Effect of Temperature on Antibody Synthesis in the Reptile, *Dipsosaurus dorsalis.** (24989)

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Although studies of antibody synthesis in mammals and birds have been confined to relatively few species, considerably more is known about the process in these 2 classes than about antibody synthesis in lower vertebrates. That lower classes of vertebrates are poikilothermic makes these animals logical choices for studies involving effects of temperature on antibody synthesis. In addition, a comparative study of antibody synthesis among various classes of vertebrates promises to provide much interesting information(1). The work of others has established that antibodies can be produced by fish(1) and frogs (2) in response to injection of particulate antigens, and in both an effect of environmental temperature on the process was noted. Evidence that reptiles are capable of forming antibodies is seen in experiments of Downs(3) on anaphylaxis in the turtle. The present paper reports studies on antibody synthesis at 25°C and 35°C in the reptile, *Dipsosaurus dorsalis* (desert iguana) in response to injections of formalin-killed *Salmonella typhosa* antigen.

Materials and methods. Forty adult specimens of *D. dorsalis* captured in Riverside County, Calif.(4), were maintained in laboratory on a diet of lettuce and mealworms, *ad lib.* Immunization was initiated within 3 weeks after capture to insure that animals would remain in good physical condition for duration of experiment. The antigen employed was a suspension of *Salmonella typhosa* prepared from 13 liters of BHI broth culture. After 24 hr of growth at 37°C, 0.3% formalin was added. Cultures remained at room temperature for 3 hr followed by overnight refrigeration. The cells were centrifuged and suspended in 500 ml of saline containing 0.3% formalin. To provide antigen for immunization, this stock was centrifuged and the cells diluted with sterile saline to optical density of 0.824 (540 mμ). During first week of immunization all 40 animals received 0.2 ml of antigen in subcutaneous tissue of thigh. Such injections were made daily in alternate hind legs for the first 3 days of the week. During following week 0.2 ml of antigen was given intraperitoneally on first 3 days of week, total 6 injections. During immunization, some animals were maintained at 25°C and some at 35°C. After completion of the 2 week immunization period, the animals were held for 7 or 14 days before bleeding as shown in Table I. The animals were bled by cardiac puncture and blood expelled into centrifuge tubes where it clotted. To measure agglutinin content of serum, the test was set up as in(5) except that 0.2 ml quantities of serum and antigen were used. The antigen was prepared from stock (H) suspension employed for immunization by diluting it to correspond to tube 3 of McFarland barium sulfate standard. The agglutination was read after 2 hours in 37°C bath.

Results. A 2 week immunization period was sufficient to produce good antibody titers in animals maintained at 35°C (Table I). Agglutinations were of the H variety and were strong and easy to read. There appeared no significant difference in groups maintained at 35°C for 7 and 14 days post-immunization.

In striking contrast the lizards held at 25° failed to produce any detectable antibody at 7 days post-immunization and only titers of 1:10 or less after 14 days. Slightly higher titers were obtained in a group immunized at 25° and held at this temperature for one week post-immunization followed by an additional week at 35°C. This latter experiment was repeated in another series (see footnote to Table I) with similar results. Pre-immunization sera were uniformly negative so that this species does not appear to possess a natural agglutinin for *S. typhosa*(H).

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TABLE I. Comparison of Antibody Levels in 25 Animals Maintained at 25°C and 35°C.

Temp during immunization	Temp and duration of post-immunization period	Reciprocal of titer
25°C (Group I)*	7 days at 25°C	<10
		"
		"
		"
25°C (Group II)	14 days at 25°C	10
		"
		<10
		10
		"
25°C (Group III)*	7 days at 25°C	20
	followed by 7	10
	days at 35°C	20
		40
		"
35°C (Group IV)	7 days at 35°C	80
		160
		"
		640
		160
35°C (Group V)	14 days at 35°C	640
		"
		160
		320
		80

* Group I was repeated by immunizing 10 additional animals exactly as in first experiment. Titers were all below 1:10.

Group III was repeated with 5 additional animals. Titers were, respectively: 40, 80, 80, 80, 20. (Note: in this repetition, the second temperature was 37° rather than 35°C.)

Discussion. These data indicate that antibody synthesis like other metabolic activities in reptiles(4,6,7) is temperature-dependent. At 25°C little or no antibody was synthesized in 7 to 14 days postimmunization, while at 35°C antibody titers of 1:80 to 1:640 were demonstrable after comparatively short period of immunization. It is of course possible that the upper limit for antibody synthesis has not been reached since maximum temperature(4) for *D. dorsalis* is well above 35°C. Further experiments on synthesis and release of antibody in poikkilothermic vertebrates are in progress.

Summary. Antibody synthesis at 25°C was compared with that at 35°C in the lizard, *Dipsosaurus dorsalis*. After 2 weeks immunization against *S. typhosa,* H-agglutinin titers of 1:80 to 1:640 were demonstrable in animals maintained at 35°C. At 25°C, on the other hand antibody level did not exceed 1:10 if detectable. Increasing temperature of 25°C group to 35°C for one week caused a relative increase in antibody synthesis. Thus, antibody synthesis in this species appears dependent on environmental temperature.

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1. Cushing, J. E., *J. Immunol.,* 1942, v45, 123. Cushing, J. E., Campbell, D. H., *Principles of Immunology,* 1957, McGraw-Hill, N. Y.

2. Bisset, K. A., *J. Path. Bact.,* 1948, v60, 87.

3. Downs, C. M., *J. Immunol.,* 1928, v15, 77.

4. Cowles, R. B., Bogert, C. M., *Am. Mus. Nat. Hist.,* 1944, v83, 261.

5. Evans, E. E., in *Manual of Microbiological Methods,* 1957, p207, McGraw-Hill, N. Y.

6. Maher, Michael J., *Effect of Thyroid Gland on Oxidative Metabolism of Lizard Anolis Carolinensis,* 1958, Thesis, Zoology Dept., Univ. of California, Los Angeles.

7. Dawson, W. R., Bartholomew, G. A., *Physiol. Zool.* 1958, v31, 100.

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