The total concentration of fecal neutral steroids (cholesterol + coprostanol + coprostanone) in group A was 3.19 ± 0.33 mg/g dry feces and in group B, 2.13 ± 0.22 mg/g dry feces ($p < 0.01$, table). Furthermore, the concentrations of coprostanol and cholesterol were significantly lower in group B than group A. There was no significant difference between the groups in fecal coprostanone concentration.

Discussion. A number of reports have been presented concerning the depression of thyroid function with advancing age of laboratory animals[17]. Our results are similar to those reported that the serum concentration of L-T_4 declined during the period of the experiment in both animal groups. During the first 4 months of the experiment, however, animals given PTU were hypothyroid with respect to the control group.

The administration of PTU for 24 weeks to rats given weekly doses of azoxymethane significantly decreased the carcinogenic effect of this compound. The hypothyroid rats developed less intestinal cancers, smaller in size and less malignant as indicated by the decreased metastatic spread of the disease. A decrease in thyroid hormone level may affect intestinal carcinogenesis in a variety of ways. The amount and nature of an animal's diet have been found to influence the genesis of chemically induced and transplanted tumors[18]. In our experiment less total food was consumed per rat and body growth was virtually arrested in rats ingesting PTU. Thus the reduced carcinogenic effect of azoxymethane may be due to a restriction in total calories in the PTU-treated rats. However, these animals consumed more food per 100 g b.wt than did the normal group. The relationship between tumor incidence and total and/or relative calorie-intake is a concept that requires much further study. On the other hand, recent evidence has indicated that L-T_4 is directly mitogenic to certain cell lines in vitro[19], while a reduction of growth by lowered serum L-T_4 can be demonstrated for an estrogen-dependent rat pituitary tumor[20]. Thus, it could be that the hypothyroid state has affected the intestinal cell proliferation in a manner reducing the susceptibility of the mucosa to neoplastic transformation[21] or the growth rate of the developing tumors.

Treatment with PTU also resulted in a significant decrease in the total concentration (mg/g dry feces) of the major fecal bile acids as well as in the neutral steroids, cholesterol and coprostanol. Earlier investigations have indicated that increased fecal concentrations of bile acids enhance the carcinogenic affect of azoxymethane[13,14]. Certain bile acids have been found to promote the carcinogenic effect of N-methyl-N'-nitro-N-nitrosoguanidine in colonic mucosa[22,23]. On the other hand, fewer tumors were observed in non-functional large bowel segments where luminal bile acids were absent[24].

It cannot be ascertained from the present data whether lower thyroid hormone levels affected intestinal carcinogenesis directly or if the effect were mediated by the influence of the hormone upon other physiological functions. In view of previous findings, however, the effect of the thyroid hormone on liver bile acid metabolism, fecal bile acid concentrations or intestinal tumor growth rates may be the most important factors to explore in this animal model.

17 P. Kumaresan and C. W. Turner, Proc. Soc. Biol. Med. *124*, 752 (1967).
18 D. B. Clayson, Cancer Res. *35*, 3292 (1975).
19 W. L. Kirtland, J. M. Sorrentino and D. A. Sirbasku, J. nat. Cancer Inst. *56*, 1159 (1976).
20 J. M. Sorrentine, W. L. Kirkland and D. A. Sirbasku, J. nat. Cancer Inst. *56*, 1155 (1976).
21 W. Oehlert, Cell Tissue Kinet. *6*, 325 (1973).
22 T. Narisawa, N. E. Magadia and J. H. Weisburger, J. nat. Cancer Inst. *53*, 1093 (1974).
23 B. S. Reddy, T. Narasawa, J. H. Weisburger and E. L. Wynder, J. nat. Cancer Inst. *56*, 441 (1976).
24 R. L. Campell, D. V. Singh and N. D. Nigro, Cancer Res. *35*, 1369 (1975).

Changes in the thermal denaturation profiles of DNA from different developmental stages of the newt *Triturus vulgaris*

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Summary. The melting profiles of DNA samples from the early gastrula and early neurula of *Triturus vulgaris* are essentially the same, whereas DNA from mid to late gastrula possesses higher T_m values and shows a deviation from the regular sigmoidal shape at temperatures above T_m. The plot on normal probability paper indicates a second DNA fraction which melts at higher temperatures and, consequently, it has a higher GC-content than the bulk DNA. These facts confirm our idea that differential DNA replication occurs during gastrulation.

In early amphibian development, the RNA content of the embryo remains essentially constant. At the onset of gastrulation, a progressive enhancement of gene activity sets in[1]. In the same period, short-term variations in the nuclear DNA content in various regions of *Triturus vulgaris* embryos have been detected by cytophotometric measurements[2–4]. There are 2 facts which lead us to the conclusion that there might be a gene amplification process during gastrulation. 1. The above-mentioned changes in DNA content are correlated with the beginning of ribosomal RNA synthesis. 2. The increase in DNA content is accompanied by a considerable increase in nuclear RNA content[5]. In order to prove whether the genes for ribosomal RNA (rDNA), whose base composition differs from the bulk DNA, are amplified, we analyzed the melting behaviour of DNAs, which were isolated from different developmental stages.

