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Urinary Putrescine and Plasma Lactate Dehydrogenase as Markers of Experimental Adenocarcinoma Growth*

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Abstract—*The objective of this study was to assess, in a controlled experimental system, whether changes in urinary polyamine excretion reflect growth of a solid tumor, and whether such changes are dependent on the location of the tumor. A transplantable* N-*methyl*-N′-*nitro*-N-*nitrosoguanidine-induced adenocarcinoma* (NG-W1) *was grown intrahepatically or s.c. in male Wistar rats. Tumor size was measured at various time intervals and blood samples and* 24-hr *urines were collected. Analyses of* 24-hr *urines for their polyamine content, using a thin-layer chromatographic method for the separation of the dansylated polyamine derivatives, revealed a positive correlation between the* 24-hr *putrescine output and the increasing tumor burden. Notably, the* 24-hr *urine volume was found to parallel the increase in* 24-hr *putrescine excretion. The* 24-hr *urinary excretion of spermidine remained constant throughout tumor growth as did that of creatinine. Analyses of blood plasma for its lactate dehydrogenase activity, using a spectrophotometric technique, indicated no relationship between plasma lactate dehydrogenase activity and tumor burden, except at a large tumor mass. The increase in* 24-hr *urinary putrescine excretion in rats harboring an intrahepatic tumor preceded that which occurred in rats harboring an s.c. tumor. This time lapse was attributable to the fact that the tumor growth characteristics, including vascularization, differed between the two locations; intrahepatic tumors having more extensive growth and better vascularization than s.c. tumors. The fact that the urine putrescine excretion, particularly in a site such as the liver, is an early marker of tumor progression, indicates that this polyamine may be helpful in appraising relapse and recurrence of cancer.*

INTRODUCTION

THE POLYAMINES putrescine, spermidine and spermine are present in elevated levels in physiological fluids of patients with cancer [1–9]. It was originally believed that the quantitative analysis of these amines would become a useful adjunct in the diagnosis of cancer. However, the initial enthusiasm for polyamine analysis in general cancer screening has subsided, because of the number of false positive and false negative results that have been obtained in the general population. Nevertheless, many potential uses of polyamine analysis remain. In cases of suspected cancer, polyamine analysis may serve as a diagnostic aid in conjunction with established diagnostic techniques. Polyamine analysis may also provide valuable information in long-term evaluation of tumor growth or regression, and in short-term evaluation of the efficacy of a specific course of therapy.

The recent study by Marton *et al.* [9] proves that cerebrospinal fluid polyamines have a prognostic value in patients with medulloblastoma. Fifteen of the 16 patients studied showed absolute correlation between polyamine concentration in cerebrospinal fluid and the eventual clinical picture. In fact, the increase in polyamine levels preceded changes in the other parameters used in evaluating the patient status; namely, neurological picture,

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computerized tomography, radionuclide scan, myelography, and cerebrospinal fluid cytology.

In view of the apparent possibility of using polyamine analyses in various areas of cancer monitoring, the objective of the present investigation was to assess, in a controlled experimental system, whether changes in urinary polyamine excretion reflect growth of a solid tumor, and whether such changes are dependent on the location of the tumor. Because the plasma lactate dehydrogenase (LDH; EC 1.1.1.27) activity was found to be directly related to tumor mass [10–12] and because the polyamine level in physiological fluids was found to exhibit a high positive correlation with the activity of tumor-derived LDH [13], the plasma LDH activity was analyzed in parallel.

MATERIALS AND METHODS

Tumor growth

Male Wistar rats weighing approximately 170 g were used as tumor hosts. They were provided with standard laboratory feed and tap water, and were allowed to eat and drink *ad libitum*. One group of rats, subjected to ether anesthesia, received an intrahepatic inoculation of a transplantable *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced adenocarcinoma (NG-W1) cell suspension in the central lobe of the liver through a midline incision. Another group of rats received an s.c. inoculation of the same tumor cell suspension in the back. Each inoculum corresponded to 1.0×10^6 tumor cells.

