Jpn. J. Cancer Res. 82, 1277–1283, November 1991

N^1-Methylnicotinamide Level in the Blood after Nicotinamide Loading as Further Evidence for Malignant Tumor Burden

Koji Nakagawa,[1] Masaru Miyazaki,[1] Katsuji Okui,[1] Noriko Kato,[2] Yoichi Moriyama[2] and Shinji Fujimura[2,3]

Departments of [1]Surgery and [2]Biochemistry, Chiba University School of Medicine, 1-8-1 Inohana, Chiba 280

Nicotinamide methyltransferase (Nmd CH_3transferase) activity increased in the liver of mice after i.p. transplantation of Ehrlich ascites tumor (ascitic form), but not in the liver of mice with acute inflammation induced by the i.p. administration of D-galactosamine, and it rather showed a decrease together with necrosis after carbon tetrachloride administration. When Nmd CH_3transferase activity of rat hepatocytes in primary culture was investigated with the addition of dexamethasone, epidermal growth factor, transforming growth factor-β, tumor necrosis factor-α and N^1-methylnicotinamide (1-CH_3Nmd), changes in activity were not correlated with DNA synthesis, suggesting that the increase of this enzyme activity in the tumor host liver was not directly related to liver cell proliferation. Thus, in order to make use of the increase of this enzyme activity as a tumor burden marker, a procedure for its estimation by measuring the blood level of 1-CH_3Nmd, a metabolite of Nmd produced by Nmd CH_3transferase, was established. The 1-CH_3Nmd level in the blood of mice bearing Ehrlich ascites tumor 4 h after s.c. loading of Nmd (500 mg/kg body weight) was closely correlated with this enzyme activity in the liver ($r=0.835$, $P<0.00001$) from the early to the terminal stage of tumor development. Furthermore, similar correlations were seen in the animal groups bearing various other tumors, such as s.c. implanted Ehrlich ascites tumor (solid form) and i.p. implanted sarcoma S-180, hepatoma MH-134, Yoshida ascites sarcoma and leukemia L-1210, but not solid tumors such as Lewis lung carcinoma and melanoma B-16, although almost all of the animals bearing these tumors showed a higher enzyme activity than their control normal animals.

Key words: N^1-Methylnicotinamide — Nicotinamide methyltransferase — Tumor burden marker

Nicotinamide: *S*-adenosylmethionine methyltransferase (EC 2.1.1.1; nicotinamide methyltransferase; Nmd CH_3transferase)[4] catalyzes the transfer of the methyl group from *S*-adenosylmethionine (SAM) to nicotinamide (Nmd) to produce N^1-methylnicotinamide (1-CH_3Nmd) and *S*-adenosylhomocysteine,[1] and is localized predominantly in the mammalian liver.[2,3] 1-CH_3Nmd is mostly excreted into urine and partly further converted via catalysis by 1-CH_3Nmd oxidase to N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-5-carboxamide, which are also excreted into urine.[4–6]

In rats bearing Walker 256 carcinosarcoma, it was postulated that the observed elevation of urinary excretion of 1-CH_3Nmd was caused by the increase of Nmd CH_3transferase activity in the tumor rather than in the liver, probably because the enzyme activity in the tumor was significantly higher than that in the host liver, in which the activity was only slightly higher than that in normal rat liver, although many metabolic changes can be seen in animals bearing tumors, especially in the liver.[7] Later, we showed that Nmd CH_3transferase activity gradually increased in the liver of the mouse with tumor development after the transplantation of Ehrlich ascites tumor, and also that this phenomenon was general in various kinds of experimental tumors.[8,9] The activity of Nmd CH_3transferase was distinguished by its continuous increase up to the terminal stage of tumor growth, in contrast to catalase activity in the liver, which is well known to decrease gradually throughout tumor growth.[10,11] Measurement of an enzyme activity which increases in the tumor host would be much more reliable for the diagnosis of tumor burden, since a decrease of enzyme activity could be a result not only specifically of the tumor burden but also just due to the decay of the tumor host.[12]

In this study, in order to utilize the 1-CH_3Nmd blood level as a tumor burden marker instead of the Nmd CH_3transferase activity in the liver in the tumor host, we examined the relationship between both values after Nmd loading, since the 1-CH_3Nmd level in the blood was undetectably low *per se* even in the tumor-bearing animals. The physiological significance of the enzyme

[3] To whom correspondence should be addressed.

[4] Abbreviations used are: Nmd CH_3transferase, nicotinamide methyltransferase; Nmd, nicotinamide; SAM, *S*-adenosylmethionine; 1-CH_3Nmd, N^1-methylnicotinamide; EGF, epidermal growth factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; CCl_4, carbon tetrachloride; DTT, dithiothreitol.

activity increase in the liver and the clinical applicability of this increase as a tumor burden marker are discussed.

