

# *Acta Medica Okayama*

---

*Volume 36, Issue 1*

1982

*Article 1*

FEBRUARY 1982

---

## Factors inhibiting cell proliferation in rat liver cytoplasm.

Makoto Kuramitsu\*

Hideki Matsui<sup>†</sup>

Masaaki Tokuda<sup>‡</sup>

Osamu Hatase\*\*

\*Kochi Medical College,

<sup>†</sup>Okayama University,

<sup>‡</sup>Okayama University,

\*\*Kagawa Medical School,

# Factors inhibiting cell proliferation in rat liver cytoplasm.\*

Makoto Kuramitsu, Hideki Matsui, Masaaki Tokuda, and Osamu Hatase

## Abstract

Two factors from normal rat liver cytoplasm inhibited the proliferation of cultured L-929 fibroblasts. One was arginase, the other was a small molecular weight inhibitor stable to trypsin and heat treatment. The small molecular weight inhibitor inhibited the protein and DNA synthesis of L-cells. Inhibition of DNA synthesis was thought to be secondary to the inhibition of protein synthesis.

**KEYWORDS:** cell proliferation, growth factor, inhibiting factor, rat liver cytosol, L-cells

---

\*PMID: 7064729 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 36, (1), 1–10 (1982)

## FACTORS INHIBITING CELL PROLIFERATION IN RAT LIVER CYTOPLASM

Makoto KURAMITSU, Hideki MATSUI\*, Masaaki TOKUDA\*,  
and Osamu HATASE\*\*

*Department of Pediatrics, Kochi Medical College, Kochi 781-51, Japan; \*Department of Physiology,  
Okayama University Medical School, Okayama 700, Japan and \*\*Department of Physiology,  
Kagawa Medical School, Kagawa 761-07, Japan*

*Received August 25, 1981*

**Abstract.** Two factors from normal rat liver cytoplasm inhibited the proliferation of cultured L-929 fibroblasts. One was arginase, the other was a small molecular weight inhibitor stable to trypsin and heat treatment. The small molecular weight inhibitor inhibited the protein and DNA synthesis of L-cells. Inhibition of DNA synthesis was thought to be secondary to the inhibition of protein synthesis.

**Key words :** cell proliferation, growth factor, inhibiting factor, rat liver cytosol, L-cells.

In previous studies of rat liver cytoplasm, we showed the co-existence of inhibitory and stimulatory factors modulating L-cell proliferation. Ethanol fractionation separated the stimulant and inhibitor from each other; 45 % saturation precipitated the inhibitor (N45) and 70 % saturation precipitated the stimulant (N70). We also showed that inhibitory activity was stronger in normal rat liver than in regenerating liver, and that stimulatory activity was enhanced in regenerating rat liver. We suggested that these factors would be regulators of cell proliferation (1).

In the next study, another strong inhibitory factor was found in the supernatant of 70 % ethanol saturation (N-sup). The properties of these two inhibitory factors were investigated. The two inhibitory factors were separated by DEAE-cellulose chromatography into an unbound fraction and a bound one. The former inhibitor was shown to be arginase. The other inhibitor was further purified by gel chromatography. In the present paper, we describe the properties and activities of inhibitors and discuss endogenous regulators of cell proliferation.

### MATERIALS AND METHODS

**Preparation of hepatic extract.** Wistar rats (60-100 g) were partially hepatectomized by the method of Higgins and Anderson (2). The resected livers were used as normal livers. Regenerating livers were harvested 24 h after the operation.

Normal and regenerating livers were washed in cold STE solution that contained 0.25

M sucrose, 10 mM Tris-HCl, pH 7.4, and 1 mM ethylenediaminetetraacetic acid (EDTA), and stocked at  $-80^{\circ}\text{C}$ . Stocked normal or regenerating livers were thawed, and homogenized in STE solution. The post-mitochondrial supernatant was centrifuged at 105,000  $\times g$  for 1 h, and the supernatant was intensively dialysed against dialysis medium that contained 1 mM Tris-HCl, pH 7.4, and 5 mM 2-mercaptoethanol and lyophilized.