Materials and methods. All studies were carried out with *Triturus vulgaris* embryos from early gastrula to early neurula stage (Harrison stage 10/11a, 12a/b, 15) which

1 E. M. Deuchar, Adv. Morph. *10*, 175 (1973).
2 K. Lohmann and W. Vahs, Experientia *25*, 1315 (1969).
3 K. Lohmann, Wilhelm Roux' Arch. *169*, 1 (1972).
4 K. Lohmann, Wilhelm Roux' Arch. *177*, 285 (1975).
5 K. Lohmann and U. Jansen, Experientia *32*, 380 (1976).

were compared to the late tailbud stages (stages 35, 36). For each preparation, 50–60 dejellied gastrulae and 40 neurulae were pooled and afterwards the nuclei were isolated according to the procedure of Imoh and Minamidani[6], and Faulhaber[7], whose methods had to be modified to some extent. DNA was prepared from the nuclei fractions by the method of Marmur[8]. By using a Zeiss PMQ II spectrophotometer UV absorbance-temperature profiles of Triturus DNA were obtained as described by Mandel and Marmur[9]. The DNA samples were dissolved in 0.1 SSC (0.015 M NaCl, 0.0015 M Na_3 citrate, pH 7.0).

Results and discussion. As has been pointed out earlier, the DNA content of nuclei isolated from various tissues of Triturus embryos is not constant in the course of development, but varies in dependence on the developmental stage and region. Thus, from the beginning of

6 H. Imoh and T. Minamidani, J. Embryol. exp. Morph. *30*, 647 (1973).
7 I. Faulhaber, Wilhelm Roux' Arch. *171*, 87 (1972).
8 J. Marmur, J. molec. Biol. *3*, 208 (1961).
9 M. Mandel and J. Marmur, Meth. Enzym. *12b*, 195 (1968).

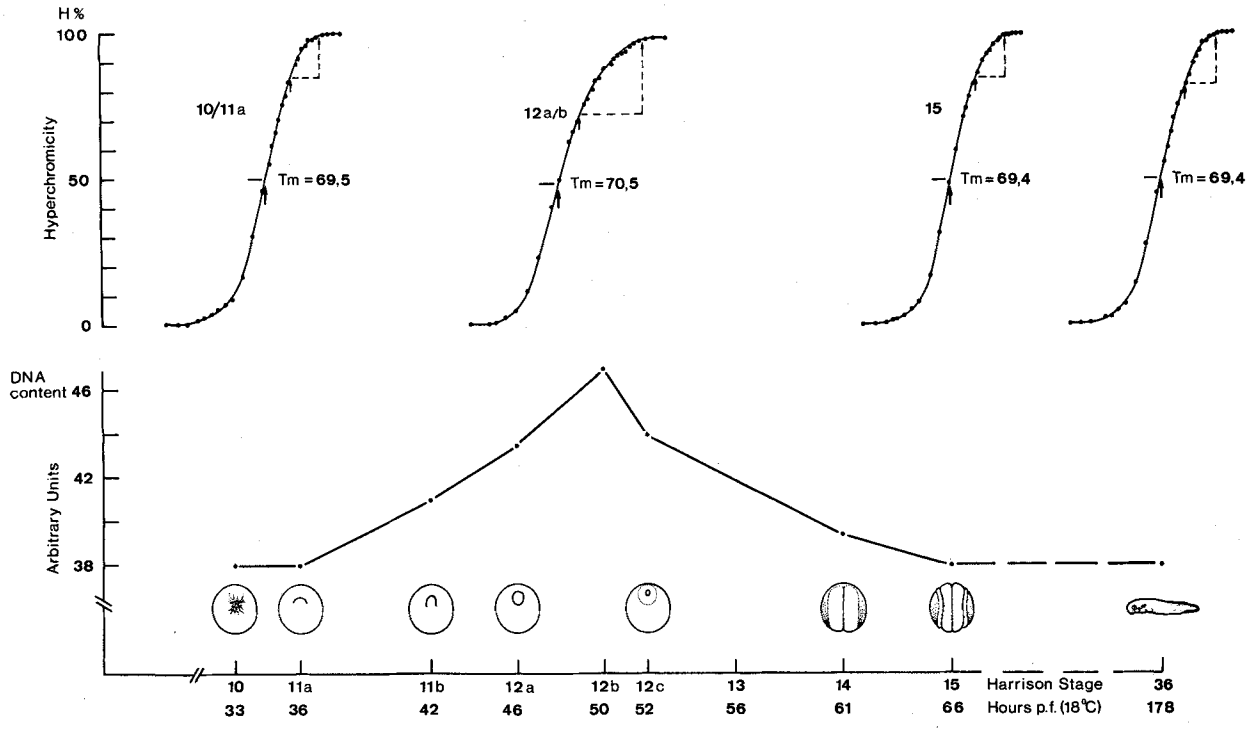

Fig. 1. Lower part: changes in Feulgen-DNA content of neuroectoderm nuclei during gastrulation of *Triturus vulgaris*. Ordinate: DNA content in arbitrary units.
Upper part: thermal denaturation profiles of DNA from different stages. Ordinate: relative absorbance (hyperchromicity) in percent. Abscissa: developmental stages (Harrison) and h post fertilization (18 °C).