At various times after inoculation, the size of each tumor was measured on ether anesthetized rats by vernier calipers at two perpendicular axes. Since the NG-W1 tumor has not been observed to metastasize, these measurements permitted an estimation of the actual tumor volume. The tumor volume was estimated according to the formula:

$$V = \frac{\pi \text{ (mean diameter)}^3}{6} \quad [14].$$

In order to maintain constant experimental conditions, rats that served as donors of plasma and urine were not subjected to tumor size measurements.

Collection and preparation of urine and blood

Twenty-four hour urines were collected in acid in Econo Model E-1100 metabolism units (Maryland Plastics, New York) modified to minimize evaporation. After measuring the total urine volume, aliquot samples were centrifuged at 1000 *g* for 10 min. The supernatants were stored at −20°C until creatinine and polyamine analyses were performed.

Blood samples, obtained from the tail of the rats, were collected in heparinized test tubes and immediately centrifuged at 2000 *g* for 30 min. The blood plasma was stored at −20°C until assayed for total LDH activity.

Creatinine determination

Urinary creatinine concentrations were determined colorimetrically with a Gemsaec Fast-Analyzer according to the Jaffé reaction, in which creatinine is reacted with an alkaline picric acid solution to form an amber-colored compound. The absorbance of this compound was measured at 520 nm. The Department of Clinical Chemistry at the University of Lund is gratefully acknowledged for running these analyses.

Polyamine analysis

Polyamines were quantitatively measured in 1.00 ml aliquots of the 24-hr urine collections using a thin-layer chromatographic method, the principle of which has been described by Seiler [15]. The details of the method as applied in our laboratory have been described [7, 16]. In the present study, however, the thin-layer chromatographic plate was developed in an elution medium that was slightly altered in its proportions of chloroform and triethylamine (1:1 by volume). The plate was not sprayed with the mixture that was previously used to increase and stabilize the fluorescence. Better reproducibility and sufficient sensitivity was achieved without spraying.

LDH assay

The total LDH activity in plasma was determined spectrophotometrically [17]. The substrate solution consisted of 2 M lithium lactate, 0.05 M Tris buffer and distilled water in proportions of 1:1:8 (pH 8.9). Prior to analysis, 0.67 mg of NAD was added per ml of the substrate solution, and this reaction mixture was pre-heated to 30°C. A 50 µl aliquot of plasma was added to 3 ml of the reaction mixture. The conversion of NAD^+ to NADH at 30°C over a 1-min-period was measured at 340 nm, and the LDH activity expressed as enzyme units per ml.

Statistical analysis

The significance of the differences between control and experimental groups were assessed statistically by Student's *t*-test, with the level set at 5% ($P < 0.05$).

RESULTS

Tumor growth

The intrahepatic and the s.c. tumors showed roughly the same growth rate and grew to about the same size over the initial 14 days of the experiment (Fig. 1). After this period there was a marked increase in tumor burden for rats bearing an intrahepatic tumor as compared to those bearing an s.c. tumor. This difference lasted until Day 24, at which time rats with intrahepatic tumors began to succumb to their tumors.