The supernatant fractions from the normal and regenerating livers were named NS<sub>2</sub> and RS<sub>2</sub> respectively.

*Ethanol fractionation.* The supernatants were slowly saturated with chilled absolute ethanol up to 45% and 70% in the presence of 0.5 mM 2-mercaptoethanol under continuous stirring.

After being left to stand for 1 h, the precipitates were centrifuged at 6,000 rpm for 10 min. The precipitates from NS<sub>2</sub> and RS<sub>2</sub> after 45% ethanol saturation were named N45 and R45 respectively: the precipitates after 70% ethanol saturation were named N70 and R70, and the supernatants after 70% saturation were named N-sup and R-sup.

*DEAE-cellulose and Sephadex G-150 column chromatography.* Samples were dissolved in TM buffer which contained 10 mM Tris-HCl, pH 7.8, and 5 mM 2-mercaptoethanol. They were charged on DEAE-cellulose column or Sephadex G-150 column equilibrated with TM buffer after homogenization and centrifugation at 10,000  $\times g$ , for 10 min. In DEAE-cellulose column chromatography, fractionation was performed by step-wise elution.

All fractions were intensively dialysed against 1 mM Tris-HCl, pH 7.4, and 5 mM 2-mercaptoethanol, then lyophilized.

*Assay of the biological activity on cell proliferation of hepatic factors.* The inhibitory and stimulatory effects of hepatic factors on cell proliferation were assayed in the cultured L-929 mouse fibroblast system. All samples were dissolved in phosphate buffered saline (PBS(-)) and sterilized just prior to use. The amount of sample to be added was determined from the protein concentration of the sample assayed by the method of Lowry *et al.* (11).

The assay procedure was as follows: L-cells were seeded in plastic dishes (7.55 cm<sup>2</sup>) (Toyoshimaseisakusho) with seeding medium (abbreviated as SM) that contained 2% tryptose phosphate broth (TPB) and minimum essential medium (MEM). The assay was started at 48 h after the medium change with RM by replacing the RM with fresh growing medium (GM) that contained RM plus 5% calf serum and samples for the assay of inhibitory activity, or by replacing with fresh RM and samples for the assay of stimulatory activity. The number of cells was counted by a Coulter counter after 48 h culture at 37°C in a CO<sub>2</sub> gas incubator. Three identical dishes were used for each experimental point. The percent of inhibition was calculated as:

$$(\text{Cell count in GM} - \text{cell count in (GM + sample)}) \div \text{Cell count in GM} \times 100$$

Percent stimulation was calculated as:

$$(\text{Cell count in (RM + sample)} - \text{Cell count in RM}) \div \text{Cell count in RM} \times 100$$

*Assay of inhibitory activity on the synthesis of nucleic acids and proteins in L-cells.* The assay of DNA synthesis was carried out by the method of Rudland (3): Induction of G<sub>1</sub>-S phase transition of resting cells was performed by replacing RM with GM (turning-on the resting cells) (4) and labeling was performed with <sup>3</sup>H-thymidine (0.5 Ci mmol<sup>-1</sup>, 2.5 μCi ml<sup>-1</sup>, New England Nuclear, NEN) for 4 h in the S phase, from 16 h after turning-on to 20 h. Radioactivity on glass filters was counted in a toluene based scintillator by a Packard scintillation counter. The assay of protein synthesis was carried out by the method of Rudland (3).

The assay of protein synthesis was performed with <sup>14</sup>C-leucine (0.63 Ci mmol<sup>-1</sup>, 0.25 μCi ml<sup>-1</sup>, Radiochemical Centre, Amersham) in the G<sub>1</sub>-S transitional phase.

The assay of RNA synthesis was carried out as follows: labeling was performed with  $^3\text{H}$ -uridine ( $0.6 \text{ Ci mmol}^{-1}$ ,  $2 \mu\text{Ci ml}^{-1}$ , NEN) in the  $G_1$ -S transitional phase for a certain period, and after extracting the acid soluble radioactivity by the method of Rozengurt (5), cells were treated with  $0.5 \text{ M NaOH}$ . The radioactivity in the acid insoluble fraction was counted in a scintillator containing Triton X-100 (6).

