

sistent with those of Talmage(9) and others, relating the avidity of an antibody to the phase of immunization which it represents, and suggest that an attempt to quantitate the hemagglutination test is valid only when the reference serum in historically comparable to the test serum. Studies are under way to differentiate the mechanism.

**Conclusion.** Whole-body irradiation of dogs immunized with an alum precipitated tetanus toxoid results in a delay in appearance of antitoxin if the first toxoid is administered after radiation. Dogs irradiated 30 days after a first dose of toxoid and receiving a booster injection 24 hours after irradiation presented a good antitoxin response when measured by the hemagglutination test. However, a poor antibody response was found if the antitoxin was measured by the

toxin neutralization technics.

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### Comparative Immunology. Antibody Response in *Dipsosaurus dorsalis* at Different Temperatures.\* (28096)

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A lizard from the California desert, *Dipsosaurus dorsalis* has been found to produce antibodies for the H-antigen of *Salmonella typhosa* after a short period of immunization provided that ambient temperature was maintained at 35°C(1). At 25°C antibody response was minimal or absent. This observation suggested that the process could be regulated by changing the ambient temperature. The present report concerns a study of the influence of temperature on antibody response in this group of animals.

**Materials and methods.** Details of the capture and maintenance of animals, preparation of *S. typhosa* vaccine and titration of antibodies have been described(1). The antigen used in the present experiments differed in that one volume of *S. typhosa* H-antigen ( $2 \times 10^9$  killed cells per ml) was diluted

with an equal volume of Pentex rabbit gamma globulin (RGG) dissolved in 0.9% NaCl at a concentration of 20 mg/ml. The RGG was included to study simultaneously the antibody response to a soluble protein. Measurement of antibodies to RGG has presented difficulties due to the small volumes of serum available. These studies will be reported separately.

Daily injections of antigen (0.2 ml) were given subcutaneously into the thigh for the first 3 days of the first week. During the second week, the same dose was given intraperitoneally on each of the first 3 days.

During the 10-day immunization period animals were maintained at constant ambient temperatures of 25°C, 35°C or 40°C. Cloacal temperatures fell within the range: ambient temperature  $\pm 1^\circ\text{C}$ .

During the post-immunization period, various groups were exposed to different com-

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TABLE I. Agglutination Titers\* of Animals Maintained at 25°C During 10-day Immunization Period Plus Varying Post-immunization Treatment.

| Group | Temp of post-immunization period (°C) | Duration of post-immunization period (days) | Titers  |                |
|-------|---------------------------------------|---------------------------------------------|---------|----------------|
|       |                                       |                                             | Range†  | Geometric Mean |
| A     | 25                                    | 7                                           | all<10  | 0              |
| B     | 25                                    | 14                                          | <10- 10 | 2              |
| C     | 25                                    | 21                                          | <10- 40 | 7              |
| D     | 35                                    | 7                                           | 40-320  | 138            |
| E     | 35                                    | 14                                          | 10-320  | 65             |
| F     | 25                                    | 7                                           | 10- 80  | 26             |
|       | 35                                    | 7                                           |         |                |
| G     | 25                                    | 7                                           | 20-160  | 40             |
|       | 35                                    | 14                                          |         |                |

\* With *S. typhosa* H antigen.

† 6 to 8 animals per group.

binations of time and temperature as shown in Tables I, II, and III. Six to 8 animals were included in each group.

**Results.** Animals represented in Table I were maintained at 25° during the immunization period. When Groups A and B were held for an additional 7 and 14 days at 25° there was no significant antibody response. When the time was extended to 21 days in Group C low titers were noted in 4 serums out of 6. When *D. dorsalis* immunized at 25° were transferred to 35° for a post-immunization period of 7 or 14 days (Groups D and E), a marked increase was seen in the antibody response. Animals in Groups F and G were held at 25° for the first 7 days post-immunization, then transferred to

TABLE II. Agglutination Titers\* of Animals Maintained at 35°C During 10-day Immunization Period Plus Varying Post-immunization Treatment.

| Group | Temp of post-immunization period (°C) | Duration of post-immunization period (days) | Titers  |                |
|-------|---------------------------------------|---------------------------------------------|---------|----------------|
|       |                                       |                                             | Range†  | Geometric Mean |
| H     | 35                                    | 7                                           | 20-320  | 65             |
| J     | 35                                    | 14                                          | 40-320  | 100            |
| K     | 35                                    | 21                                          | 40-320  | 79             |
| L     | 35                                    | 28                                          | 20-160  | 98             |
| M     | 25                                    | 7                                           | 20-320  | 50             |
| N     | 25                                    | 14                                          | <10- 40 | 13             |
| P     | 35                                    | 7                                           | 80-640  | 200            |
|       | 25                                    | 7                                           |         |                |
| Q     | 35                                    | 7                                           | 40-160  | 90             |
|       | 25                                    | 14                                          |         |                |

\* With *S. typhosa* H antigen.

