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Received August 22, 1962. P.S.E.B.M., 1962, v111.

The Immune Response in Cold-Acclimated Mice.* (27796)

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Recent studies of the effects of cold exposure upon animals have indicated that changes in the immune response may occur. Rats exposed to cold demonstrate an increase in the alpha and beta globulin fractions of the blood and a corresponding decrease in the gamma globulin fraction(1). Similar results have been reported for cold-acclimated rabbits(2). In guinea pigs exposed to low temperature, there is an increase in the complement-titer and serum bactericidal activity for *Staphylococcus pyogenes*(3).

The rate of decay of passively administered antibodies is greater in depilated rabbits exposed to −15°C(4,5) than in controls maintained at room temperature (20-25°C). Cold exposure is also accompanied by an increased rate of decay of actively produced antibodies(6), and a slower rate of increase in the level of circulating antibodies(5).

It is well established that bacteriophages are good antigens; coliphage has been shown to serve as suitable antigen in rabbits(7) and actinophage as a good antigen in mice(8). One definite advantage in using phage as an antigen is that it allows for very early detection of antibody production(8) which can be accurately and rapidly determined from the decrease in plaque formation.

In this study the effect of cold-acclimation upon production of active coliphage antibodies in mice was investigated. The temperature of acclimation was kept above zero (4°C) and the pelage left intact to reduce stress.

Materials and methods. One hundred and twenty 8-week-old albino Swiss mice were divided into 4 groups. Two were control groups and were kept at room temperature (23°C). The other 2 groups were cold-acclimated at 4°C for 4 weeks. After the 4-week acclimation period, immunization with coliphage was begun on one group. The remaining acclimated group was then placed at room temperature for an additional 4 weeks of de-acclimation before being immunized. A control group was immunized at the same time as each experimental group. All animals were given Purina Chow pellets and water *ad libitum.*

Immunization and collection of blood samples. All animals were injected every other day for 6 days intraperitoneally with 1 ml of 5×10^{10} plaque-forming units of coliphage strain T_2r^+.‡ Blood samples were obtained from mice killed on the 2nd, 6th, and 10th days after the final immunization injection.

* This investigation was supported by research grants from U. S. Public Health Service and Am. Cancer Soc.

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‡ The coliphage and bacteria used in this experiment were generously supplied by Dr. R. L. Nutter, Loma Linda University.

TABLE I. Percentage of Inactivation of Coliphage T_2r^+ by Anti-Coliphage Antibodies in Mice.

Days after last inj	Cold-acclimated (4°C)	Control (23°C)	De-acclimated (23°C)	De-acclimated control (23°C)
6	74.5 ± 3.2*	70.1 ± 3.6	61.6 ± 3.8	62.1 ± 4.0
10	95.8 ± 4.1	95.0 ± 5.0	92.3 ± 4.2	93.5 ± 4.3

* ± stand. error of mean.

In each case, the heart of the etherized mouse was exposed surgically and blood was removed by direct heart puncture. Sterile needles and syringes rinsed with a 0.5% heparin solution were used. The sera samples were refrigerated in sterile vials and stored until assayed.

Assay of anticoliphage sera. The sera were assayed for coliphage antibodies according to standard technic as outlined by Adams(9). Coliphage strain T_2r^+ and *Escherichia coli,* strain B, were used. The bacteria were grown in Frazier's broth§ and the plaque counts made on Frazier's agar. The phage were suspended and injected in standard phage buffer.‖

Six plates were poured for each of the 120 sera samples to obtain an average count for each sample. To facilitate plaque-counting, the assay phage was diluted to 3.3×10^6 plaque-forming units/ml rather than to 1×10^7/ml as described by Adams(9). Total plaque counts were made for each plate using a Quebec Colony Counter.

Results and discussion. The percentage of phage inactivation was the same in sera samples obtained on the 2nd and 6th days after immunization, but was markedly higher in samples obtained on the 10th day at which time the peak (or near peak) level of circulating antibody was obtained (96% inactivation).

There was no significant difference in the levels of active coliphage antibodies between cold-acclimated and control mice (Table I). Thus the elevated metabolic rate of cold-exposed animals with the accompanying increase in protein turnover(4,6) apparently does not result in an increased rate of synthesis of active antibodies in mice. Trapani(5) has pointed out, however, that even if there were an increase in rate of antibody production, there might also be a corresponding increase in rate of antibody decay which could obscure any actual change.