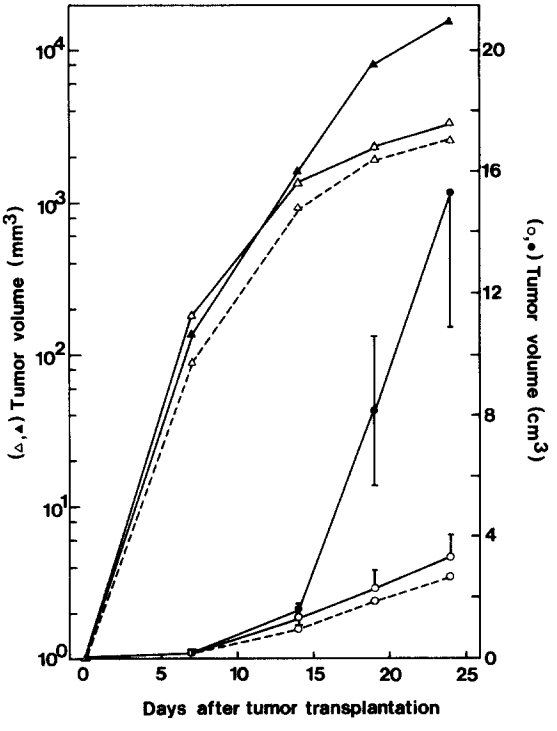

Fig. 1. NG-W1 adenocarcinoma growth following intrahepatic (filled symbols) or s.c. (open symbols) transplantation of 1×10^6 *tumor cells. Tumor size was monitored semiquantitatively by caliper measurements in two dimensions and the volume estimated according to the formula* $V = \frac{\pi \cdot (\text{mean diameter})^3}{6}$. *Triangular symbols refer to the logarithmic scale and circular symbols refer to the linear scale.* ●, *Mean* ± *S.E.M.* (n = 5); ○, *mean* ± *S.E.M.* (n = 4). *The actual s.c. tumor growth was estimated by subtracting a constant of* 1.5 mm, *corresponding to the double skin thickness, from each dimension measurement (dashed lines).*

Urine volume

The 24-hr urine volume excreted from rats bearing an intrahepatic tumor was found to increase steadily until a maximum volume was reached on Day 17 (Fig. 2A). The urinary output stayed at about this volume during the rest of the experimental period. The increase in urine volume recorded was found to be statistically significant ($P < 0.05$) from Day 7 and on ($P < 0.01$ on Days 11 and

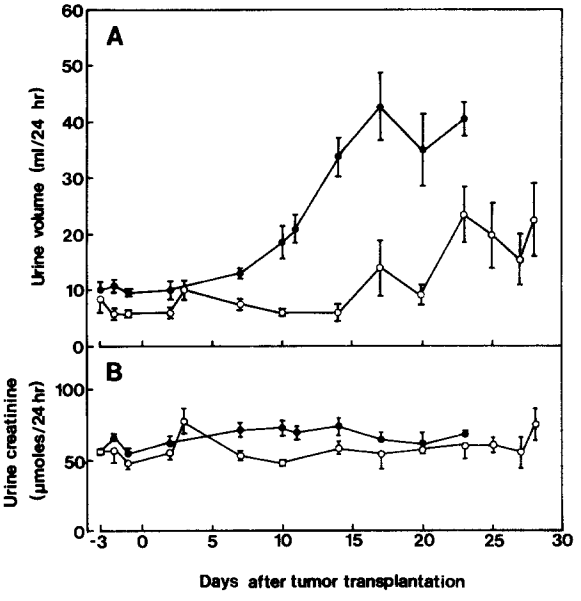

Fig. 2. Twenty-four hour urine volume (A) and 24 hr *creatinine excretion (B) at various times of adenocarcinoma growth. Intrahepatic* (●); *s.c.* (○) *transplantation.* ●, *Mean* ± *S.E.M.* (n = 5); ○, *mean* ± *S.E.M.* (n = 4).

20; $P < 0.001$ on Days 14, 17 and 23). Towards the end of the experiment the 24-hr urine volume was roughly 40 ml as compared to 10 ml at the time of tumor transplantation. The largest 24-hr urine volume excreted was 58 ml.

The 24-hr urinary output from rats bearing an s.c. tumor remained at a normal level during the first 14 days of growth (Fig. 2A), despite the fact that the growth rate of this tumor approximated that of the intrahepatic tumor (Fig. 1). With continued growth of the s.c. tumor, there was a marked increase in tumor burden and in urine volume. A significant increase in urine volume ($P < 0.05$) was observed from Day 23 and on. Towards the end of the experimental period the 24-hr urinary output reached a level that was roughly 4-fold higher than that observed at the time of tumor transplantation.

Urinary creatinine excretion

The 24-hr urinary creatinine excretion remained essentially constant throughout the experimental period, both for rats with an intrahepatic tumor and for rats with an s.c. tumor (Fig. 2B).

Urinary polyamine excretion

The 24-hr urinary excretion of putrescine increased continuously from the time of intrahepatic tumor transplantation until a maximum level was reached on Day 17, with a major increase occurring between Days 10 and

14 (Fig. 3A). The increase in putrescine excretion was statistically significant ($P<0.05$) from Day 11 and on ($P<0.01$ on Days 14, 17 and 23). Towards the end of the experiment the amount of putrescine excreted remained essentially constant at a level that was 4- to 5-fold higher than at the time of tumor transplantation.