MATERIALS AND METHODS

Chemicals L-[Methyl-^{3}H]SAM (75.2 Ci/mmol) and [methyl-^{3}H]thymidine (43.0 Ci/mmol) were purchased from Amersham International plc, Buckinghamshire, England. Nmd, 1-CH_3Nmd, aprotinin and dexamethasone were purchased from Sigma Chemical Co., St. Louis, Mo.; sodium 1-octanesulfonate was from Regis Chemical Co., Morton Grove, Ill.; epidermal growth factor (EGF) was from Takara Shuzo Co., Ltd., Kyoto; insulin and transforming growth factor-β (TGF-β) were from Biomedical Technologies Inc., Stoughton, Mass.; and tumor necrosis factor-α (TNF-α) was from R&D Systems Inc., Minneapolis, Minn. All other chemicals used were obtained from commercial sources.

Animal and tumor dd/Y strain male mice, 6 weeks old, were provided by Funabashi Farm Co., Ltd., Funabashi. Ehrlich ascites tumor had been maintained i.p. in this strain by weekly serial transplantations of the tumor ascites (1×10^7 cells), which was implanted i.p. and s.c. to prepare ascitic and solid forms, respectively. C3H/He, ICR/J and DBA/2J strains of mice (male, 6 weeks old) were obtained from Shizuoka Agricultural Cooperative Ltd., Shizuoka, to maintain i.p. hepatoma MH-134, sarcoma S-180 and leukemia L-1210, respectively, by serial transplantations of the ascites (1×10^7 cells). Six-week-old male Donryu strain rats obtained from Nihon Rat Co., Tokyo, were used to maintain Yoshida ascites sarcoma by i.p. weekly serial transplantations of the tumor ascites (1×10^6 cells). Solid Lewis lung carcinoma and melanoma B-16 (1×10^7 cells) were inoculated s.c. into 6-week-old male BDF 1 and C 57 BL/6 mice purchased from Shizuoka Agricultural Cooperative Ltd., respectively.

Induction of hepatic disorders One model of hepatic disorder with inflammation was prepared by the administration of D-galactosamine to mice. Male dd/Y mice, 6 weeks old, were injected i.p. with D-galactosamine dissolved in saline at a dose of 250 mg/kg body weight 3 times every 4 h. The mice were used for experiments 48 h after the first injection.[13, 14] The other model of hepatic disorder with necro-cytosis was prepared by the administration of carbon tetrachloride (CCl_4) to mice. CCl_4 dissolved in olive oil at a concentration of 0.05% was given at a dose of 4 mg/kg body weight intragastrically into the same mice as above. After 24 h, necrosis of hepatocytes was induced with increased serum GOT, GPT and LDH activities as described before,[15, 16] and the mice were used for experiments.

Primary culture of rat hepatocytes The liver of male 7-week-old Wistar strain rats (Shizuoka Agricultural Cooperative Ltd.) was perfused *in situ* with Hanks' solution containing collagenase (230 units/mg; Wako Pure Chemical Industries, Ltd., Osaka) at a concentration of 0.5 mg/ml and the liver cells were collected. Parenchymal hepatocytes which were isolated by centrifugation 3 times at 50*g* for 1 min each from the collected cells were cultured for 4 h in Williams medium-E (Flow Laboratories, Irvine, Scotland) with 5% fetal calf serum (Gibco Co., Grand Island, N.Y.) and 10^{-9} *M* insulin[17] at an initial cell density of 1×10^5 cells/cm^2. Then the medium was changed to serum- and insulin-free Williams medium-E with aprotinin at the concentration of 0.2 μg/ml. Twenty h later, test substances were added to the medium and the culture was continued for another 24 h, and finally DNA synthesis and Nmd CH_3transferase activity were assayed.

Assay of Nmd CH_3transferase activity The enzyme activity was assayed by using the supernatant obtained by centrifugation at 105,000 *g* for 60 min of the liver homogenate prepared in 4 volumes of 50 m*M* Tris-HCl buffer (pH 8.0) containing 2 m*M* DTT (dithiothreitol). In the case of hepatocytes in the primary culture, the cells were washed with ice-cold phosphatebuffered saline, harvested with a rubber policeman, and suspended in 50 m*M* Tris-HCl buffer (pH 8.0) containing 2 m*M* DTT. Then the cells were disrupted by three cycles of freezing and thawing to prepare the homogenate, and the supernatant of the homogenate was obtained as described above.

The enzyme assay was done by the method of Hoshino *et al.*[18] with some modifications. The reaction mixture (50 μl in total) consisted of 50 m*M* Tris-HCl buffer (pH 8.0), 2 m*M* DTT, 10 m*M* Nmd, 0.5 μCi [CH_3-^{3}H]SAM and the enzyme preparation. After incubation for 5 min at 37°C with the subsequent addition of 3 μl of concentrated perchloric acid, 20 μl of the resulting supernatant was developed on Whatman 3MM filter paper in a solvent system consisting of isopropyl alcohol:formic acid: H_2O (70:10:20; v/v/v). The radioactivity of the area on the paper corresponding to 1-CH_3Nmd was measured.[19] Activity was determined as an initial velocity under the condition that radioactivity was increasing linearly as a function of time, and is represented by cpm/h/mg protein.