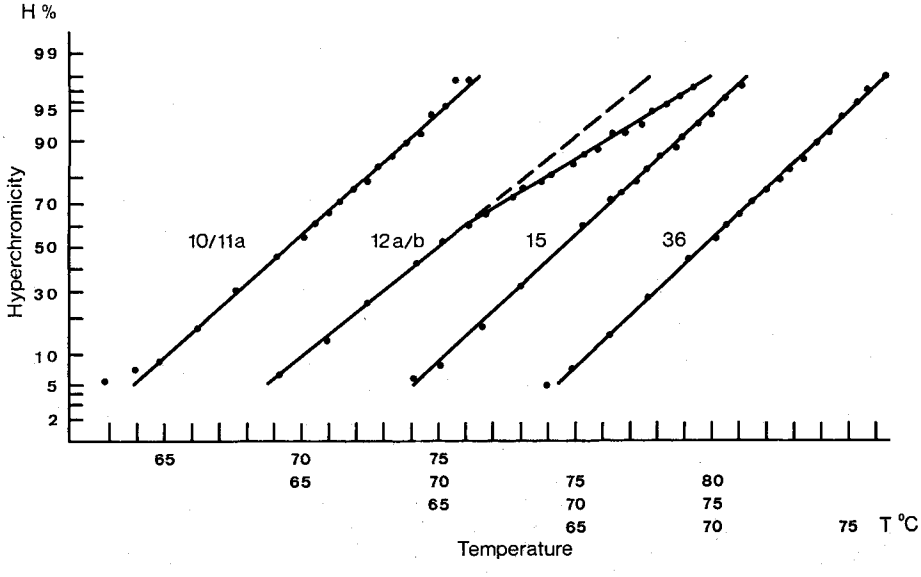

Fig. 2. Thermal denaturation curves of DNA (stages as in figure 1) plotted on normal probability paper. Ordinate: Hyperchromicity in percent. Abscissa: Temperature (°C).

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.

10 I. B. Dawid, D. D. Brown and R. H. Reeder, J. molec. Biol. *51*, 341 (1970).

Mitogenic action of neuraminidase

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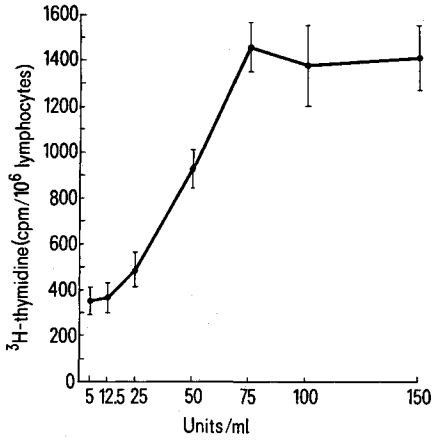
Summary A new effect of NCV on lymphocytes is demonstrated. This property is the capacity to act as a mitogen in and of itself. The possible mechanisms of this phenomenon are discussed.

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.

1 E. Watkins, Behring Inst. Mitt. *55*, 355 (1974).
2 Han Tin, Clin. exp. Immun. *18*, 95 (1974).
3 A. Rios and R. L. Simmons, J. nat. Cancer Inst. *51*, 637 (1973).
4 A. de Mouzon, E. Ohayon, F. Oksman, F. Oksman-Domejean and J. Ducos, Ann. Immun. (Inst. Pasteur) *127C*, 687 (1976).
5 E. Grothaus-Reisner and D. B. Amos, Transplantation *14*, 455 (1972).
6 P. K. Ray, H. Gewurz and R. L. Simmons, Clin. exp. Immun. *11*, 441 (1972).
7 G. Ludgren and R. L. Simmons, Clin. exp. Immun. *9*, 915 (1971).
8 G. Semenzato, G. Amadori and G. Gasparotto, unpublished observations.
9 Z. Bentwich, S. D. Douglas, E. Skutelsky and H. G. Kunkel, J. exp. Med. *37*, 1532 (1973).
10 M. S. Weiner, C. Bianco and V. Nussenzweig, Blood *42*, 939 (1973).
11 U. Galili and M. Schlesinger, J. Immun. *112*, 1628 (1974).
12 G. L. Nicolson, J. nat. Cancer Inst. *50*, 1443 (1973).
13 A. Novogrodsky and E. Katchalski, Proc. nat. Acad. Sci. USA *70*, 2515 (1973).