To assay inhibitory effects on nucleic acid and protein synthesis, hepatic inhibitors were added to the medium at the time of turning-on in the assay system of DNA synthesis, and at the same time as the addition of radioactive precursors in the assay system of RNA and protein synthesis.

Three identical dishes were used to obtain the mean value for radioactivity at each experimental point. The percent variation from the control system was calculated as the inhibitory activity by the formula:

$$(\text{c.p.m. in GM} - \text{c.p.m. in (GM + sample)}) \div \text{c.p.m. in GM} \times 100.$$

*Arginase activity assay.* The arginase activity was examined by the method of Van Slyke (7)

## RESULTS

Table 1 shows that the cytoplasm of normal rat liver contained at least two inhibitors of L-cell proliferation (N45 and N-sup). Inhibitory activity of N45 was found in the unbound fraction of DEAE-cellulose chromatography (N45-TM fr.). The bound fraction (N45-NaCl fr.) did not show any inhibitory activity on cell proliferation (Tables 2 and 3). The unbound fraction of DEAE-cellulose chromatography of N70 also had slight inhibitory activity (N70-TM fr.).

Stimulatory activity was detected in the bound fraction of N70 eluted by  $0.5 \text{ M NaCl}$  from the DEAE-cellulose column (N70-NaCl fr.; Tables 2 and 3).

The unbound fraction of DEAE-cellulose chromatography of rat cytoplasm (N-A) had inhibitory activity (Fig. 1 and Table 4), which contained N45-TM fr. and N70-TM fr. The inhibitor in the N-A fraction was shown to be arginase.

Strong arginase activity was detected in the N-A fraction, and its inhibitory activity for cell proliferation was competitively and quantitatively inhibited by 1-arginine (Figs. 2 and 3). The second inhibitory factor in N-sup was fractionated by Sephadex G-150 column chromatography (Fig. 4). The lower molecular

TABLE 1. EFFECTS OF HEPATIC INHIBITORY FACTORS ON L-CELL PROLIFERATION<sup>a</sup>

| Systems                                       | Cell number <sup>b</sup><br>in $10^{-2}$ | Percent control <sup>b</sup> |
|-----------------------------------------------|------------------------------------------|------------------------------|
| Growing system (GM)                           | $3374 \pm 81$                            | 100                          |
| GM + NS <sub>2</sub> ( $100 \mu\text{g/ml}$ ) | $2902 \pm 461$                           | 86.0                         |
| GM + N45 ( $100 \mu\text{g/ml}$ )             | $2172 \pm 263$                           | 64.4                         |
| GM + N-sup ( $100 \mu\text{g/ml}$ )           | $2258 \pm 235$                           | 66.9                         |

<sup>a</sup> The assay procedure and the codes used are described in Materials and Methods.

<sup>b</sup> Calculation of cell number and percent control was done by the formula described in Materials and Methods.

TABLE 2. EFFECTS OF INHIBITORY AND STIMULATORY FACTORS ON L-CELL PROLIFERATION<sup>a</sup>

| Systems                                   | Cell number <sup>b</sup><br>in 10 <sup>-2</sup> | Percent control <sup>b</sup> |
|-------------------------------------------|-------------------------------------------------|------------------------------|
| Growing system (GM)                       | 1567 ± 45                                       | 100                          |
| GM + N45-TM fr. <sup>c</sup> (40 µg/ml)   | 265 ± 37                                        | 16.9                         |
| GM + N70-TM fr. <sup>c</sup> (40 µg/ml)   | 1169 ± 102                                      | 74.6                         |
| Resting system (RM)                       | 466 ± 38                                        | 100                          |
| RM + N45-NaCl fr. <sup>c</sup> (40 µg/ml) | 496 ± 52                                        | 106.4                        |
| RM + N70-NaCl fr. <sup>c</sup> (40 µg/ml) | 519 ± 47                                        | 112.8                        |

<sup>a</sup> The assay procedure is described in Materials and Methods.

<sup>b</sup> Calculation of cell number and percent control was done by the formula described in Materials and Methods.