Determination of 1-CH_3Nmd level in the blood After the s.c. injection of Nmd (500 mg/kg body weight) into the back of mice, blood was taken from the femoral vein under diethyl ether anesthesia. Ninety μl of the serum filtered through an ultrafiltration membrane (Mr. 30,000 sieve, Ultrafree C3TK; Millipore Japan Co., Ltd., Tokyo) was mixed with 10 μl of 0.5 *M* phosphate buffer (pH 7.0) containing 0.25 *M* 1-octanesulfonate, with adjustment of the pH to 7.0 by adding H_3PO_4 or KOH if necessary. Then the prepared sample was used for analysis. The HPLC system consisted of a Multipump (CCPM

and PX-8010), reverse-phase analytical column (TSKgel ODS-80TM, C18, 250 mm × 4.6 mm in diameter; particle size, 5 μm) and detector (UV-8011, Tosoh Co., Ltd. Tokyo), and the method of Kutnink *et al.*[20] was employed with some modifications. The mobile phase contained 10 m*M* K_2HPO_4 and 10 m*M* 1-octanesulfonate in 8% acetonitrile, with the pH adjusted to 7.0. Conditions were isocratic with a flow rate of 1 ml/min, the temperature of the column and mobile phase was 25°C, and the detection wavelength was 264 nm. The blood level of 1-CH_3Nmd was quantitated in terms of the peak area of HPLC in comparison with that of the 1-CH_3Nmd standard.

Assay of DNA synthesis DNA synthesis was assayed by measuring the incorporation of [^{3}H]thymidine into the trichloroacetic acid-insoluble fraction of hepatocytes in primary culture as described before.[21]

Protein determination Protein was estimated by the method of Lowry *et al.*[22] with bovine serum albumin as a standard.

Statistical analysis Statistical significance of differences was evaluated by using Student's two-tailed *t* test.

RESULTS

Nmd CH_3transferase activity in livers of tumor-bearing, diseased and normal mice Nmd CH_3transferase activity in the liver of mice 7 days after i.p. inoculation of Ehrlich ascites tumor was 3-fold higher than that of normal mice. In the liver of mice with hepatitis induced by the i.p. administration of D-galactosamine, Nmd CH_3transferase activity was almost the same as in normal mice. Meanwhile, in mice with necro-cytosis induced by CCl_4 p.o. administration, the activity was significantly lower than in the control mouse liver (Table I).

Effects of dexamethasone, EGF, TGF-β, TNF-α and 1-CH_3Nmd on Nmd CH_3transferase activity and DNA synthesis in rat hepatocytes in primary culture DNA synthesis in the hepatocytes was measured 24 h after the addition of the various agents to the culture medium. As shown in Fig. 1, the addition of dexamethasone and TGF-β resulted in 70% and 60% decreases in DNA synthesis, respectively; in contrast, EGF resulted in an 11-fold increase compared with the control. Neither TNF-α nor 1-CH_3Nmd had any significant effect. No significant change of Nmd CH_3transferase activity occurred after the addition of any of the above agents.

Time course of 1-CH_3Nmd level in the blood and Nmd CH_3transferase activity in the liver of tumor host mice after Nmd injection When Nmd (500 mg/kg body weight) was injected s.c. into male dd/Y mice 7 days after inoculation of Ehrlich ascites tumor, the 1-CH_3Nmd level in the blood increased, reached a plateau at 12 h and maintained this level at least until 24 h after Nmd injection. At the same time, the level in normal mice also increased with a peak at 9 h and then decreased to the initial level at 24 h (Fig. 2A). Accordingly, Nmd CH_3transferase activity in the liver was measured. As shown in Fig. 2B, the activity in the tumor host liver increased gradually until 24 h after the Nmd injection, becoming more than 2-fold higher than the initial activity, while the activity in the normal mouse liver stayed almost constant until 24 h. From the above findings, 4 h was chosen as the most suitable time to measure the activity and the blood level after Nmd injection.

Time course of 1-CH_3Nmd blood level and Nmd CH_3-transferase activity in the liver under Nmd loading in mice after tumor inoculation The activity in the liver of

Table I. Nmd CH_3transferase Activity in Tumor Host Liver and Injured Liver

Groups	Nmd CH_3transferase activity (cpm × 10^{-5}/h/mg protein)	
	Treated	Control
Ehrlich ascites tumor-implanted	36.3±7.0**	11.1±1.3
D-Galactosamine-treated	13.7±2.5	13.4±3.0
CCl_4-treated	7.7±1.6*	11.3±1.0

** Significant vs. control ($P<0.01$).
* Significant vs. control ($P<0.05$).
Saline and olive oil were used as controls for D-galactosamine and CCl_4 treatments, respectively. Each value represents the mean and SE of three or four mice.

