Materials and methods. Venous blood was obtained from 20 healthy human volunteers. Mononuclear cells were separated by gradient centrifugation on a layer of Ficoll-Hypaque, density 1077 (report of a WHO/IARC sponsored Workshop[14]). The lymphocytes were washed with Hanks's balanced salt solution and suspended in TC-199 Medium (Difco-Laboratories). The effect of NCV was analyzed by incubating the lymphocytes for 30 min in various doses of the enzyme (Behringwerke AG) at 37 °C in an atmosphere with 5% CO_2 (see the dose-response curve, figure). Triplicate cultures of 1×10^6 NCV-treated and untreated lymphocytes in 0.75 ml of TC-Medium and 0.25 ml of autologous plasma were made in 16×95 mm sterile tubes. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere for 3 days. In 12 experiments, the action of NCV on lymphocyte cultures stimulated with 50 µl/ml of PHA-M (Difco-Laboratories) was also studied. DNA synthesis was quantified by incorporation of 1 µCi of 3H-labelled thymidine (specific activity 26 mCi/mM) for the last 12 h of culture. Radioactivity was counted in a Packard Liquid Scintillation Counter. The results are expressed as the mean cpm/10^6 lymphocytes of triplicate tubes ± SEM. Student's t-test was used to estimate the significance.

Results and discussion. In '6 cases, studied previously (figure), note that the lymphocyte response to NCV is optimal at the dose of 75 units/ml. The table demonstrates that pretreated lymphocyte cultures have a net increase in spontaneous blastigenesis. The lymphocytes incubated with the enzyme exhibit higher levels of 3H-thymidine incorporation (1224 ± 146 cpm) with respect to non-pretreated lymphocyte cultures (457 ± 101 cpm) with a significant statistical difference ($p < 0.001$). There were no differences in pretreated and non-pretreated cultures when stimulated by PHA.

From our data it is clear that NCV has mitogenic properties on circulating lymphocytes. This has also been shown for other enzymes (trypsin and chymotrypsin[15], papain[16] and galactose oxidase[17]). Furthermore it should be noted that NCV is capable of stimulating the growth of fibrobasts, HeLa cells and FL amnion cells[18].

It is known that the treatment of lymphocytes with NCV greatly modifies the properties of these cells, and particularly the immunological properties[19]. NCV has been shown capable of removing sialic acid from the cell membranes[20] but it is difficult to correlate this effect with the immunological properties of the enzyme.

The hypothesis of the reduction of surface electric charge with consequent facilitation of cell contact, and the hypothesis of unmasking active sites seem most suited to explain other effects of NCV (such as the influence on the formation of E rosettes or the increase in cellular immunogenicity). With regard to the mitogenic properties of NCV, it can be hypothesized that the enzymatic reactions on the lymphocyte cell membrane are capable of inducing cellular activation.

Neuraminidase effect on lymphocyte blastigenesis

	Number of experiments	Untreated lymphocytes	NCV-treated lymphocytes
Unstimulated	20	457 ± 101*	1224 ± 146*
PHA-stimulated cultures	12	13936 ± 1474**	13830 ± 1373**

*$p < 0{,}001$; **not significant.

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Effect of splenectomy on the in-vitro migration inhibition response to sheep erythrocytes in the lizard, *Calotes versicolor*[1]

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Summary. Splenectomy completely erased the PFC response to SRBC in lizards. In contrast, it has very little effect on the degree of MI to all doses ranging from 10^4 to 10^9, excepting the lowest dose, 10^3 SRBC.

Both cell-mediated (CMI) and humoral immune (HI) responses can be specifically induced to sheep erythrocytes (SRBC) in the lizard, *Calotes versicolor*, under appropriate conditions[3,4]. Both types of immune responses are inversely related to the amount of SRBC-injected[3,4]. Low dose SRBC-induced CMI as measured by the in-vitro capillary migration inhibition (MI) technique[3], and high dose induced predominantly plaque-forming cells (PFC) with a minimal MI level[4]. In an attempt to study the modulation of MI response to SRBC in *Calotes versicolor*, spenectomy was used as a tool to elucidate the role of antibody-producing cells on MI response, since it was known that splenectomy completely abrogated the HI response to varying antigens[5–7]. The data obtained indicate that

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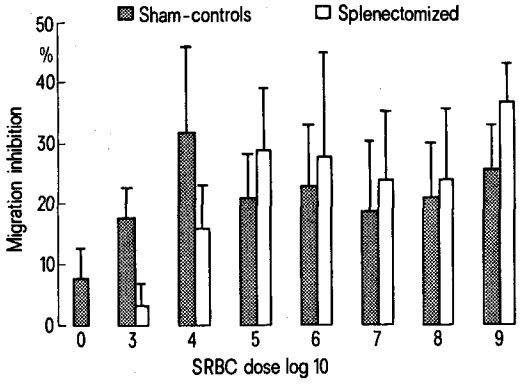
splenectomy created no dearth of competent lymphocytes that mediate MI while removing the major source of antibody production.