<sup>c</sup> The unbound fractions of N45 and N70 ethanol fractions in DEAE-cellulose chromatography were named N45-TM fr. and N70-TM fr., respectively. Fractions eluted by the addition of 0.5 M NaCl were named N45-NaCl fr. and N70-NaCl fr., respectively.

TABLE 3. EFFECTS OF INHIBITORY AND STIMULATORY FACTORS ON DNA SYNTHESIS OF L-CELLS<sup>a</sup>

| Systems                                   | <sup>3</sup> H-thymidine<br>incorporated <sup>b</sup><br>(c.p.m.) | Percent control <sup>b</sup> |
|-------------------------------------------|-------------------------------------------------------------------|------------------------------|
| Growing system (GM)                       | 26,100 ± 2430                                                     | 100                          |
| GM + N45-TM fr. <sup>c</sup> (50 µg/ml)   | 20,400 ± 994                                                      | 78.2                         |
| GM + N45-NaCl fr. <sup>c</sup> (50 µg/ml) | 28,500 ± 2070                                                     | 109                          |
| Resting system (RM)                       | 4,320 ± 260                                                       | 100                          |
| RM + N70-TM fr. <sup>c</sup> (50 µg/ml)   | 4,046 ± 503                                                       | 93.7                         |
| RM + N70-NaCl fr. <sup>c</sup> (50 µg/ml) | 6,777 ± 252                                                       | 157                          |

<sup>a</sup> The assay procedure is described in Materials and Methods.

<sup>b</sup> <sup>3</sup>H-thymidine incorporated in acid insoluble fractions was detected in a scintillator by the procedures described in Materials and Methods. Calculation was done by the formula described in the same section.

<sup>c</sup> The codes used are described in Table 3.

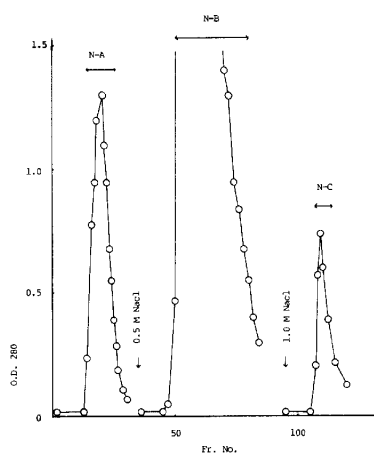


Fig. 1. Pattern of step-wise elution of NS<sub>2</sub> from DEAE-cellulose chromatography.

The unbound fraction was named N-A, fractions eluted by the addition of 0.5 M NaCl and 1.0 M NaCl were named N-B and N-C, respectively.

TABLE 4. EFFECTS OF INHIBITORY FACTORS IN  $\text{NS}_2$  ON L-CELL PROLIFERATION FRACTIONATED BY DEAE-CELLULOSE CHROMATOGRAPHY<sup>a</sup>

| Systems                                    | Cell number<br>in $10^{-2}$ | Percent control |
|--------------------------------------------|-----------------------------|-----------------|
| Growing system (GM)                        | $596 \pm 43$                | 100             |
| GM + $\text{NS}_2$ (100 $\mu\text{g/ml}$ ) | $512 \pm 55$                | 85.9            |
| GM + N-A (100 $\mu\text{g/ml}$ )           | $216 \pm 32$                | 36.2            |
| GM + N-B (100 $\mu\text{g/ml}$ )           | $536 \pm 50$                | 89.9            |
| GM + N-C (30 $\mu\text{g/ml}$ )            | $572 \pm 44$                | 96.0            |

<sup>a</sup> The assay procedure and calculation method are described in Materials and Methods. Samples were fractionated by DEAE-cellulose chromatography, and were named N-A, unbound fraction; N-B, eluted by TM buffer plus 0.5 M NaCl; N-C, eluted by TM buffer plus 1.0 M NaCl, respectively.

