fluorophores in section A were effective as visualization aides. The compounds in section B were not effective agents, primarily because colonies could not be distinguished from the background due to high agar fluorescence or lack of colony fluorescence.

Both ANS and 2-p-toluidino-6-naphthalenesulfonic acid are practically non-fluorescent in aqueous solutions but in association with proteins or in non-polar (hydrophobic) environments, very large increases in the quantum yields occur (quantum efficiencies, non-polar/polar of 245 and 712, respectively[7,8]).

Acridine orange is another compound which works well in our system. The reason for its efficacy seems to be the frequently observed 'blue shift'[9] in its fluorescence emission maximum from orange in an aqueous environment to yellow in a more hydrophobic environment, i.e. in the bacterial envelope. This compound is known to affect the expression of plasmids[10], although we observed no effect on cultural characteristics. Pyrenebutyrate is useful due to another interesting phenomenon. The fluorescence of this compound is strongly quenched by dissolved oxygen. Vaughn and Weber[11] found, however, that if pyrenebutyrate were conjugated or adsorbed to bovine serum albumin, then fluorescence was unaffected by the presence of oxygen. This may be the mechanism by which fluorescent colonies occur on this agar.

1 A.M. Paton and S.M. Jones, in: Methods in Microbiology, vol. 5A, p. 135. Ed. J.R. Morris and D.W. Ribbons. Academic Press, New York 1971.
2 R.W. Weaver and L. Zibilske, Appl. Microbiol. *29*, 287 (1975).
3 L. Brand and J.R. Gohlke, A. Rev. Biochem. *42*, 843 (1972).
4 W.A. Cramer and S.K. Phillips, J. Bacteriol. *104*, 819 (1970).
5 W.S. Ramsey, C.A. Lepp and R.D. Mason, A. Meet. Am. Soc. Microbiol. Abstrs. p. 122 (1977).
6 W.S. Ramsey, E.D. Nowlan, L.B. Simpson, R.A. Messing and M.M. Takeguchi, A. Meet. Am. Chem. Soc. Microbiol. Abstrs. p. 4 (1978).
7 S. Undenfriend, in: Fluorescence Assay in Biology and Medicine, vol. II, p. 248. Academic Press, New York 1969.
8 W.O. McClure and G.M. Edelman, Biochemistry *5*, 1908 (1966).
9 T.L. Pasby, in: Fluorescence Spectroscopy, p. 65. Ed. A.J. Pesce, C.-G. Rosen and T.L. Pasby. Marcel Dekker Inc., New York 1971.
10 S. Sonea, J. de Repentigny and A. Frappier, J. Bacteriol. *84*, 1056 (1962).
11 W.M. Vaughn and G. Weber, Biochemistry *9*, 464 (1970).

Effect of splenectomy on the humoral immune response in the lizard, *Scincus scincus*[1]

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Summary. Adult splenectomy in the lizard, *Scincus scincus*, did not affect humoral immune response to rat erythrocytes until 30 days post-immunization, but severely depressed subsequent antibody production.

It is well-known that adult splenectomy depresses but does not abolish humoral immune response in amphibians[2-4], birds[5,6] and mammals[7-12]. In contrast, splenectomy completely abrogates antibody production in lizards, *Calotes versicolor*[13-15]. No other study concerned with effect of splenectomy on reptilian humoral reactivity is available. Such shortage incited us to investigate the effect of splenectomy on humoral response of the lizard, *S. scincus*. Immune system of *S. scincus* in the different seasons has been described[16], and it was shown that lymphoid complex and capacity for antibody production are fully developed in summer and autumn. Splenectomy experiments were, therefore, performed in autumn.

Materials and methods. Adult male and female *S. scincus* (Scincidae, oviparous, hibernating[17]) weighing 20–40 g, were collected from the desert margin. Lizards were kept in large terraria, with 30 cm deep sand in a sunny animal room where the temperature in autumn ranged from 20 to 27 °C. Live insects and water were given ad libitum.

