The effects of nitrogen mustard were examined by addition of 0·1–10 μg/ml. to leucocyte suspensions containing PHA (2 μl./ml.) and counts were made in 6 days. The addition of 5 μg/ml. of HN2 allowed the survival of about one-half of the lymphocytes and lymphoblastoid cells. In contrast, this concentration of HN2 killed all the lymphocytes in control suspensions without PHA. The finding indicates that PHA protected lymphocytes against the radiomimetic agent HN2.

This work was supported by a grant from the National Cancer Institute, U.S. Public Health Service.

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IMMUNOLOGY

Relationship of Antigenicity and Degree of Tolerance to Heterologous Serum Albumins in C57BL/6 Mice

IN an attempt to examine the determining factor(s) for induction and duration of tolerance it had been shown that there was no correlation between persistence of labelled antigen and duration of tolerance, and therefore it was concluded that persistence of antigen *per se* is not the determining factor for maintaining the tolerant state[1,2]. In the work recorded here another parameter was examined, namely, the antigenicity of several heterologous proteins. Antigenicity was compared with the previously obtained results on the duration of neonatally induced immunological unresponsiveness[1].

Groups of 5 *C57BL/6* mice (20 g, males) were given a single injection of either rabbit (RSA), human (HSA), chicken (CSA), sheep (SSA), equine (ESA), or bovine serum albumin (BSA) incorporated into incomplete Freund's adjuvant. A total amount of 0·05 ml. adjuvant mixture containing various amounts of antigen was injected and distributed among three foot-pads[3].

Antibodies were determined quantitatively by the ammonium sulphate technique described by Farr[4]. Individual mouse sera were assayed by using five-fold dilutions beginning at 1:40.

The diluent was 10 per cent normal mouse serum in borate buffer (ionic strength 0·1, *p*H 8·3–8·5). Antigen labelled with iodine-131 was used in a concentration of 0·02 μg N/ml. Antigen binding capacity (*ABC*–33) is given as μg AgN bound per ml. of undiluted serum.

Table 1 shows the antibody response to a single immunizing dose of 100 μg BSA 15, 30, 50, and 84 days after injection. By this criterion HSA is the most effective antigen followed by RSA, CSA, BSA, SSA, and ESA. Table 2 shows the minimal amounts of antigen necessary to induce an immune response. Again HSA, RSA, and CSA appear to be the most effective antigens, since all animals responded to 1 μg of these antigens, whereas only 2 out of 5 animals responded to 1 μg SSA and there was no response with BSA or ESA at that concentration. At a concentration of 0·1 μg, the only animals which responded were those which had been injected either with HSA or with CSA.

Previous results[1] have indicated that on neonatal injection of 20 mg protein there is the following order of duration of tolerance: SSA and BSA caused most prolonged tolerance, followed by ESA and HSA; RSA and CSA were the antigens with which it was most difficult to induce a tolerant state. When challenged at 12 weeks of age, 25 per cent of the mice injected with SSA were still fully tolerant. Taking this as a criterion, tolerance to BSA lasted approximately 8 weeks, to ESA and HSA 6, and to RSA 4, whereas none of the mice injected with CSA was fully tolerant when challenged 4 weeks later. As can be seen from these results and the present findings, there is no correlation between the capacity of an antigen to induce the formation of antibody and its capacity to induce and maintain the tolerant state. This suggests that those processes are mediated by different properties of the antigen.

Table 1. ANTIBODY RESPONSE TO HETEROLOGOUS SERUM ALBUMINS IN *C57BL/6* MICE (*ABC*-33 AFTER INJECTION OF 100 μg IN 0·05 ML. OF INCOMPLETE FREUND'S ADJUVANT, 3 FOOT-PADS)

Antigen	Days after injection 15	30	50	84
RSA	1·55 ± 0·75	6·90 ± 3·24	16·91 ± 5·28	12·32 ± 6·80
HSA	1·89 ± 0·41	10·75 ± 4·00	28·00 ± 8·16	29·87 ± 11·73
CSA	0·99 ± 0·16	2·92 ± 0·59	6·12 ± 0·46	10·76 ± 4·20
SSA	0·86 ± 0·18	2·54 ± 1·00	3·68 ± 1·25	5·11 ± 3·55
ESA	0·84 ± 0·31	1·97 ± 0·64	2·71 ± 1·10	3·56 ± 1·60
BSA	0·47 ± 0·41	4·07 ± 0·93	7·19 ± 3·52	6·17 ± 2·06

Table 2. ANTIBODY RESPONSE TO HETEROLOGOUS SERUM ALBUMINS IN *C57BL/6* MICE (*ABC*-33, 40 DAYS AFTER INJECTION OF ANTIGEN IN 0·05 ML. OF INCOMPLETE FREUND'S ADJUVANT, 3 FOOT-PADS)

Antigen	μg injected 10·0	1·0	0·1
RSA	9·02 ± 0·52	2·46 ± 1·49	not done
HSA	7·07 ± 3·02	4·27 ± 1·57	1·27 (2)
CSA	6·83 ± 1·76	1·68 ± 1·44	0·13 (1)
SSA	2·27 ± 1·76	0·21 (2)	—
ESA	0·76 ± 0·32	—*	—
BSA	1·58 (2)	—	—

Figures in parentheses show the total number of mice which gave a measurable *ABC*-33.
* No detectable antibody.

This work was performed during a research fellowship at the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California, and was supported by a U.S. Public Health Service grant.

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Apparent Inability of Polypeptides constructed from D-Amino-acids to stimulate Antibody Formation

Gill, Gould and Doty[1] have demonstrated that while a synthetic polypeptide containing the L-amino-acids of lysine and glutamic acid stimulates antibody formation, a similar polypeptide constructed from D-amino-acids does not. Two possible explanations were offered for the inability of the latter to stimulate antibody formation: (1) some failure in the sequence of steps leading to antibody production, including the possibility that the 'D-polypeptide' cannot be transported to the site of antibody production, or that some prerequisite hydrolysis cannot be performed by the usual enzymes; (2) the inability of the γ-globulin chain to fold around the determinant portion of the 'D-polypeptide'.

An alternative explanation for this phenomenon is presented here. Antibody formation is generally stimulated by repeated injections of antigen. However, it is well known that the presence of a continuous excess antigen inhibits the synthesis of an antibody[2]. Continuous excess of antigen may be brought about in two ways: (1) by the administration of large and repeated doses of antigen; (2) by the administration of an antigenic substance which the animal has no means of breaking down or eliminating. It seems likely that the 'D-polypeptides' persist for long periods of time without being broken down or eliminated, since there are few enzymes which