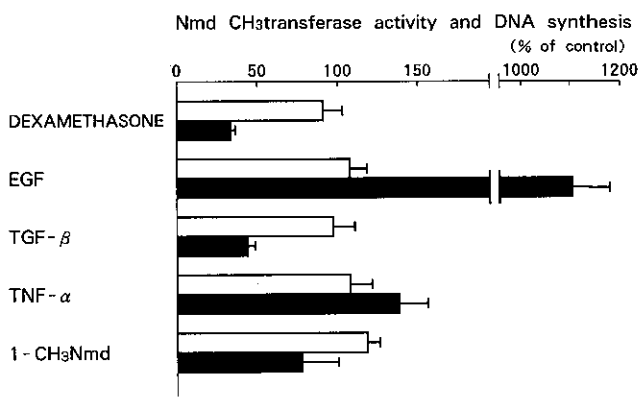
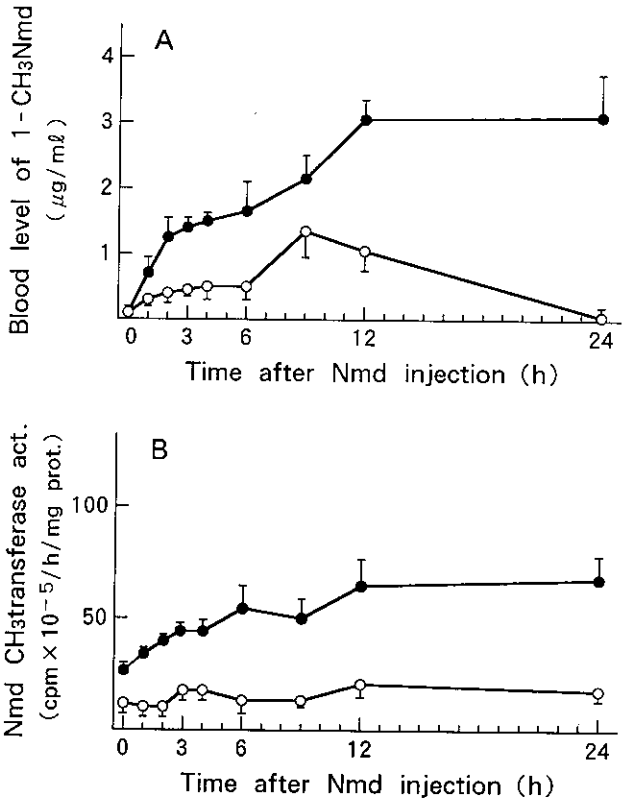


Fig. 1. Effects of various reagents on Nmd CH_3transferase activity and DNA synthesis in rat hepatocytes in primary culture. Nmd CH_3transferase activity (□) and DNA synthesis (■) were measured as described in "Materials and Methods" 24 h after the addition of dexamethasone (10^{-7} *M*), EGF (20 ng/ml), TGF-β (100 pg/ml), TNF-α (20 ng/ml) or 1-CH_3Nmd (1 m*M*) to the medium. Each column represents the mean±SE (bar) of values of four cultures, expressed as percentage of the control (100%).



mice under Nmd loading was more than 3-fold higher at 2 days after the inoculation of Ehrlich ascites tumor than that of the normal mouse (at day 0), and continued to increase until the expiration of the host mouse. This alteration of activity paralleled that without Nmd loading (Fig. 3A). The 1-CH_3Nmd blood level with Nmd loading also showed an increase after tumor inoculation (Fig. 3B). However, 1-CH_3Nmd was not detectable in blood at any time after tumor inoculation without Nmd loading. The correlation between the enzyme activity of the liver and 1-CH_3Nmd blood level under Nmd loading was very high, with a correlation coefficient of 0.835 ($P<0.00001$), throughout the tumor development.

Fig. 2. Time course of 1-CH_3Nmd level in the blood and Nmd CH_3transferase activity in the liver of the tumor host mice after Nmd s.c. injection. A: 1-CH_3Nmd levels in the blood of normal mice (○) and mice 7 days after i.p. inoculation of Ehrlich ascites tumor (ascitic form)(●) were measured as a function of time after s.c. injection of Nmd as described in "Materials and Methods." B: Nmd CH_3transferase activities of normal mice (○) and mice 7 days after i.p. inoculation of Ehrlich ascites tumor (ascitic form) (●) were measured as a function of time after s.c. injection of Nmd. Each point represents the mean±SE (bar) of the values of three mice.