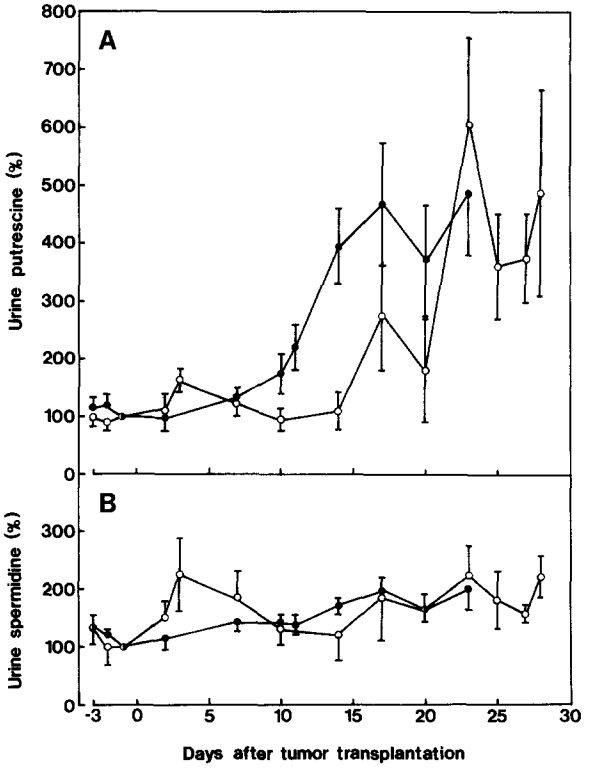

Fig. 3. Twenty-four hour urinary excretion of putrescine (A) and spermidine (B) at various times of adenocarcinoma growth. Intrahepatic (●); s.c. (○) transplantation. One hundred per cent (Day 0) corresponds to 1.68 ± 0.22 *and* 0.50 ± 0.15 *μmoles of putrescine per* 24 hr *in rats with intrahepatic and s.c. tumors, respectively; and* 1.65 ± 0.21 *and* 0.49 ± 0.11 *μmoles of spermidine per* 24 hr *in rats with intrahepatic and s.c. tumors, respectively.*
●, *Mean*$\pm$*S.E.M.* (n=5); ○, *mean*$\pm$*S.E.M.* (n=4).

In rats harboring an s.c. tumor the 24-hr urinary putrescine output remained roughly constant for about 14 days (Fig. 3A). Subsequently, and in parallel with the increase in tumor burden, there was a marked increase in the rate of putrescine excretion. A significant increase in putrescine excretion ($P<0.05$) was observed from Days 23 through 27 ($P<0.01$ on Days 25 and 27). At the end of the experimental period the 24-hr urinary putrescine excretion was 4- to 6-fold greater than at the time of tumor transplantation. This increase in putrescine output is equivalent to that observed for rats bearing an intrahepatic tumor. Comparing Figs. 2A and 3A, one finds that the increase in urinary putrescine excretion was greater than the increase in urine volume in rats with an s.c. tumor, whereas these parameters changed in parallel in rats with an intrahepatic tumor.

The 24-hr urinary spermidine output did not change significantly during the experimental period, neither when the tumor grew intrahepatically nor when it grew s.c. (Fig. 3B). Thus, the urinary excretion of spermidine was similar to that of creatinine. The 24-hr urinary spermine excretion was not recorded, because its level was barely detectable.

When comparing the absolute amounts of the 24-hr urinary polyamines excreted from the two groups of rats that were used as tumor (intrahepatic or s.c.) hosts, one finds that there is a significant difference between the basal levels (shown in the legend to Fig. 3). A corresponding difference is true for the 24-hr urine volume (Fig. 2A). In fact, there is proportionality between putrescine output and urine volume throughout adenocarcinoma growth, as is evident from Fig. 5 in which these parameters are linearly fitted. Whether the difference in the slopes of the linear regression lines is a function of the site of tumor growth or is due to other factors is not known at present.

Plasma LDH activity

The plasma LDH activity remained at a low and constant level for at least 11 days in rats with an intrahepatic or an s.c. tumor (Fig. 4). After this period the plasma LDH activity showed about a 7-fold increase in rats harboring an intrahepatic tumor, whereas it remained unchanged in rats with an s.c. tumor. The increase in plasma LDH activity was statistically significant on Day 17 ($P<0.05$) and on Day 23 ($P<0.001$).

