky, A. B., and Arquilla, E. R., *J. Immunol.*, **74**, 306 (1955). Fiset, L. G., *Immunology*, **5**, 580 (1962).