free in the serum, were either absent or were of too weak a titre to be demonstrated.

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IMMUNOLOGY

Quantity and Quality of Antibody produced following the Termination of Tolerance in *C57BL/6* Mice

The mechanisms involved in the termination of immunological tolerance are still obscure. Basic to the understanding of this phenomenon is an evaluation of the degree to which the immune capacity of the previously tolerant animal has returned to normal. Such information would also be necessary in comparing the results obtained in the termination of the tolerant state produced under a diversity of experimental situations.

Previous investigations in mice in which the tolerant state terminated spontaneously suggested that the immune response in these animals may differ from that of normal mice since no precipitating antibody could be observed in the previously tolerant group[1]. The experiments to be reported were performed in order to determine the amount of antibody produced in *C57BL/6* mice in which tolerance to bovine serum albumin (BSA) was lost spontaneously 8–10 weeks after the neonatal injection of antigen. Also, a parameter of the quality of antibody produced in normal and previously tolerant animals was studied, namely, the dissociation rate of antigen–antibody complexes made in antigen excess. A modification of the Farr ammonium sulphate salting-out technique[2] was used both for quantitating[3] the immune response and for the estimation of the bond-strength of antigen–antibody complexes by means of dissociation rate measurements[4]. *C57BL/6* mice were made tolerant to BSA by neonatal subcutaneous injection of 20 mg BSA. At 10 weeks of age these animals and a group of controls of the same age were immunized with 0·1 mg BSA in incomplete Freund's adjuvant. Table 1 compares the anti-BSA activity of the previously tolerant group to the normal controls 25 and 74 days following the immunizing dose of BSA. The figures under the *ABC*–33 column refer to the μg BSA nitrogen bound per ml. of undiluted serum when tested at an antigen concentration of 0·02 μg ^{131}I–BSA N/ml. On day 25 the sera of the control group contained slightly more antibody than the tolerant group, but by day 74 the amount of antibody in the sera of both groups was the same. The figures under the $T_{1/2}$ column represent the half-dissociation time of antigen–antibody complexes, which is a function of the rate at which the complexes dissociated. On day 25 the antibodies of the tolerant group had a somewhat faster rate of dissociation than the antibody of the control group, but by day 74 the dissociation rates were the same. The slight differences observed in quantity and quality of antibody present on day 25 were not statistically significant but might represent some residual tolerance in a few animals at the time of the early bleeding. These results demonstrate that, as judged by the amount and quality of antibody produced, there is a complete return of immunological competence when tolerance is terminated spontaneously in the mouse. It remains to be shown whether the same findings can be obtained in other species and under different conditions of tolerance.

Table 1. Quantity and Quality of Antibody following Termination of Tolerance

	Days after challenge* 25		74	
	ABC-33	$T_{1/2}$	*ABC*-33	$T_{1/2}$
Tolerant	1·6 ± 1·7 (17)†	3·8 ± 2·5 (9)	8·5 ± 5·0 (17)	36·0 ± 8·4 (15)
Control	2·5 ± 1·0 (5)	7·6 ± 3·9 (5)	8·8 ± 4·0 (5)	35·3 ± 2·1 (5)

* Challenged at the age of 10 weeks.
† No. of mice.

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Activity of Homologous and Heterologous Antisera prepared against the Same Antigen

The type of antibody activity present in antisera from different species directed towards the same antigen has important implications for the selection of the most effective antibody-producing animal for any given antigen–antibody system. In preparing antisera against mouse histocompatibility antigens, the use of mice of different histocompatibility types as antibody producers has proved very effective in differentiating these antigens[1].

It has been suggested[2] that the effective antigenicity of a given antigen is influenced by the taxonomic relationship the antigen bears to the antibody producing animal.

In previous work[3] we reported that rabbit anti-12-day chick embryo red blood cell serum after absorption with adult chicken red blood cells had no residual activity for other pools of adult red blood cells, but retained excellent activity against 12-day chick red blood cells, as assayed by hæmagglutination.

It is the purpose of this communication to indicate that rabbit antiserum and chicken antiserum, both against the same antigen—embryonic chick red blood cells—have strikingly different properties.

Twelve-day-old New Hampshire chick embryos were bled from the chorioallantoic vessels. Four parts of blood were mixed with one part of ACD solution. The blood was centrifuged and the cells washed three times with cold 0·9 per cent sodium chloride, after which they were packed and made up to a final concentration of 50 per cent in 0·9 per cent saline.

Adult New Hampshire chickens and adult New Zealand rabbits were injected intravenously every other day with the following amounts (c.c.) 0·25, 0·50, 0·75, 1·00, 1·25 and 1·50. After a 7-day interval these animals were given an intravenous booster injection of 2·50 c.c. and bled for serum 7 days subsequent to this last injection. The cells from 148 embryos were used for the injection of adult chickens and 142 for the injection of rabbits. The antisera obtained were heated to 56° C for 30 min and stored frozen until used. Normal rabbit serum and normal