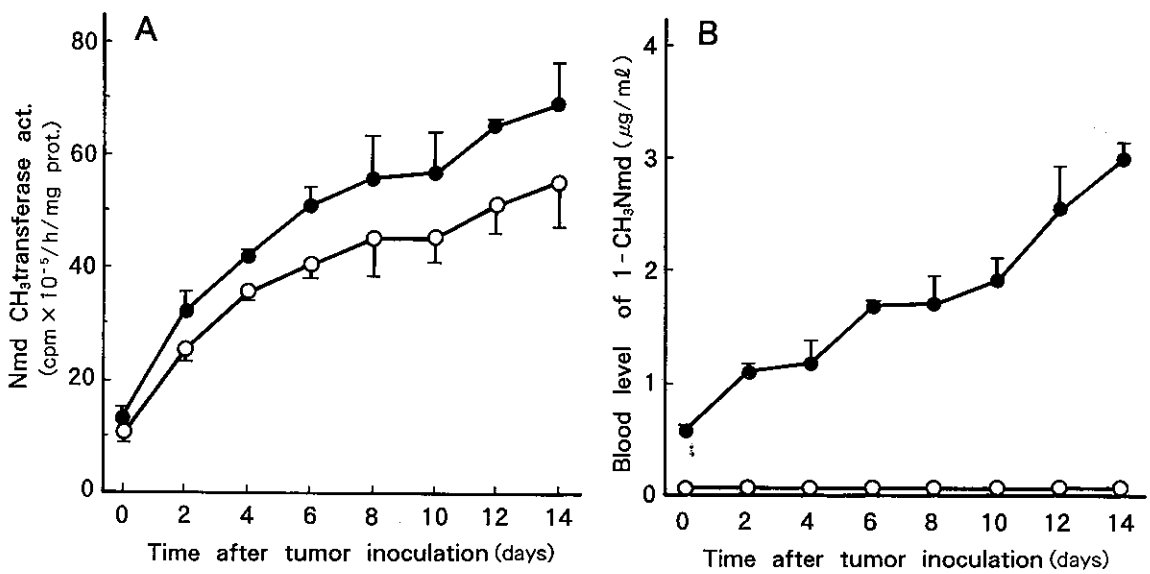


Fig. 3. Time course of 1-CH_3Nmd level in the blood and Nmd CH_3transferase activity in the liver under Nmd loading after i.p. inoculation of Ehrlich ascites tumor. A: Nmd CH_3transferase activities in the liver of mice without (○) and with s.c. injection of Nmd (4 h later) (●) were measured as a function of time after i.p. inoculation of Ehrlich ascites tumor as described in "Materials and Methods." B: 1-CH_3Nmd levels in the blood of mice without (○) and with s.c. injection of Nmd (4 h later) (●) were measured as a function of time after i.p. inoculation of Ehrlich ascites tumor. Each point represents the mean±SE (bar) of the values of three mice.

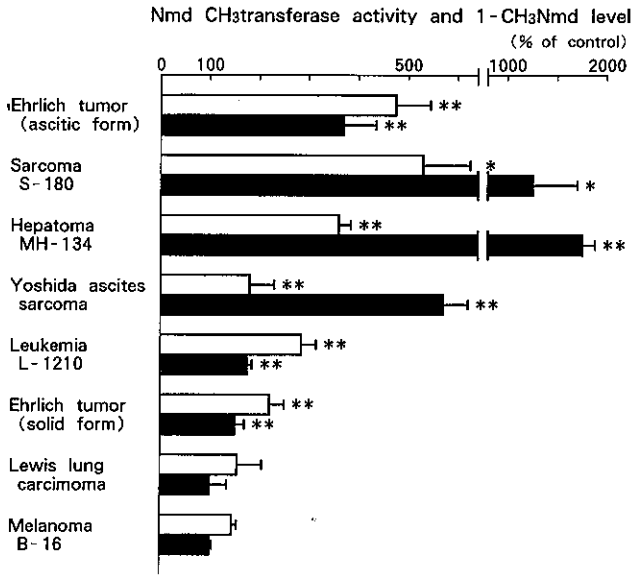


Fig. 4. 1-CH_3Nmd level in the blood and Nmd CH_3transferase activity in the liver of mice with various tumors under s.c. loading of Nmd. 1-CH_3Nmd level (■) and Nmd CH_3transferase activity (□) were measured as described in "Materials and Methods" 4 h after s.c. injection of Nmd. The intervals after tumor inoculation were 5 days in the case of Yoshida ascites carcinoma, 18 days in Lewis lung carcinoma, 10 days in melanoma B-16 and 7 days in the other tumors. Each column represents the mean±SE (bar) of values of three to five mice, expressed as percentages of the mean of the corresponding normal control (100%). A statistically significant difference vs. control is expressed by asterisks: * $P<0.05$ and ** $P<0.01$. 1-CH_3Nmd level and Nmd CH_3transferase activity of controls under Nmd loading varied according to the strain or species of the animal used for each tumor as follows, in the same order as that in which the tumors are listed: 498±133, 105±61, 67±99, 200±50, 1717±22, 498±133, 870±139 and 880±44 mg of 1-CH_3Nmd/ml of the blood, and 11.9±2.1, 5.1±0.8, 7.2±2.2, 39.2±4.9, 9.7±2.3, 11.9±2.1, 17.9±3.4 and 41.8±5.6 cpm $\times 10^{-5}$ of 1-CN_3Nmd formed/h/mg of the liver protein.