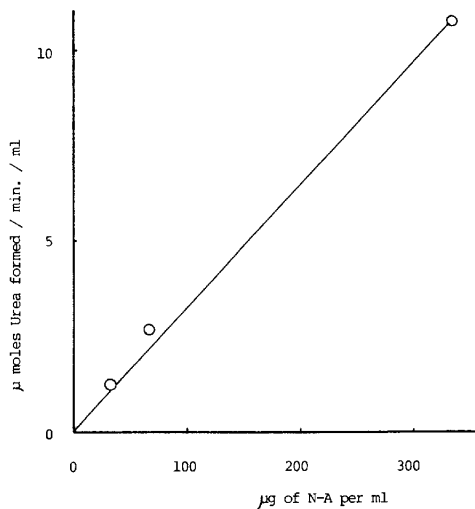


Fig. 2. Arginase activity in N-A.

The arginase activity was examined by the Method of Van Slyke (7).

Curve shows the proportionality between the amount of arginine hydrolyzed and the amount of enzyme present in N-A.

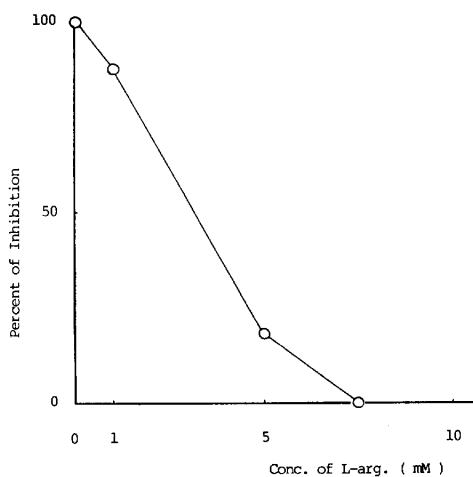


Fig. 3. Competitive effect of L-arginine on the inhibitory effect of N-A on cell proliferation.

The percent of inhibition was calculated by the following formula:  $\frac{[(\text{cell number in GM} + \text{L-arg.}) - (\text{cell no. in GM} + \text{N-A (50 } \mu\text{g/ml)} + \text{L-arg.})]}{[(\text{cell no. in GM}) - (\text{cell no. in GM} + \text{N-A (50 } \mu\text{g/ml)})]}$ . The abscissa indicates concentrations of L-arg. in GM. Assay procedure for cell proliferation is described in Materials and Methods.

weight fraction showed strong inhibitory activity (N-sup-fr. II; Fig. 4 and Table 5).

The apparent molecular weight of N-sup-fr. II was estimated as being less than 10,000 dalton by SDS poly-acrylamide gel electrophoresis.

The N-B fraction, step-wisely eluted by the addition of 0.5 M NaCl on the DEAE-cellulose column chromatography of NS<sub>2</sub>, was refractionated by Sephadex G-150 column chromatography (Fig. 5). Strong inhibition of DNA synthesis was found in the fraction of the lowest molecular weight peak (N-B-5; Fig. 5 and Table 6). The other four fractions did not have any inhibitory effect on the DNA synthesis of L-cells.

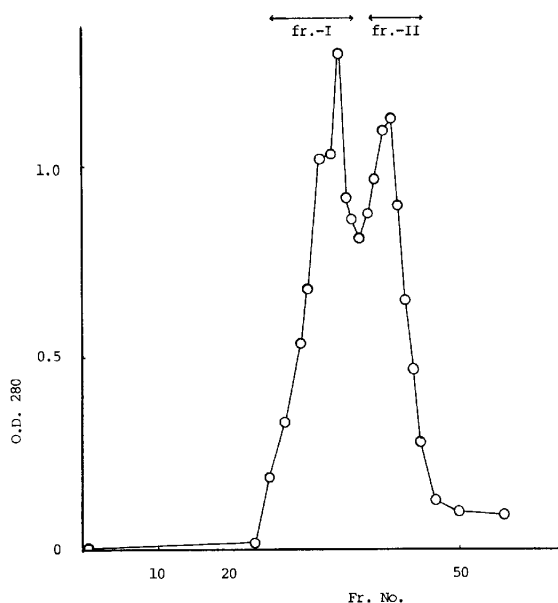


Fig. 4. Sephadex G-150 chromatography pattern of N-sup.  
The procedure is described in Materials and Methods.