Materials and methods. Adult lizards were maintained[8] and splenectomized[5] as detailed elsewhere. Different dilutions of SRBC in phosphate-buffered saline (PBS) were given i.m. to both sham-operated and splenectomized lizards 7 days after surgery. 9 parts of blood in Alsever's solution was mixed with one part of 6% Dextran (mol wt 5,000,000; Sigma Chemical Co., USA) and kept at room temperature for 1.5 h to separate peripheral blood white cells of *Calotes*[9]. Peritoneal exudate cells (PEC) were obtained by washing the peritoneum with calcium-magnesium-free PBS (with antibiotics and 5.0 IU heparin/ml) 4 days after the i.p. injection of 4–6 ml of heavy paraffin oil[9]. 14 days after immunization, PFC[8] and MI assays were performed. The method for MI assay utilizing PEC was similar to that described for *Calotes* spleen cells[3]. After 24 h of incubation at 37 °C, the areas of migration of PEC cultured with and without antigen were compared and the degree of MI was calculated. Statistical assessment of data was by Student's t-test.

Effect of splenectomy on PFC response to SRBC*

Group	Spleen	Peripheral blood white cells	Peritoneal exudates
Unsensitized controls	8.5±4.4** (5)***	1.0±0.7** (5)***	14.2±5.4** (11)***
Sham-operated controls	210.5±31.8 (6)	107.3±20.4 (6)	46.0±22.0 (6)
Splenectomized	–	11.1±5.3 (5)	15.5±8.7 (5)

*PFC assay was performed 14 days after immunization. **Arithmatic mean ± SE. ***Number of animals investigated.



Effect of splenectomy on MI response to SRBC. Both sham-operated and splenectomized lizards were immunized with varying dilution of SRBC and assayed 14 days later for MI response. Each point represents the mean ± SE of 4–10 animals.

Results and discussion. Previous studies have shown that the intact spleen is an absolute necessity for *Calotes* to produce antibodies to i.p. injection of SRBC[5] or bovine serum albumin[7] and also to manifest anaphylaxis to an intracardiac injection of egg albumin[6]. In the present study, i.m. route was chosen to immunize the lizards since this route favours the efficient elicitation of both CMI and HI to SRBC[3,4]. Hence, an experiment was performed to ensure that splenectomy would erase the HI response irrespective of the route of injection of SRBC. PFC were monitored in peripheral blood and PEC, since it was already known that splenectomy abrogated the humoral antibody production[5,7]. I.m. injection of 10^8 SRBC failed to induce PFC generation in peripheral blood and PEC (table), thereby confirming the earlier reports[5–7]. The next experiment was conducted to analyse the status of CMI in animals that were deprived of HI by splenectomy. The data (figure) indicate that splenectomy did not impair the ability of lizards to mount CMI to SRBC doses ranging from 10^4 to 10^9. However, the lowest dose, 10^3 SRBC, failed to sensitize the splenectomized lizards ($p < 0.05$) for MI response.

The observation that splenectomy affected the CMI response to very low SRBC dose is in keeping with the findings in mice[11]. Similarly, in toads the antibody synthesis to SRBC and human gamma-globulin was affected by splenectomy only to low but not to high threshold antigen levels[12]. A plausible interpretation could be that the low antigen dose has minimal chances to interact with competent lymphocytes in the absence of spleen[11]. Splenectomized lizards did not show any increment in the level of MI to high doses of SRBC ($p > 0.5$), unlike mice[11]. Similar results were obtained with Cyclophosphamide and Freund's complete adjuvant treatment[9]. This evidence suggests the total independence of MI on HI in lizards.

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Temperature variation and the function of complement and antibody of amphibia[1]

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Summary. Temperature treatment of sera of erythrocyte immunized adults of 3 amphibian species has shown that cooling enhances antigen-antibody complexing and does not alter complement function in vitro. These findings bear on understanding in vivo temperature immunosuppression in poikilotherms.

Cohen[2] has recently discussed the consequence of large daily or seasonal environmental temperature fluctuations on immunity and pathogenesis in poikilotherms, placing particular emphasis on growth rates of bacteria and viruses as they affect reptiles. It is known that temperature depression will inhibit allograft rejection in fish[3,4] and amphibia[5–8], as well as humoral immunity of fish[9–11], amphibia[12–17] and reptiles[18]. It is conceivable then that different parts of the adaptive immune response may represent temperature sensitive events. It has recently