gastrulation onwards, the DNA content increases up to the late gastrula and gets reduced in the following stages down to the normal level, as is outlined in figure 1. The lower curve of figure 1 illustrates the changes in DNA content of neuroectoderm nuclei, the maximum values of which range from 15 to 20% above the final diploid amount (stage 36) in stage 12b. The thermal denaturation curves of DNAs which have been derived from 4 different stages are shown in the upper part of figure 1. Standard Triturus DNA (stage 36) has a regular melting profile with a T_m-value of 69.4 °C. Corresponding melting curves have been recorded in the stages 10/11a and 15. However, in stage 12a/b, when nuclear DNA content reaches maximum values, the thermal denaturation profile deviates from the regular sigmoidal shape at temperatures above T_m being accompanied by an increase of the T_m value. In several samples, the temperature shift to higher degrees varied between 0.8 and 1.2 °C. The deviation from the normal shape is demonstrated by the marked space below the upper part of the curves. The larger space in stage 12a/b results from the flattening of the curve at lower hyperchromicity values (70% H) than usual (85% H), and moreover it depends on the fact that the thermal denaturation of DNA is completed at approximately 3–4° later than in other stages. The latter can be most clearly seen in figure 2. The use of normal probability paper for the purpose of plotting the changes in hyperchromicity demonstrates (figure 2) that the melting behaviour of DNAs from the stages 10/11a, 15 and 36 corresponds to the Gaussian distribution with respect to the mean base composition. In contrast, the DNA melting profile in the mid-gastrula reflects a heterogeneity of the DNA sample which indicates the presence of 2 DNA fractions. Since the second DNA fraction melts at higher temperatures, we have to conclude that this portion of DNA consists of sequences with higher GC-contents than the bulk DNA, as is known in the case of ribosomal RNA genes. For example, rDNA in Xenopus possesses a mean GC-content of 67%[10], whereas bulk DNA has a mean GC-content of about 40%.

These results confirm our conception that the stage-specific increase of DNA during Triturus development is caused by differential replication of DNA. The question whether the thermal satellite DNA represents amplified rDNA or any other GC-rich fraction is being proved now by other biochemical methods.

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Mitogenic action of neuraminidase

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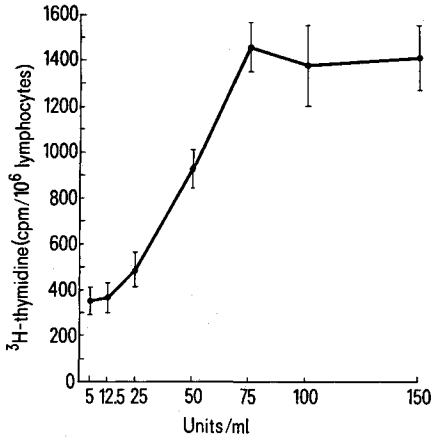
Summary A new effect of NCV on lymphocytes is demonstrated. This property is the capacity to act as a mitogen in and of itself. The possible mechanisms of this phenomenon are discussed.

Treatment of lymphocytes with vibrio cholerae neuraminidase (NCV) enhances the immunogenicity[1,2] and antigenicity[3,4] of normal lymphoid cells, fetal tissue and tumor cells and increases the reactivity of lymphocytes in the cytotoxicity test[5,6]. It has also been shown that treatment of the stimulatory cells (but not responding cells) with NCV, in allogeneic human one-way mixed lymphocyte reaction, significantly augments the DNA synthetic response by the responding cells[7,8]. NCV is known to increase the capacity of normal human lymphocytes to form sheep red blood cell rosettes – more red cells are bound[9,10] and the rosettes are more stable[11]. These changes might result from the exposure of new sites on the cell surface[12,13], a reduction in the net surface charge of the cells or a combination of the 2 effects[11]. The aim of this study was to investigate the effect of NCV on the in vitro blastogenic response.



Dose-response curve. Each point represents the mean ± SE of 6 experiments in which lymphocytes were incubated for 30 min in varying concentrations of neuraminidase. The response is analyzed in counts/min (^{3}H-thymidine) per 10^6 lymphocytes versus Units/ml of NCV.

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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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