Correlation between 1-CH_3Nmd level in the blood and Nmd CH_3transferase activity in the liver of animals with various tumors under Nmd loading As shown in Fig. 4, hepatic Nmd CH_3transferase activities in the hosts of all of the tumors investigated were 1.5- to 5-fold higher on average than the respective controls. The blood level of 1-CH_3Nmd was also significantly higher (1.5- to 17-fold) than the controls, except in 2 of the 3 solid tumors, i.e., Lewis lung carcinoma and melanoma B-16. There was a significant correlation between the activity and the blood level for each kind of tumor, except in the 2 solid tumors, as shown in Table II.

Table II. Correlation between 1-CH_3Nmd Level in the Blood and Nmd CH_3transferase Activity in the Liver of Various Tumor-Host Animals under s.c. Loading of Nmd

	$r^{a)}$	$(P)^{b)}$
Ehrlich tumor (ascitic form)	0.932	(<0.001)
Sarcoma S-180	0.981	(<0.001)
Hepatoma MH-134	0.985	(<0.001)
Yoshida ascites sarcoma	0.871	(<0.05)
Leukemia L-1210	0.932	(<0.01)
Ehrlich tumor (solid form)	0.694	(<0.05)
Lewis lung carcinoma	0.349	(>0.05)
Melanoma B-16	0.592	(>0.05)

1-CH_3Nmd level and Nmd CH_3transferase activity were measured as described in the legend to Fig. 4.
a), *b*) Correlation coefficient (*r*) between the activity and the blood level in the same tumor case for each kind of tumor with *P* value.

DISCUSSION

In this study, a method for evaluation of the increase of the 1-CH_3Nmd blood level instead of the increase of Nmd CH_3transferase activity in the liver of tumor host animals was established for the purpose of non-surgically detecting the tumor burden in cancer patients.

In the mouse models of hepatic inflammation and damage, ordinarily observed disorders in the human liver, no increase in the activity of Nmd CH_3transferase was observed (Table I). This suggested that the increase of activity in the liver of tumor host mice was at least not caused by these disorders. In the regenerating rat liver after partial hepatectomy, Nmd CH_3transferase activity increased together with an increase of DNA synthesis[18,23]; furthermore in RLC cells (cell line of well differentiated rat hepatocellular carcinoma), 1-CH_3Nmd stimulated DNA synthesis.[24] Thus, Nmd CH_3transferase activity is probably related to hepatocellular proliferation. However, in the present study, it was clearly demonstrated by the use of hepatocytes in primary culture that 1-CH_3Nmd did not stimulate DNA synthesis. Furthermore, EGF, which stimulated DNA synthesis of cultured hepatocytes,[25] did not alter the Nmd CH_3-transferase activity at all. The enzyme activity increase in the regenerating liver could very well be caused by a compensatory hyperfunction as discussed in connection with another enzyme after partial hepatectomy.[26] The addition of dexamethasone, which is known to induce differentiation and to reduce proliferation,[25,27] did not have any effect on Nmd CH_3transferase activity (Fig. 1). Thus, this enzyme, unlike other enzymes such as tryptophan 2,3-dioxygenase (EC 1.13.11.11)[28] and tyrosine

aminotransferase (EC 3.6.1.5),[29] should not be related to differentiation of the hepatocytes in primary culture.

A variety of tests concerned with detection of malignant disease have been reported [30–32] but their results were not necessarily specific to the tumor burden. Overall, these tests might reflect aspects of altered metabolism in a certain tumor, including disruption and proliferation. Therefore, it is difficult for these tests to distinguish cancer from inflammatory diseases and to detect a low tumor burden.[33, 34] However, the increase of Nmd CH_3transferase activity in the liver should reflect the metabolic alteration of the tumor host itself. The mechanism of the increase of this enzyme in the tumor host liver is not clear. We are now searching for the factor(s) that stimulates Nmd CH_3transferase activity in the liver of tumor hosts.

Liver biopsy is not used in clinical investigations to repeatedly measure hepatic enzyme activity. At least for the tumors investigated in this study, Nmd CH_3-transferase activity in the tumor was negligible.[8, 9] Moreover, 1-CH_3Nmd oxidase activity, which catalyzed the oxidation of 1-CH_3Nmd, remained constant in the liver, at least after inoculation of Ehrlich ascites tumor. Thus, we could estimate the Nmd CH_3transferase activity in the liver in terms of 1-CH_3Nmd, the reaction product formed by this enzyme. Urinary excretion and blood level of 1-CH_3Nmd are influenced by niacin intake via food, and the latter is very low, probably due to its prompt excretion into urine.[35, 36] However, by s.c. injection of Nmd as the substrate of Nmd CH_3transferase, the 1-CH_3Nmd blood level could be made high enough to be detectable, even in normal mice (Fig. 2A). Such Nmd loading also stimulated Nmd CH_3transferase activity in the liver of the tumor hosts but not so much as in the normal mouse (Fig. 2B). Therefore, the 1-CH_3Nmd blood level can reasonably be expected to increase specifically in the tumor host upon Nmd loading. In fact, the blood level of 1-CH_3Nmd steadily increased even in the early stage of tumor growth (Fig. 3B).