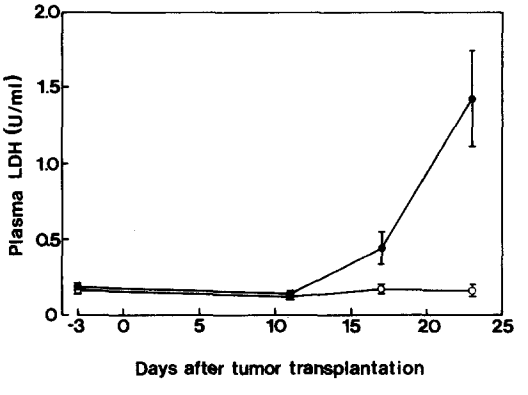

Fig. 4. Total plasma LDH activity at various times of adenocarcinoma growth. Intrahepatic (●); s.c. (○) transplantation.
●, *Mean*$\pm$*S.E.M.* (n=5); ○, *mean*$\pm$*S.E.M.* (n=4).

DISCUSSION

Because of the difficulty in estimating the amount of tumor mass in clinical cancer, no attempts have been made to compare the urine levels for the polyamines with total tumor burden. Therefore, to facilitate the comparison of these parameters, we have used an experimental tumor model that permits serial estimations of tumor volume during the course of intrahepatic as well as s.c. tumor growth.

Only one of the urinary polyamines (putrescine) was found to reflect the changes observed in tumor mass. In rats with an intrahepatic adenocarcinoma there was a close correspondence between the increase in tumor mass and the increase in urinary putrescine excretion. In rats with an s.c. adenocarcinoma, however, the increase in putrescine excretion was markedly delayed. A possible explanation for this delay is that a certain tumor size (about $2\,cm^3$) is required for the putrescine excretion to increase, and that this size is reached at a later time in rats with an s.c. tumor. In fact, the size of the s.c. tumor may be somewhat overestimated, because skin thickness makes a certain contribution to the diameter measurements. By subtracting an appropriate constant (1.5 mm double skin thickness) from each dimension measurement [14], a growth curve is obtained that may better represent the actual s.c. tumor growth (Fig. 1). Results obtained from rats with 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas are consistent with the notion that about a $2\,cm^3$ tumor size is required to produce a significant increase in the urinary putrescine excretion [18].

In mice with experimental s.c. tumors induced by a single injection of 3,4-benzopyrene, the urinary putrescine excretion was found to increase at a very early stage of carcinogenesis and to change in parallel with the tumor mass, whereas the spermidine and spermine excretion showed no significant change [19]. In rats with experimental tumors of the glandular stomach, induced by multiple intragastric administrations of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, the urinary excretion of putrescine, but not that of spermidine and spermine, was markedly elevated [3]. In fact, a majority of studies of physiological fluid polyamines, including clinical ones [3–5, 9], suggest that putrescine is a better marker of tumor growth than are spermidine and spermine. In some cases, however, a combination of putrescine and spermidine may further enhance the usefulness of polyamine analyses [4].

It is evident from an analysis of the growth data for the NG-W1 adenocarcinoma that the urinary putrescine excretion correlates to the total mass (or tumor burden) and not to the growth rate of the tumor. The growth rate was maximal immediately after tumor transplantation and subsided steadily with increasing tumor mass.

Interestingly, the 24-hr putrescine output shows a high positive correlation with the 24-hr urine volume, with correlation coefficients of 0.94 and 0.86 for rats with an intrahepatic and an s.c. tumor, respectively (Fig. 5). The possibility of an osmotic influence of putrescine and other metabolites originating from the tumor and producing the diuresis will be investigated.

Apparently, the increase in urine volume and in putrescine excretion is not a function of the increasing tumor mass only. Despite the fact that the intrahepatic tumor mass almost doubled between Days 14 and 24, both the output of urine and putrescine remained at a plateau level. Whether this situation causes an accumulation of putrescine in the serum remains to be determined. Interestingly, this is the period during which the plasma LDH level increases in rats harboring an intrahepatic tumor. Moreover, serum polyamines and LDH have been found to change in parallel in a heterologous system of ascites tumor regression [13].

