Manifestation of Anaphylaxis to Egg Albumen in the Lizard, *Calotes versicolor*

In recent years, there has been much emphasis on the study of immunological competence in lower vertebrates. There are many reports on the efficacy of reptiles, demonstrating the immunological potency to produce antibodies against varying antigens and to reject tissue allografts[1]. While extensive data are available in mammals and in chicken concerning the hypersensitivity reaction type I, anaphylaxis[2], very little is known in other vertebrates[3–7]. The earliest study on the induction of anaphylaxis in reptiles was that of DOWNS[8] in turtles, using mammalian serum. Since we have been interested in characterizing the various types of immune reactions in the lizard, *Calotes versicolor*[9,10], the present study on anaphylaxis was undertaken. Since spleen plays an important role in the production of humoral antibodies and splenectomy abrogated the humoral immune function to sheep red blood cells (SRBC) in *Calotes versicolor*[9,11], experiments were conducted to study the effect of splenectomy on the manifestation of the anaphylactic response.

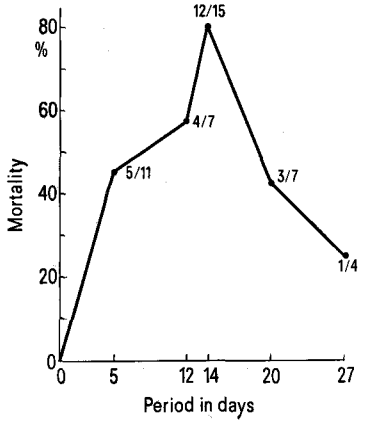
Materials and methods. Lizards of both sexes, weighing 30–50 g, were used and maintained in the laboratory as described earlier[12]. They were sensitized by a single intra-cardiac injection of 100 mg/kg body weight of egg albumen (EA; The British Drug Houses Ltd., Poole, England) in 0.85% normal saline. Shocking was performed by a single injection of 2 g/kg body weight of EA by the same route. Animals were shocked on days 5, 12, 14, 20 and 27 after sensitization. Symptoms and the time of occurrence of death were recorded. Autopsy was performed immediately after respiratory cessation to identify gross anatomical changes.

Splenectomy was carried out as described earlier[11]. Both splenectomized and sham-operated lizards were sensitized 7 days after operation. Shocking dose was given on 14th and 21st day after sensitization and results were recorded as described above.

Results. In cases of severe anaphylaxis, the following symptoms were observed: frequent gasping for breath, laborious breathing, popping of eyes, dragging the body feebly with the head tilted toward one side, stretching the head flat on the floor, loss of righting reflex, spilling of frothy secretions from mouth, outstretching of limbs in association with convulsive movements, defecation, urination and complete respiratory cessation. All these symptoms were produced within 2 min after the injection of shocking dose and death occurred 3 to 10 min later. Mild anaphylactic shock was characterized by gasping for breath, weakness, motionless condition for a few moments, resulting in recovery of these animals within 15 min.

At autopsy, it was observed that the heart was beating irregularly but faintly and was engorged with blood. Lungs were highly inflated and usually at inspiratory phase at the time of death. In some cases, the hind part of the intestine and rectum was bloated with air. As shown in Table I, all the control animals (groups II and III) were normal without any adverse symptoms for the next 48 h. Failure to respond to BSA by EA sensitized lizards reveals the specificity of the reaction. Mortality occurred in EA sensitized lizards that received the shocking dose of EA. The rate of mortality increased from 45% on day 5, to a maximum of 80% on day 14 and subsided to 25% on day 27 after sensitization (Figure). As shown in Table II, all splenectomized lizards did not show anaphylactic response to the shocking dose given on day 14 or 21 after sensitization. On the contrary, sham-operated lizards (Ic and IIc) manifested anaphylaxis to EA.

Discussion. The gross symptoms observed in *Calotes versicolor* have remarkable similarities to those found in chicken[5] and mammals[13]. Preliminary studies showed that the tissue mast cells degranulated due to anaphylaxis (unpublished observations). The role of antibodies in anaphylaxis is also evidenced by the results of splenectomy experiments (Table II). Unlike in *Xenopus laevis*[14], splenectomy in the lizard abrogated humoral immunity to SRBC[11] and to BSA (MUTHUKKARUPPAN, unpublished). Splenectomized animals failed to show anaphylactic symptom (Table II) indicates: that 1. this response is



Correlation between the post-sensitization period and the intensity of anaphylactic reaction to the shocking dose of EA in *Calotes versicolor*. The number of positive cases/total number is given for each period tested.

