Immunological Competence in the Newly Hatched Lizard, *Calotes versicolor*[1] (36387)

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In recent years increased emphasis has been placed on the analysis of the phylogenetic and developmental aspects of immunity. While considerable information is available on the pattern of immunological maturation in mammals [cf. (1, 2)] relatively little is known concerning the development of immunological capacities in lower vertebrates. Ontogenic information is available primarily for amphibians (3–9) with only limited reports for other poikilothermic classes (10–12). The present study provides information concerning the immune capacity of the hatchling lizard of the species *Calotes versicolor*.

Materials and Methods. Eggs of the garden lizard *C. versicolor* were procured and incubated under the laboratory conditions as described earlier (13). Hatchlings obtained from laboratory-incubated eggs and relatively older ones collected from the field were used in the present investigation. Animals in different age groups (Table I) were challenged intraperitoneally with a single injection of either 0.03 ml (Group I to IV) or 0.05 ml (Group V and VI) of 25% SRBC in saline. Control animals in all these groups received an appropriate quantity of saline. Animals were maintained at ambient temperature ranging from 20 to 28°.

In order to detect the presence of plaque-forming cells (PFC) in the spleen, the modified plaque-forming cell technique of Cunningham and Szenberg (14) was followed. Spleen cell suspensions were prepared in 10% serum–saline. Concentration and viability of these cells were determined by the trypsin blue dye exclusion method as modified for chick (15), counting viable white cells in a hemacytometer. A reaction mixture consisting of 0.06 ml of 10% serum–saline, 0.01 ml of 10% SRBC in saline, 0.01 ml of Calotes complement and 0.1 ml of cell suspension to be tested was prepared and plated in a monolayer between two microslides [(12); cf. also (16)]. The slides were incubated for 30 min at 37°. The plaques were counted under 18× magnification and the number of plaques per million viable white cells was calculated.

Serological analysis of antibody response could not be carried out due to very limited quantity of blood available in the hatchlings. For histological studies, spleens were fixed in Bouin's fluid, sectioned at 6 μ and stained in Heidenhain's hematoxylin and eosin.

Results. The spleen of the 1-day old lizard is an oval reddish structure measuring about 0.5 mm in length and containing about 0.8×10^6 white blood cells. Histologically, lymphocytes are present, and these are distributed uniformly, without organization into definite follicles.

As shown in Table I, spleens of immunized animals of all ages studied were found to contain significant numbers of plaque-forming cells, ranging from 25 to 2186 PFC/10^6 white blood cells. Even animals immunized within 24 hr of hatching (Groups I and II) responded well to SRBC. Control, unimmunized animals were uniformly negative. The specificity of anti-SRBC activity was demonstrated by the lack of significant cross-reactivity to rat red blood cells, when these were substituted in the slide assay.

Attempts were made to induce a graft-

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TABLE I. Number of Plaque-Forming Cells (PFC) Against Sheep Red Blood Cells in the Spleen of Immunized and Control Lizards of Different Age Groups.

Group no.	Wt (g)	Age	Postimmunization period (days)	No. of positive[a]/total	PFC/10^6 white cells
Immunized					
I[b]	0.5	12 hr	7	3/6	233, 100, 2000
II[b]	0.6–0.7	1 day	14	4/5	1000, 800, 2186, 75
III[b]	1–2.5	1–1.5 months	14	9/10	456, 221, 980, 1680, 125, 84, 25, 16, 400
IV	3	2–3 months[c]	7	4/4	125, 145, 117, 46
V	5–8	4–5 months[c]	14	5/6	1072, 1470, 1850, 1500, 1572
VI	8–10	6 months[c]	7	8/8	113, 40, 410, 344, 1563, 1063, 142, 116
Controls					
I–III	0.5–2.5	12 hr–1.5 months	7/14	0/20	0
IV–VI	3–10	2–6 months	7/14	0/10	0

[a] Ten PFC and above were treated as positive.
[b] For both controls and experiments sibling hatchlings were used.
[c] Animals collected from the field and approximate age calculated on the basis of weight.

versus-host reaction by injecting newly hatched lizards (20 animals) intraperitoneally with 0.6×10^6 adult lizard spleen cells. Additional animals were injected with 0.6×10^6 adult thymus cells or with control solution of phosphate-buffered saline. There was no sign of wasting disease in any animals. Thirteen of the spleen cell-injected animals were killed 16 days after treatment and examined for splenomegaly or histological manifestations of a graft-versus-host reaction but none was observed.

Discussion. It is clear from the results described above that antibody-forming capacity as judged by the ability to respond to sheep red blood cells is already developed at the time of hatching in *Calotes versicolor*. Moreover, the rate of reaction to antigen seen in hatchlings is comparable to that of adult lizards and the actual number of PFC/10^6 cells is indeed higher than that observed in adults (16, 17).

The fact that such an immunological competence already exists at time of hatching serves as a possible explanation for the inability to demonstrate a graft-versus-host reaction; confirmation for the validity of such an explanation could presumably be obtained by use of irradiated host animals.

In lower vertebrates in general maturation of immunological potential takes place at different time intervals after hatching. Thus the ambiotocid teleost *Cymatogaster aggregata* matures immunologically in its capacity to reject scale allografts during the first 2 weeks after parturition (10) while maturation of immunological responsiveness occurs in early or late larval stages in both urodele and anurant amphibians. Among the reptiles, maturation has been described only for snapping turtles, and here it appears to occur very slowly over a 6-month period following hatching (11, 12). To what extent this maturation is influenced by hibernation which sets in shortly after hatching in the snapping turtle is not known (12).

Summary. Studies are reported on the ability of the hatchling of the lizard *Calotes versicolor* to engage in immunological reactions. The experiments demonstrate that even within the first 24 hr after hatching the lizard is able to mount an immune reaction against sheep red blood cells, in a manner not readily distinguished from adult animals.

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