It is noteworthy that the total plasma LDH activity remained at a normal level during the initial period of rapid adenocarcinoma growth. In rats with an intrahepatic tumor, the plasma LDH activity showed a substantial increase from Day 17 and on. This period of tumor growth is characterized by a moderate growth rate but a marked increase in tumor burden. In rats with an s.c. tumor, the plasma LDH activity exhibited a normal level throughout s.c. tumor growth. It should be pointed out, however, that the 24-day s.c. tumor had a size comparable to that of a 15-day intrahepatic tumor, a tumor that produced only a small increase in plasma LDH activity. Therefore, the present results may indicate that the plasma LDH activity is a useful marker of tumor growth only when the tumor has reached a rather large mass. This is in reasonable agreement with the finding by DiPersio *et al.* [12] that human LDH becomes detectable in the plasma of athymic nude mice when a human tumor xenograft reaches a size of 200–800 mm^3. Subsequently, the

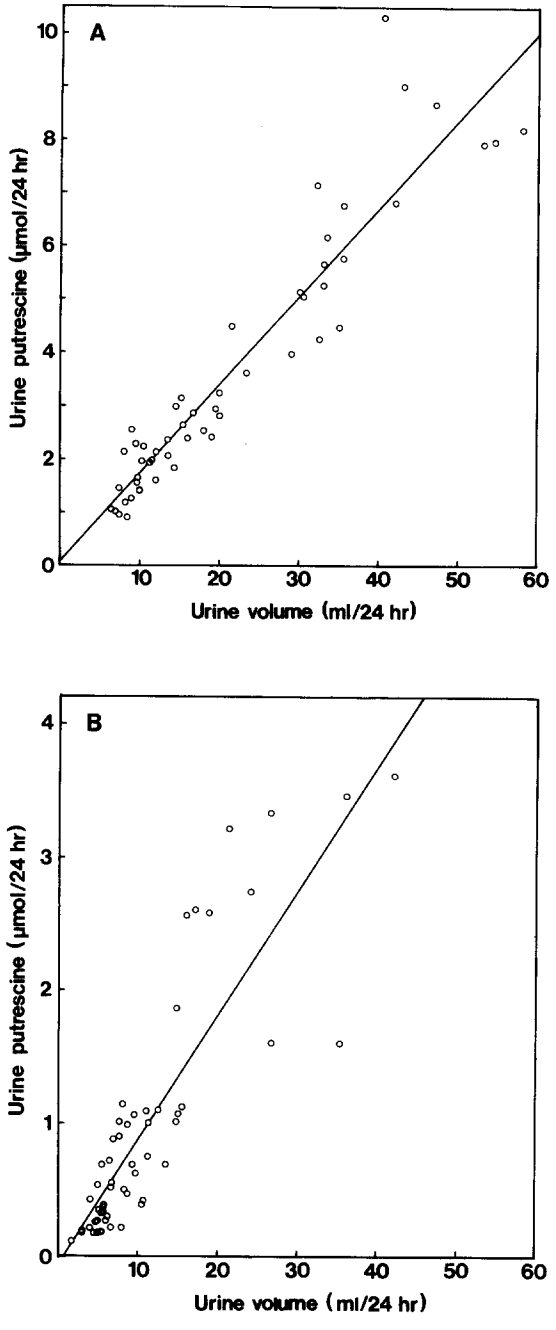

Fig. 5. Correlation between 24-hr *urinary putrescine excretion and* 24-hr *urine volume in rats harboring an intrahepatic (A) or an s.c. (B) tumor. The linear regression lines are least-square fits to each set of data points. The correlation coefficients are* 0.94 *and* 0.86, *respectively. Each point has been obtained by combining the urinary putrescine output (experimental data as shown in Fig.* 3A) *with the corresponding urine volume (Fig.* 2A) *at various times after tumor transplantation. These relationships are thus representative for the entire growth period of the intrahepatic as well as the s.c. rat adenocarcinoma.*

LDH activity increased in parallel with tumor size [12]. Furthermore, we cannot exclude the possibility that the increase in plasma LDH activity observed in rats harboring an intrahepatic NG-W1 adenocarcinoma might be a consequence of local damage to the liver. Conceivably, plasma LDH could be a marker of liver involvement rather than tumor mass. Unfortunately, the tissue LDH isozyme profiles for the NG-W1 adenocarcinoma and the normal liver were not sufficiently different from that of normal blood plasma to render a change in the plasma LDH isozyme pattern. This would have permitted us to disclose the source of the LDH accumulating in the plasma of rats with an intrahepatic tumor.