In our study, antibody production took place over a maximal period of only 16 days and, therefore, antibody decay as a source of error was probably minimized. Nevertheless, this source of error must be evaluated before the possibility of a balanced increase in rate of synthesis and degradation of antibody can be eliminated. In any case, however, our experiments show the net result is that the circulating antibody-titer of cold-acclimated mice is no higher than that of controls.

There was no difference between the circulating antibody levels in the de-acclimated animals and their controls, which is not surprising in light of the fact that there was no change in the case of the cold-acclimated mice.

It is of interest that at the 6th day the levels of circulating antibody in both groups of the 12-week-old mice (the cold-acclimated animals and their controls) were higher than in the de-acclimated animals and their controls which were 4 weeks older when their immunization was started (Table I). The 4-week difference in age represents an appreciable period in the life-span of mice, and apparently the older mice take longer to reach peak antibody production. However, by the 10th day after final injection, there is no difference in serum antibody-titer in the two age groups. In the younger mice, the higher 6th-day response probably is a consequence of a more active general metabolic activity and protein turnover.

Summary. A study of the effects of cold-

§ KH_2PO_4, 4.5g; Na_2HPO_4, 10.5g; NH_4Cl, 3.0g; $MgSO_4 \cdot 7H_2O$, 0.3g; casein hydrolysate (Nutritional Biochemicals Corp.) 5.0g; glycerol, 24.0 ml; $CaCl_2$ (1% sol.) 0.3 ml; gelatin (1% sol.) 0.3 ml; distilled H_2O, 1 liter.

‖ KH_2PO_4, 3.0g; Na_2HPO_4, 7.0g; NaCl, 4.0g; $MgSO_4 \cdot 7H_2O$, 0.2g; distilled H_2O, 1 liter.

acclimation and de-acclimation on the immune response in 8-week-old mice was conducted. The mice were exposed to an ambient temperature of 4°C for 4 weeks and some of these were then de-acclimated at room temperature (23°C) for an additional 4 weeks. In each case, controls were maintained at 23° C. The antigen used was coliphage T_2r^+ and the levels of circulating antibody were determined in terms of the remaining serum plaque-forming units of coliphage. Neither acclimation nor de-acclimation, under these conditions, resulted in significant change in the levels of circulating coliphage antibodies.

We wish to thank Dr. Eugene H. Cota-Robles for his valuable suggestions during this research.

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Received August 27, 1962. P.S.E.B.M., 1962, v111.

Modification of Complement Fixation Test for Estimation of Rous Sarcoma Virus Antibodies in Turkey and Chicken Serums. (27797)

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Bushnell and Hudson(1) and Rice(2) have reported on attempts to use the classical complement fixation (C-F) test for chicken serum but found that some heated serums were anticomplementary at dilutions of 1:50 and lower. More recently Brumfield and Pomeroy(3), in tests with ornithosis antigen and unheated turkey serums, reported adequate qualitative results by increasing the amount of complement to overcome the anticomplementary effects; however, many serums could be used only at relatively high dilutions. Later they(4) found that enhanced fixation of guinea pig complement occurred when heat inactivated serums were supplemented with small amounts of fresh, normal chicken or turkey serums. Boulanger and Bannister(5) reported good fixation of guinea pig complement with heated bovine serum and vesicular stomatitis virus when fresh, normal calf serum was added. Recently Benson, *et al.*(6) and Brumfield, *et al.*(7) showed that the factor in normal, unheated chicken serum was probably the C'1 component of chicken complement.

This is a brief report on the detection and quantitation of Rous sarcoma virus antibody in heated turkey and chicken serum with a modified C-F test.

Materials and methods. Antigen. Rous sarcoma virus (RSV) antigen used in these studies was prepared according to the procedures described by Moloney(8), except that protamine sulfate precipitation was not done and the 40,000 × *g* pellet was brought back into suspension with 0.05 M sodium citrate, pH 6.7, to a concentration of one gram of original tumor per milliliter of final material.

Antiserums. The anti-Rous virus chicken serums were produced by the method of Fink and Rauscher(9). The anti-Rous virus turkey serums were produced by subcutaneous inoculation of 7-day-old turkey poults with

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