TABLE 5. EFFECTS OF INHIBITORY FACTORS IN N-SUP ON DNA SYNTHESIS FRACTIONATED BY SEPHADEX G-150 CHROMATOGRAPHY <sup>a</sup>

| Systems                                    | <sup>3</sup> H-thymidine incorporated <sup>b</sup><br>(c.p.m.) | Percent control <sup>b</sup> |
|--------------------------------------------|----------------------------------------------------------------|------------------------------|
| Growing system (GM)                        | 20,900 ± 2,350                                                 | 100                          |
| GM + N-sup fr. I <sup>c</sup> (200 μg/ml)  | 17,100 ± 750                                                   | 81.8                         |
| GM + N-sup fr. II <sup>c</sup> (100 μg/ml) | 12,200 ± 871                                                   | 58.4                         |

<sup>a</sup> The assay procedure is described in Materials and Methods.

<sup>b</sup> Calculation was done by the formula described in Materials and Methods.

<sup>c</sup> Fractionation pattern is shown in Fig. 4.

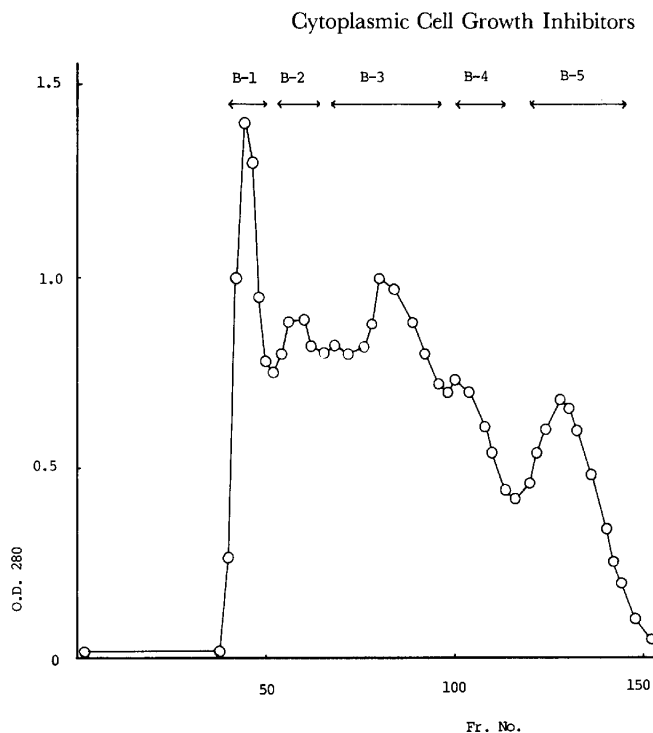


Fig. 5. Sephadex G-150 chromatography pattern of N-B. The procedure is described in Materials and Methods.

TABLE 6. INHIBITORY EFFECTS OF N-B-5 ON DNA SYNTHESIS OF L-CELLS<sup>a</sup>

| Systems                 | Percent control |
|-------------------------|-----------------|
| Growing system (GM)     | 100             |
| GM + N-B-5              |                 |
| Exp. 1 (10 $\mu$ g/ml)  | 11.6            |
| Exp. 2 (10 $\mu$ g/ml)  | 43.1            |
| Exp. 3 (40 $\mu$ g/ml)  | 53.7            |
| Exp. 4 (100 $\mu$ g/ml) | 45.4            |

<sup>a</sup> Assay system is described in Materials and Methods. The mean value of the incorporated <sup>3</sup>H-thymidine was calculated from 3 identical experimental dishes in each system. The standard deviations did not exceed 10% of the mean values.

The apparent molecular weight of N-B-5 was estimated to be less than 10,000 dalton by gel chromatography.

These results suggest that the inhibitory factors in N-sup-fr. II and N-B-5 might be the same inhibitor. We investigated the action mechanism of the second inhibitor (N-B-5).

Synchronized L-cells were treated by N-B-5 according to the time schedule shown in Fig. 6, and the inhibition of DNA synthesis was studied. N-B-5 showed much stronger inhibition of DNA synthesis when the cells were treated

in the G<sub>1</sub>-S transitional phase than when they were treated in the S phase (Fig. 6).