Despite the correlation between tumor burden and urinary putrescine excretion (and plasma LDH activity) it is apparent that these parameters become elevated at an earlier time in rats with an intrahepatic tumor than in rats with an s.c. tumor. Conceivably, there is a more rapid release of polyamines, LDH and other cell components from the intrahepatic than from the s.c. tumor because of its better vascularization. Thus, when the size of an s.c. NG-W1 adenocarcinoma exceeds 1.5 g (by Day 14) its blood flow is considerably slower than that of an intrahepatic adenocarcinoma of a comparable size. In tumors weighing 2.5–3.0 g the flow rate ($ml \cdot min^{-1} \cdot g^{-1}$) was 0.52 and 0.16 in the periphery and center of the intrahepatic tumor, compared to 0.14 and 0.05 in the s.c. tumor [20]. The fact that the intrahepatic tumor has a better vascularization than the s.c. tumor is probably also the reason for its greater growth potential.

Consistent with the idea presented above are the results of Kyriazis *et al.* [11]. In a study of human tumors grown in nude athymic mice, i.p. tumors were found to produce a much larger increase in human plasma LDH than did the less vascularized s.c. tumors [11]. Growth of the s.c. tumor was considerably slower compared to that of the i.p. tumor, and a large part of the s.c. tumor was necrotic whereas necrosis was minimal in i.p. growing tumors.

The underlying mechanism for the release of intracellular polyamines, resulting in elevated extracellular levels, remains elusive. Cultured BHK 21/C13 cells were found to release spermidine into the culture medium when their growth rate was slowed down by serum depletion [21] or 6-thioguanosine treatment [22]. On the contrary, the extracellular putrescine content showed a positive correlation with the labeling index of Burkitt's lymphoma cells in culture [23], and the urinary excretion of putrescine in multiple myeloma patients receiving chemotherapy was correlated with the *in vitro* labeling index of marrow plasma cells [6]. Yet in other studies

physiological fluid putrescine and spermidine were found to be related to the extent of tumor cell death [13, 24].

The present data do not give any further information about the mechanism behind the increase in urinary putrescine excretion occurring during tumor growth, but they reveal an interesting relationship between putrescine output and tumor burden. This suggests that urine putrescine determinations may be valuable in monitoring the progression of a tumor. The fact that urine putrescine excretion, particularly in a site such as the liver, is an early indicator of tumor progression, indicates that this polyamine may be helpful in appraising relapse or recurrence of cancer prior to overt evidence of tumor growth by existing clinical means. This is in analogy with what has been observed for cerebrospinal fluid polyamines in patients with brain tumors [9].