Table I. Anaphylactic response to egg albumen in *Calotes versicolor*

Post sensitization period (days)	Group I				Group II				Group III			
	No. animals	—	+	D	No. animals	—	+	D	No. animals	—	+	D
5	11	5	6	5	3	3	0	0	2	2	0	0
12	7	3	4	4	3	3	0	0	2	2	0	0
14	15	2	13	12	5	5	0	0	3	3	0	0
20	7	3	4	3	3	3	0	0	2	2	0	0
27	4	2	2	1	3	3	0	0	2	2	0	0

Group I: Sensitized lizards shocked with EA. Group II: Sensitized lizards shocked with BSA. Group III: Saline-injected lizards shocked with EA. —, no symptoms; +, mild to severe symptoms; D, death due to anaphylaxis.

Table II. Effect of splenectomy on the anaphylactic response to EA in *Calotes versicolor*

Group treatment[a]	Time of shocking dose	No. animals	−	+	D[b]
Ie splenectomy	14 days after sensitization	10	10	0	0
Ic sham	14 days after sensitization	10	2	8	8
IIe splenectomy	21 days after sensitization	5	5	0	0
IIc sham	21 days after sensitization	5	2	3	1

[a] Animals were sensitized 7 days after operation. [b] Symbols as in Table I.

antibody-mediated and 2. splenectomy deprives the animal of the ability to synthesize antibodies that are responsible for the manifestation of anaphylaxis. In higher animals, spleen plays a definite role in humoral antibody synthesis, especially when small doses of antigen and i.v. routes are employed[15]. It is probable, therefore, that in the lizard the spleen plays a definite role in the production of antibodies that mediate anaphylaxis, since small dose of antigen and intra-cardiac route have been employed.

That the maximum mortality occurs at the end of the 2nd week after sensitization (Figure) may be due to the persistence of more antibodies in the tissues. This is in correlation with the maximum fatal sensitivity observed in birds on 14th day[16]. The specificity of the antigen-antibody interaction is revealed by the unresponsiveness of EA sensitized lizards to the shocking dose of bovine serum albumen (BSA) as reported in fishes[3] and in other species[5]. Further studies are underway to characterize the nature of antibody, mediators and the immunological mechanisms involved in anaphylaxis.

Summary. Anaphylaxis was induced in the lizard, *Calotes versicolor* by egg albumen. The symptoms were similar to those found in other species. The maximum mortality occurred 14 days after sensitization and the reaction was specific. Splenectomy before sensitization abrogated the manifestations of anaphylaxis.

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Opposite Effects of Vasotocin Injected Intrapituitarly and Intraventricularly on Corticotropin Release in Mice

We have previously reported that the mammalian pineal, including that of man, contains[1,2] and synthesizes[3] the specific octapeptide arginine vasotocin (AVT), and that minute amounts of AVT injected into the 3rd ventricle of the mouse inhibit gonadotropin[4] and corticotropin[5] release. Since in vitro AVT has ACTH-releasing activity[6], and when injected into the 3rd ventricle, on the contrary, inhibits ACTH release[5], the present study compares the effects of AVT injected intrapituitarly and intraventricularly on the compensatory adrenal hypertrophy (CAH) in unilaterally adrenalectomized mice. Male RAP (Rockland for All Purposes) mice weighing 18–22 g were used. The mice were unilaterally adrenalectomized under Evipan anesthesia. The uninjected controls were sham adrenalectomized under the same anesthesia. 1 h later, synthetic AVT in a volume of 2 µl was injected into the left lobe of the pituitary or into the 3rd ventricle. Controls received 2 µl saline. The substances were injected via a 26 gauge needle with a micrometer syringe attached to a Horsley-Clark rat stereotactic apparatus, as described[5]. All animals were killed 3 days postoperatively and the remaining (right) adrenal was cleaned and weighed fresh to the nearest 0.1 mg on a torsion balance. Adrenal weights were expressed as mg/100 body weight. The data were evaluated statistically by the Student *t*-test. As shown in the Figure, a single injection of 10 pg AVT/µl into the anterior pituitary of the mouse on the day of surgery, significantly potentiated CAH measured 3 days later, whereas the same concentration of AVT injected into the 3rd ventricle produced adrenal atrophy. Lower concentrations of AVT between 0.001 and 0.00001 pg/µl injected into the pituitary were

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