We studied the inhibitory effect of N-B-5 on RNA and protein synthesis in the G<sub>1</sub>-S transitional phase. The results showed that N-B-5 inhibited protein synthesis soon after treatment and continued its inhibitory effect (Fig. 7), but did not inhibit RNA synthesis during 120 min of treatment (not shown). The sensitivity of the inhibitory factors in N-B-5 to the trypsin and heat treatment was investigated. The inhibitor was insensitive to both treatments (Table 7).

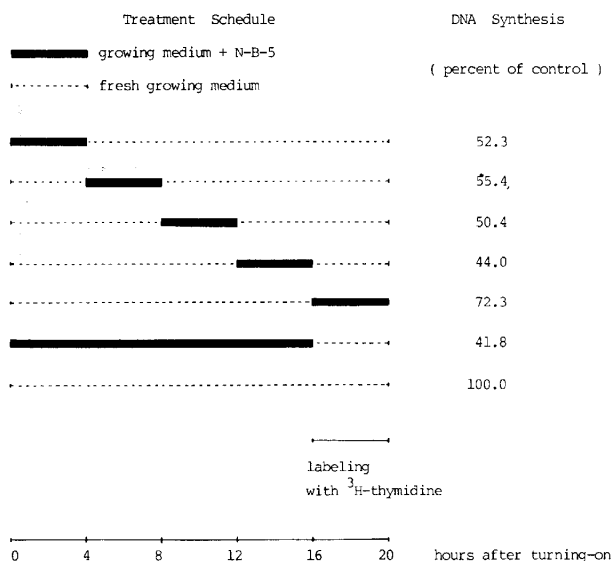


Fig. 6. Effect of N-B-5 on DNA synthesis in S phase by the treatment in G<sub>1</sub>-S transitional phase.

N-B-5 (14  $\mu$ g/ml) was added to GM at each time indicated in the treatment schedule, and after 4 h the medium was changed with fresh GM. Labeling with <sup>3</sup>H-thymidine was performed for 4 h from 16 h after turning-on to 20 h.

The percentage of the control was calculated by the formula described in Materials and Methods.

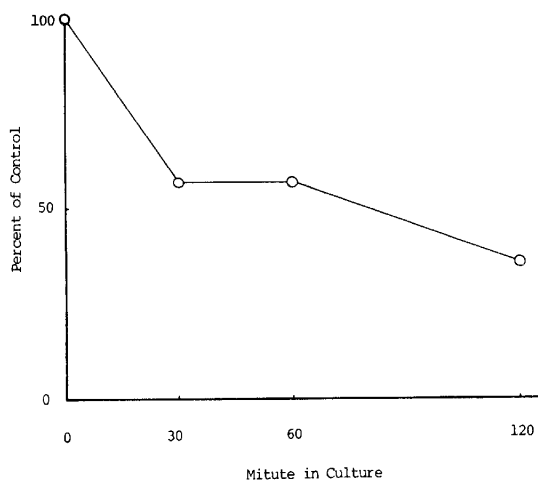


Fig. 7. Kinetic effect of N-B-5 on protein synthesis in the G<sub>1</sub>-S transitional phase.

N-B-5 (30  $\mu$ g/ml) and <sup>14</sup>C-leucine were added to the medium at 8 h after turning-on, and incubated for 30, 60 and 120 min. as indicated. The assay system of the incorporated <sup>14</sup>C-leucine is described in Materials and Methods.

The percentage of the control was calculated by the formula described in Materials and Methods.

TABLE 7. EFFECTS OF TRYPSIN- AND HEAT-TREATMENT ON INHIBITORY ACTIVITY OF N-B-5 IN DNA SYNTHESIS<sup>a</sup>

| Systems                                 | <sup>3</sup> H-thymidine incorporated<br>(c.p.m.) |
|-----------------------------------------|---------------------------------------------------|
| Growing system (GM)                     | 8,270 ± 910                                       |
| GM + N-B-5 (30 µg/ml)                   | 230 ± 42                                          |
| GM + N-B-5 boiled <sup>b</sup>          | 240 ± 38                                          |
| GM + N-B-5 trypsin treated <sup>b</sup> | 250 ± 61                                          |

<sup>a</sup> The assay procedure is described in Materials and Methods.