Since the 1-CH_3Nmd blood level was correlated to Nmd CH_3transferase activity in the liver under Nmd loading in mice bearing any of the ascitic tumors investigated (Table II), the 1-CH_3Nmd level may be available as a general tumor burden marker. However, in 2 of 3 solid tumors, the 1-CH_3Nmd blood level did not increase significantly. This might be caused by the smaller increase of Nmd CH_3transferase activity in the solid tumor host livers (1.5-fold compared with the normal control) than in the ascites tumor host liver (2- to 5-fold), as shown in Fig. 4. However, since in the case of the solid form of Ehrlich ascites tumor, the 1-CH_3Nmd level did increase even though the increase of the enzyme activity was small (2-fold), the response of the 1-CH_3Nmd level might also reflect the character of the tumor in the host. As most human tumors are solid, the application of this method for cancer patients might not be warranted. However, in surgical specimens of human breast cancer, gastric cancer and especially hepatocellular carcinoma, high activity of Nmd CH_3transferase has been observed (data not shown). Accordingly, this method may provide a reliable measure of tumor burden in certain patients.

(Received April 13, 1991/Accepted August 2, 1991)

REFERENCES

1) Cantoni, G. L. Methylacceptor systems. A. Nicotinamide methylpherase, liver. *Methods Enzymol.*, **2**, 257–260 (1957).
2) Seifert, R., Hoshino, J. and Kröger, H. Nicotinamide methylation. Tissue distribution, developmental and neoplastic changes. *Biochim. Biophys. Acta*, **801**, 259–264 (1984).
3) Alton, T. A. and Abeles, R. H. Substrate specificity of nicotinamide methyltransferase isolated from porcine liver. *Arch. Biochem. Biophys.*, **260**, 601–608 (1988).
4) Ohkubo, M. and Fujimura, S. Loss of activity of an N^1-methylnicotinamide oxidase in livers of rats fed 2-acetylaminofluorene. *Cancer Res.*, **38**, 697–702 (1978).
5) Ohkubo, M., Sakiyama, S. and Fujimura, S. Purification and characterization of N^1-methylnicotinamide oxidase I and II separated from rat liver. *Arch. Biochem. Biophys.*, **221**, 534–542 (1983).
6) Chang, M. L. W. and Jonson, B. C. N-Methyl-4-pyridone-5-carboxamide as a metabolite of nicotinic acid in man and monkey. *J. Biol. Chem.*, **236**, 2096–2098 (1961).
7) Clark, B. R., Murai, J. T., Pomeranz, A., Mills, P. A., Halpern, R. M. and Smith, R. A. Altered distribution and excretion of N^1-methylnicotinamide in rats with Walker 256 carcinosarcoma. *Cancer Res.*, **35**, 1727–1733 (1975).
8) Hanazawa, Y., Sato, K., Kuriyama, A., Ohtuka, H., Moriyama, Y. and Fujimura, S. Purification of nicotinamide methyltransferase in the liver of the mouse bearing Ehrlich ascites tumor (abstract). *Proc. Jpn. Biochem. Soc.*, **56**, 1062 (1984) (in Japanese).
9) Hanazawa, Y., Sato, K., Kuroiwa, N., Moriyama, Y. and Fujimura, S. Purification and characterization of nicotinamide methyltransferase in the liver of the mouse bearing Ehrlich ascites tumor (abstract). *Proc. Jpn. Biochem. Soc.*, **57**, 1200 (1985) (in Japanese).
10) Greenstein, J. P. and Andervont, H. B. The liver catalase activity of tumor-bearing mice and the effect of spontaneous regression and removal of certain tumors. *J. Natl. Cancer Inst.*, **2**, 345–355 (1942).
11) Matuo, Y., Nishikawa, K., Horio, T. and Okunuki, K. Effect of growth of rhodamine sarcoma in rat on some