A group of 70 lizards were anaesthetized with ether and trunk region sterilized with ethyl alcohol. A small incision was made to the left of the midline opposite to the stomach, which was turned aside and the spleen excised by cauterizing the splenic blood vessels. After complete removal of spleen, the wound area was sprayed with Diacilin powder (Misr Co. Pharm. Ind., Cairo) and then the incision was closed with autoclips (Clay Adams, Parsippany, New Jersey, USA). Sham splenectomy was performed on a group of 70 lizards, by following all surgical procedures depicted above, except removal of spleen. Completion of the operation was ascertained by autopsy at the end of the investigation.

1 week after surgery, surviving 50 splenectomized, 50 sham-operated, as well as 50 intact lizards, received each a single i.p. injection of 0.3 ml of 20% rat erythrocytes suspension. 3–5 lizards from each group were sacrificed at a 15-day interval, over a period of 2 months and serum haemagglutinins assayed as described previously[16]. Significant differences between mean peak titers of the various groups were determined by the F-test (T Programmable 58, Texas Instruments, USA).

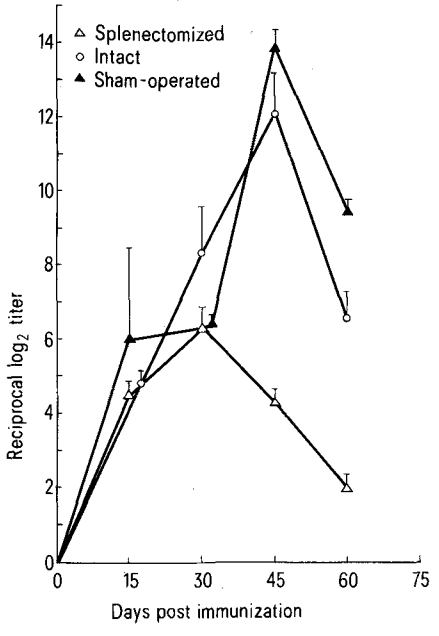
Results. Splenectomy experiments were performed in autumn 1976 and repeated in autumn 1977. Identical results were obtained and therefore pooled (figure). At 15, as well as, at 30 days post-immunization, splenectomized *Scincus* produced antibody levels close to those produced by sham-operated and intact lizards. There was no significant difference in antibody titers between the 3 groups. Serum antibody titers of intact and sham-splenectomized lizards rose rapidly, peak activity occurred at day 45, with a mean titer ($\log_2$) ranging from 12.1 to 13.8. As for the splenectomized lizards, serum antibody level began to wane and was significantly lower than antibody levels of intact and sham-operated *Scincus*, $p < 0.002$. On day 60, antibody titers of intact, sham-operated and those of splenectomized animals were still significantly different, $p < 0.005$.

Discussion. The present study indicates that in the lizard, *S. scincus*, adult splenectomy did not affect antibody production against rat red blood cells up till 30 days post-immunization. Serum haemagglutinin titer did not peak, however, and remained at a significantly lower level than in intact or sham-splenectomized lizards. Similar results were recorded in amphibians, birds and mammals, since adult splenectomy only depresses titer of circulating anti-

bodies[2-12]. Our findings are, however, contradictory with those obtained in the lizard, *C. versicolor*, where adult splenectomy entirely abolishes humoral response to sheep erythrocytes[13,15]; a thorough evaluation of results therefore appears warranted.

It is well-known that effect of splenectomy varies, depending on antigen type, dose and route of administration, experimental animal and on presence or absence of a number of peripheral lymphoid centers other than spleen[2,3,9,12,15]. In our study, ambient temperature, antigen type, dose as well as route of injection were not unlike corresponding experimental conditions in earlier investigations on lizards[13,15,18], but a different species of lizard was examined. *C. versicolor* is a species characterized by the fact that a large proportion of intact and sham-operated animals produce no circulating antibodies to i.p. injected sheep erythrocytes, and in responding lizards, titer is rather low[13,15,18]. Moreover, *Calotes* entirely lacks gut-associated lymphoid tissue except for a cloacal aggregate[15,18]. Splenectomy removes the major source of antibody-producing cells, thus leading to complete abolition of humoral response.