REFERENCES

1. Dreyfuss F, Chayen R, Dreyfuss G, Dvir R, Ratan J. Polyamine excretion in the urine of cancer patients. *Isr J Med Sci* 1975; **11:** 785.
2. Waalkes TP, Gehrke CW, Bleyer WA, Zumwalt RW, Olweny CLM, Kuo KC, Lakings DB, Jacobs SA. Potential biologic markers in Burkitt's lymphoma. *Cancer Chemother Rep* Part 1, 1975; **59:** 721.
3. Fujita K, Nagatsu T, Maruta K, Ito M, Senba H, Miki K. Urinary putrescine, spermidine, and spermine in human blood and solid cancers and in an experimental gastric tumor of rats. *Cancer Res* 1976; **36:** 1320.
4. Marton LJ, Heby O, Levin VA, Lubich WP, Crafts DC, Wilson CB. The relationship of polyamines in cerebrospinal fluid to the presence of central nervous system tumors. *Cancer Res* 1976; **36:** 973.
5. Townsend RM, Banda PW, Marton LJ. Polyamines in malignant melanoma: Urinary excretion and disease progress. *Cancer* 1976; **38:** 2088.
6. Durie BGM, Salmon SE, Russell DH. Polyamines as markers of response and disease activity in cancer chemotherapy. *Cancer Res* 1977; **37:** 214.
7. Heby O, Andersson G. Simplified micro-method for the quantitative analysis of putrescine, spermidine and spermine in urine. *J Chromatogr* 1978; **145:** 73.
8. Nishioka K, Romsdahl MM, Fritsche HA Jr., Johnston DD. Polyamines in sera of patients with solid tumors. In: Campbell RA, Morris DR, Bartos D, Daves GD, Bartos F, eds. *Advances in Polyamine Research.* New York: Raven Press, 1978: Vol. 2, p. 265.
9. Marton LJ, Edwards MS, Levin VA, Lubich WP, Wilson CB. Predictive value of cerebrospinal fluid polyamines in medulloblastoma, *Cancer Res* 1979; **39:** 993.
10. Pesce AJ, Bubel HC, DiPersio L, Michael JG. Human lactic dehydrogenase as a marker for human tumor cells grown in athymic mice. *Cancer Res* 1977; **37:** 1998.
11. Kyriazis AP, DiPersio L, Michael GJ, Pesce AJ, Stinnett JD. Growth patterns and metastatic behavior of human tumors growing in athymic mice. *Cancer Res* 1978; **38:** 3186.
12. DiPersio L, Kyriazis AP, Michael JG, Pesce AJ. Monitoring the therapy of human tumor xenografts in nude mice by the use of lactate dehydrogenase. *J Natl Cancer Inst* 1979; **62:** 375.
13. Andersson G, Bengtsson G, Albinsson A, Rosén S, Heby O. Increased polyamine concentration and tumor-derived lactate dehydrogenase activity in physiological fluids during regression of a heterologous tumor transplant. *Cancer Res* 1978; **38:** 3938.
14. Steel GG. *Growth Kinetics of Tumours.* Oxford: Clarendon Press, 1977.
15. Seiler N. Use of the dansyl reaction in biochemical analysis, *Methods Biochem Anal* 1970; **18:** 259.
16. Österberg S, Rosén S, Heby O. Urinary polyamine excretion during the menstrual cycle. *Clin Chem* 1978; **24:** 769.
17. Bengtsson GB, Karlsson BW. Lactate and malate dehydrogenase activities during the ontogeny of the Mongolian gerbil, rat, mouse and rabbit. *Comp Biochem Physiol* 1976; **54B:** 501.

18. Andersson G, Albinsson A, Anehus S, Heby O. Urinary putrescine as a marker of tumor mass during growth and regression of 7,12-dimethylbenz(a)anthracene-induced rat mammary carcinoma. *Proc Am Ass Cancer Res* 1980; **21:** 66.
19. Fujita K, Nagatsu T, Shinpo K, Maruta K, Takahashi H, Sekiya A. Increase of urinary putrescine in 3,4-benzopyrene carcinogenesis and its inhibition by putrescine. *Cancer Res* 1978; **38:** 3509.
20. Sundqvist K, Hafström L, Persson B. Measurements of total and regional tumor blood flow and organ blood flow using $^{99}Tc^{m}$ labelled microspheres. *Eur Surg Res* 1978; **10:** 433.
21. Melvin MAL, Keir HM. Polyamine metabolism in BHK 21/C13 cells: Loss of spermidine from cells following transfer to serum-depleted medium. *Exp Cell Res* 1978; **111:** 231.
22. Melvin MAL, Melvin WT, Keir HM. Excretion of spermidine from BHK 21/C13 cells exposed to 6-thioguanosine. *Cancer Res* 1978; **38:** 3055.
23. Woo KB, Perini F, Sadow J, Sullivan C, Funkhouser W. Polyamine content and release during the proliferation of Burkitt's lymphoma cells *in vitro*. *Cancer Res* 1979; **39:** 2429.
24. Heby O, Andersson G. Tumour cell death: The probable cause of increased polyamine levels in physiological fluids. *Acta Pathol Microbiol Scand* [A] 1978; **86:** 17.