<sup>b</sup> N-B-5 was boiled at 100°C for 1 min, and one milligram of N-B-5 was treated with 10 µg of trypsin at 37°C for 60 min. Trypsinization was terminated by the addition of 10 µg of trypsin inhibitor that did not show any effect on DNA synthesis by itself.

### DISCUSSION

Two inhibitors of rat liver cytoplasm on cell proliferation were studied. The first inhibitor, which was found in the N-A fr. of DEAE-cellulose chromatography fractionation and the N45 fr. of ethanol fractionation, was arginase. The inhibitory activity of rat liver arginase on cultured cells has already been reported by Sasada *et al.* (8). The cytoplasm of regenerating rat liver also had arginase activity (not shown), therefore R45 probably contained arginase activity not less than N45. It is still unclear why R45 exhibited less inhibitory activity than N45.

Arginase was reported to cause no significant inhibition when administered *in vivo* (9); therefore, the inhibitory effect of N45 on DNA synthesis *in vivo* after partial hepatectomy (1) must be due to other inhibitors.

The second inhibitory factor in N-B-5 has not been reported previously. The primary action of this N-B-5 inhibitor was thought to be inhibition of protein synthesis. The inhibition of DNA synthesis would be secondary to inhibition of protein synthesis in the G<sub>1</sub>-S transitional phase required for DNA synthesis. N-B-5 showed an inhibitory effect on DNA synthesis even in the S phase treatment (Fig. 6), and must be due to inhibition of some kind of protein synthesis (10).

The data for UV absorption spectra (not shown) and stability against the trypsin and heat treatment suggest that the N-B-5 inhibitor is a nucleotide derivative. Peptides in N-B-5 might be contamination. Further investigation and purification of the inhibitor in N-B-5 and of the stimulator in the N70-NaCl fr. are to be done.

### REFERENCES

1. Hatase, O., Fujii, T., Kuramitsu, M., Itano, T., Takahashi, F., Murakami, T. and Nisida, I.:

- Co-existence of inhibitory and stimulatory factors modulating cell proliferation in rat liver cytoplasm. *Acta Med. Okayama* **33**, 73-80, 1979.
2. Higgins, G.H. and Anderson, R.M.: Experimental pathology of the liver. *Arch. Pathol.* **12**, 186-202, 1931.
  3. Rudland, P.S.: Control of translation in cultured cells; Continued synthesis and accumulation of messenger RNA in nondividing cultures. *Proc. Natl. Acad. Sci. U.S.A.* **71**, 750-754, 1974.
  4. Temin, H.M.: Stimulation by serum of multiplication of stationary chicken cells. *J. Cell Physiol.* **78**, 161-170, 1971.
  5. Rozengurt, E. and Stein, W.D.: Regulation of uridine uptake by serum and insulin in density-inhibited 3T3 cells. *Biochim. Biophys. Acta* **464**, 417-432, 1977.
  6. Ball, C.R., Van Den Berg, H.W. and Poynter, R.W.: The measurement of radioactive precursor incorporation into small monolayer cultures. In *Methods in Cell Biology*, VII, ed. Prescott, Academic Press, N.Y. and London, pp 349-360, 1973.
  7. Van Slyke, D.D. and Archibald, R.M.: Gasometric and photometric measurement of arginase activity. *J. Biol. Chem.* **165**, 293-309, 1946.
  8. Sasada, M. and Terayama, H.: The nature of inhibitors of DNA synthesis in rat liver hepatoma cells. *Biochim. Biophys. Acta* **190**, 73-87, 1969.
  9. Lavigne, J., Lalanne, M., Thompson, J.P. and Simard, A.: Specific inhibition of the incorporation of tritiated thymidine in regenerating rat liver by a rat hepatocytes supernatant. *Rev. Can. Biol.* **36**, 27-36, 1977.
  10. Weiss, B.G.: The dependence of DNA synthesis on protein synthesis in Hela S3 cells. *J. Cell Physiol.* **73**, 85-90, 1969.
  11. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.