liver enzyme activities. *Gann,* **59,** 299–316 (1968).
12) Arigiles, J. M. and Soriano, J. L. The effects of tumour necrosis factor-α (cachectin) and tumour growth on hepatic amino acid utilization in the rat. *Biochem. J.,* **266,** 123–126 (1990).
13) Kepper, D., Lesch, R., Reutter, W. and Decker, K. Experimental hepatitis induced by D-galactosamine. *Exp. Mol. Pathol.,* **9,** 279–290 (1968).
14) Tsuda, H., Masui, T., Ikawa, E., Imaida, K. and Ito, N. Compared promoting potential of D-galactosamine, carbon tetrachloride and partial hepatectomy in rapid induction of preneoplastic liver lesions in the rat. *Cancer Lett.,* **37,** 163–171 (1987).
15) Recknagel, R. O. Carbon tetrachloride hepatotoxicity. *Pharmacol. Rev.,* **19,** 145–208 (1967).
16) Gaus, J. H. and Korson, R. Liver nuclear DNA synthesis in mice following carbon tetrachloride administration or partial hepatectomy. *Proc. Soc. Exp. Biol. Med.,* **175,** 237–242 (1984).
17) Michalopoulos, G., Cianciulli, H. D., Novotny, A. R., Kligeman, A. D., Storm, S. C. and Jirtre, R. L. Liver regeneration studies with rat hepatocytes in primary culture. *Cancer Res.,* **42,** 4673–4682 (1982).
18) Hoshino, J., Kuhne, U. and Kröger, H. Methylation of nicotinamide in rat liver cytosol and its correlation with hepatocellular proliferation. *Biochim. Biophys. Acta,* **719,** 518–526 (1982).
19) Swiatek, K. R., Simon, L. N. and Chao, K-L. Nicotinamide methyltransferase and S-adenosylmethionine: 5′-methylthioadenosine hydrase. Control of transfer ribonucleic acid methylation. *Biochemistry,* **12,** 4670–4674 (1973).
20) Kutnink, M. A., Vannucchi, H. and Sauberlich, H. E. A simple high performance liquid chromatography procedure for the determination of N^1-methylnicotinamide in urine. *J. Liq. Chromatogr.,* **7,** 969–977 (1984).
21) Kan, M., Hung, J., Mansson, P. E., Yasumitu, H., Carr, B. and McKeehan, W. L. Heparin-binding growth factor type 1 (acidic fibroblast growth factor): a potential biphasic autocrine and paracrine regulator of hepatocyte regeneration. *Proc. Natl. Acad. Sci. USA,* **86,** 7432–7436 (1989).
22) Lowry, O. H., Rosebrough, N. J., Farr, A. I. and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193,** 265–275 (1951).
23) Morley, C. D. and Boyer, J. L. Stimulation of hepatocellular proliferation by a serum factor from thioacetamide-treated rats. *Biochim. Biophys. Acta,* **477,** 165–176 (1977).
24) Hoshino, J., Kuhne, U. and Kröger, H. Enhancement of DNA synthesis and cell proliferation by 1-methylnicotinamide in rat liver cells in culture: implication for its *in vivo* role. *Biochem. Biophys. Res. Commun.,* **105,** 1446–1452 (1982).
25) Richman, R. A., Claus, T. H., Pilkis, S. J. and Friedman, D. L. Hormonal stimulation of DNA synthesis in primary cultures of adult rat hepatocytes. *Proc. Natl. Acad. Sci. USA,* **73,** 3589–3593 (1976).
26) Slater, T. F., Cheeseman, K. H., Benedetto, C., Collins, M., Emery, S., Maddix, S. P., Nodes, J. T., Proudfoot, K., Bouton, G. W. and Ingold, K. U. Studies of the hyperplasia ('regeneration') of the liver following partial hepatectomy. *Biochem. J.,* **265,** 51–59 (1990).
27) Hirvonen, A., Immonen, T., Leinonen, P., Alhonen-Hongisto, L., Janne, O. and Janne, J. Effect of dexamethasone on the activity and expression of ornithine decarboxylase in rat liver and thymus. *Biochim. Biophys. Acta,* **950,** 229–233 (1988).
28) Nakamura, T., Shinno, H. and Ichihara, A. Insulin and glucagon as a new regulator system for tryptophan oxygenase activity demonstrated in primary cultured rat hepatocytes. *J. Biol. Chem.,* **255,** 7533–7535 (1980).
29) Nakamura, T., Noda, C. and Ichihara, A. Two phase regulation of tyrosine aminotransferase activity by insulin in primary cultured hepatocytes of adult rats. *Biochem. Biophys. Res. Commun.,* **99,** 775–780 (1981).
30) Horn, Y., Beal, S. L. Walach, N., Lubich, L. S., Spigel, L. and Marton, L. J. Further evidence for the use of polyamines as biochemical markers for malignant tumors. *Cancer Res.,* **42,** 3248–3251 (1982).
31) Akinson, R. J., Lowry, W. S. and Strain, P. An analysis of the SCM test in cancer diagnosis. *Cancer,* **52,** 91–100 (1983).
32) Ashall, F., Bramwell, M. E. and Harris, H. A new marker for human cancer cells. *Lancet,* **ii,** 1–6 (1982).
33) Bagshawe, K. D. Tumor markers-Where do we go from here? *Br. J. Cancer,* **48,** 167–175 (1983).
34) Laurence, D. J. R. General cancer tests. *Tumor Biol.,* **8,** 75–77 (1987).
35) Shibata, K. Nutritional factors affecting the activity of liver nicotinamide methyltransferase and urinary excretion of N^1-methylnicotinamide in rats. *Agric. Biol. Chem.,* **50,** 1489–1493 (1986).
36) Shibata, K., Nomamoto, R. and Iwai, K. Effect of dietary protein levels on the urine excretion of nicotinamide and its metabolites in rats. *Agric. Biol. Chem.,* **52,** 1765–1769 (1988).