† 6 to 8 animals per group.

35° for 7 or 14 days. The titers were inferior to those in groups D and E both of which were maintained at 35° for comparable periods without the intervening week at 25°.

The serum titers from animals maintained at 35° during the immunization period are shown in Table II. Groups H, J, K and L, held respectively for post-immunization times of 7, 14, 21 and 28 days at 35°, showed a good antibody response although there was considerable variation among individual animals. Group M which was changed from 35° to a post-immunization temperature of 25° for 7 days displayed only moderate titers with one exception where the titer reached 320. Prolongation of the time to 14 days in Group N resulted in marked attenuation of the response.

TABLE III. Agglutination Titers\* of Animals Maintained at 40°C During 10-day Immunization Period Plus Varying Post-immunization Treatment.

| Group | Temp of post-immunization period (°C) | Duration of post-immunization period (days) | Titers  |                |
|-------|---------------------------------------|---------------------------------------------|---------|----------------|
|       |                                       |                                             | Range†  | Geometric Mean |
| R     | 40                                    | 7                                           | 10-160  | 44             |
| S     | 40                                    | 28                                          | <10- 80 | 19             |

\* With *S. typhosa* H antigen.

† 6 to 8 animals per group.

In Groups P and Q, kept at 35° for the first post-immunization week, titers were high even after an additional 7 or 14 days at 25°.

Two groups of *D. dorsalis*, shown in Table III, were immunized and maintained at 40°, primarily to ascertain whether or not this temperature would result in an antibody response superior to that at 35°. Since the data did not suggest any such affect, further studies at 40° were not attempted.

Non-immunized controls were uniformly negative at 1:10, the lowest serum dilution employed in these studies.

**Discussion.** In examining these data, one should bear in mind that all the animals were subjected to an immunization period of the same length and that post-immunization periods of different groups varied from 7 days to 28 days in duration. In certain

groups, the ambient temperatures were the same in both periods. In these groups, the data show that regardless of duration, antibody response was poor or non-existent at 25° and good at 35° with some variation among individual animals. After 7 days at 40°, the response was similar to that at 35°, but after 28 days at 40°, titers were generally lower than in the comparable 35° group.

In certain experiments, animals were immunized at one temperature, then transferred to another temperature during the post-immunization period. When Groups D and E were immunized at 25° and then shifted to 35°, antibody response was enhanced; conversely, when animals were immunized at 35° and then moved to 25°, the response was inhibited. This effect was particularly striking in Group N after 14 days.

The results with Groups F, G, P and Q suggest that within the limits of quantitation possible by the tube agglutination method, the temperature of the first post-immunization week is important in determining eventual antibody level. Even though animals are later moved to a favorable or to an unfavorable temperature, the eventual titer reached is strongly influenced by the temperature of the first post-immunization week.

These results indicate that it is possible to regulate antibody synthesis in poikilothermic vertebrates by varying ambient temperature. These and earlier experiments(1) also demonstrate for the first time synthesis of agglutinating antibody by a member of the

class Reptilia. Other studies with lower vertebrates have shown antibody formation in teleost fishes(2-4), and amphibia(4-6), but not by cyclostomes(3). Experiments to be presented elsewhere show that antibodies of *D. dorsalis* are located in an electrophoretic zone comparable to the  $\beta_2$  and  $\gamma$ -globulin areas of higher vertebrates.

**Summary.** The antibody response of the reptile, *Dipsosaurus dorsalis*, to typhoid vaccine was compared at constant ambient temperatures of 25°, 35° and 40°. Antibody response was poor or non-existent at 25°, good at 35° and moderately good at 40°. Variations in titer were noted among different individuals within a group. When animals immunized at 35° were transferred to 25°, antibody response was inhibited; in the reverse situation, the response was enhanced. Data obtained during this investigation have shown that a member of the phylogenetic class Reptilia can synthesize agglutinating antibodies and that the process can be regulated by varying the ambient temperature.

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## Developmental Changes in Glycogen Content of Primordial Germ Cells in Chick Embryo.\* (28097)

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The discovery of a high alkaline phosphatase content in the primordial germ cells

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(PGCs) of the mouse embryo(1) provided an excellent method for studying the origin and migration of these cells in mammals. Meyer(2) observed a similar phenomenon in the PGCs of chick embryos, but the substance was glycogen instead of alkaline phosphatase. This finding in the chick embryo