We have shown that splenectomy has rather marginal effects on reptilian humoral response by using the lizard, *S. scincus*, which displays consistent and powerful response to heterologous erythrocytes (figure). Besides, *S. scincus* is endowed with an extensively developed array of gut-associated lymphoid aggregates. Removal of spleen leaves alternative sites of organized lymphoid tissue, and thus would not drastically impair immunological ability. Nonetheless, splenectomy in *Scincus*, severely depressed titer of circulating antibodies; this finding suggests that spleen has a crucial role in immune defense in lizards as in higher vertebrates[19].



Humoral response in *Scincus scincus*. Vertical bars represent SE.

1 This work was supported by United States Public Health Research, grant No. 03-015-N.
2 R.J. Turner, J. exp. Zool. *183*, 35 (1973).
3 R.J. Turner, Experientia *30*, 1089 (1974).
4 B.A. Brown and E.L. Cooper, Immunology *30*, 299 (1976).
5 G.L. Rosenquist and H.R. Wolfe, Immunology *5*, 211 (1962).
6 S.D. Keily and P. Abramoff, J. Immun. *102*, 1058 (1969).
7 D.A. Rowley, J. Immun. *64*, 289 (1950).
8 W.H. Taliafero and L.G. Taliafero, Science *113*, 473 (1951).
9 J.W. Uhr and M.S. Finkelstein, Prog. Allergy *10*, 37 (1966).
10 P.A. Campbell and M.F. La Via, Proc. Soc. exp. Biol. *124*, 571 (1967).
11 B.B. Lozzio and L.B. Wargon, Immunology *27*, 167 (1974).
12 C.A. Landahl, A. Chakravarty, M. Sulman, L. Kubai and R. Auerbach, J. Immun. *117*, 151 (1976).
13 P. Kanakambika and Vr. Muthukkaruppan, Experientia *28*, 1225 (1972).
14 S. Jayaraman and Vr. Muthukkaruppan, Experientia *31*, 1468 (1975).
15 Vr. Muthukkaruppan, P.S. Pillai and S. Jayaraman, in: Immuno-aspects of the spleen, p.61. Ed. J.R. Battisto and J.W. Streilein, North Holland Publishing Co., Amsterdam 1976.
16 M.F. Hussein, N. Badir, R. El Ridi and S. El Deeb, submitted for publication.
17 N. Badir, Z. wiss. Zool. *160*, 290 (1958).
18 P. Kanakambika and Vr. Muthukkaruppan, J. Immun. *109*, 415 (1972).
19 J.R. Battisto and J.W. Streilein (ed.), Immuno-aspects of the spleen. North-Holland, Amsterdam 1976.

Chemical crypsis in predatory ants

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Summary. The repellent responses of worker termites to ants are determined by the exocrine gland secretions of the latter. Specialized termite predators produce non-repellent aliphatic alcohols as the major components of their mandibular glands, whereas unspecialized con-generics usually produce repellent ketones and aldehydes.

Our researches on ant predators of termites in West Africa have shown that chemical stimuli play an important role at several stages of the predator-prey interaction[2,3]. A successful predator must avoid early detection by its prey. Some ants have pheromones used in alarm and recruitment of nest-mates to which the termites themselves are insensitive. We believe this to be analogous to crypsis dependent upon visual concealment.

Decamorium uelense, a myrmicine ant, which was studied in the Southern Guinea savanna at Mokwa, Nigeria, is a specialized predator of small termites which forage within their food of roots, grass stems and wood[2,4]. Single workers (scouts) search for foraging termites. A successful scout returns to the colony and recruits a group of 10–30 workers who proceed to the termite foraging area. They then dig into the foraging galleries of the termites and immobilize