◎ 1999 The Japan Mendel Society Cytologia 64: 259-267, 1999

Effect of Cisplatin on the Lactate Dehydrogenase Activity and

its Isozyme Pattern in Dalton's Lymphoma Bearing Mice

Surya B. Prasad * and Anirudha Giri **

Cell and Tumor Biology Laboratory, Department of Zoology, North-Eastern Hill University, Shillong-793 022, India

Accepted May 7, 1999

Summary Lactate dehydrogenase (LDH) activity and its isozyme patterns were determined in var-
ious tissues of normal, Dalton's lymphoma (DL) bearing and cisplatin treated tumorous mice. Tumor-bearing hosts showed about two fold higher serum LDH activity than that in the normal animals and following cisplatin treatment (8 mg/kg body wt, i.p.) for 1-4. days, serum LDH activity further in-creased. In kidney, as compared to normal mice, there was no significant change in the enzyme activ-ity in tumor bearing hosts, but liver LDH activity increased in tumorous condition. After cisplatin treatment overall 20-30% decrease in the activity was noted in kidney and liver, with slight increase on the day 2 of treatment. LDH isozyme analysis revealed that in serum and kidney, all the five isozyme constituents were present, whereas, in liver and ascites tumor supernatant only LDH-3, -4 and -5 were observed with the predominance of LDH-5. In liver, after cisplatin treatment LDH-3 and -4 expression gradually decreased. In DL cells, LDH-5 was the only isozyme form present and after cisplatin treatment its activity increased.
Thus, it is suggested that LDH activity is definitely affected in the tissues of tumor bearing hosts and during tumor regression after cisplatin treatment. The changes in LDH activity could be very useful parameter in malignancy and cisplatin-mediated chemotherapy against murine Dalton's lymphoma in particular and cancer in general. LDH isozyme patterns revealed the presence of tissue specificity of different isozymes, with only LDH-5 in tumor cells and appearance of some specific isozyme variant, named here as LDH-T, in the serum of tumor bearing hosts.

Cis-diamminedichloroplatinum (II), commonly known as cisplatin, is a leading chemothera-
peutic drug being used effectively against a variety of malignancies (Kociba et al. 1970, Rosenberg
1985, Prasad and Giri 1994). However, its therapeutic efficacy is limited due to its side effects
which include nephrotoxicity, neuroxicity, gastrointestinal toxicity, ototoxicity (Prestayko et al.
1979, Roberts et al. 1988), embryotoicity (Keller and Aggarwal 1983), mutagenicity (Giri et al.
1998) etc. Many of the properties and biological effects of cisplatin have been well documented
(Rosenberg 1985, Chu 1994, Pil and Lippard 1997) with numerous reports indicating that cellular
DNA could be the primary target in its anticancer activity (Pinto and Lippard 1985, Zamble and
Lippard 1995).
In addition to its interaction with cellular DNA, the changes in various biochemical/enzymatic
parameters, immune response, cell surface etc. have also been observed which led to propose the
involvement of multistep and multilevel effects of cisplatin in the tumor cells/host during cisplatin-
mediated chemotherapy against cancers (Giri 1995). Enzymatic changes have also been implicated
in the Mechanism of action of cisplatin (Aggarwal 1993). Aggarwal and Meara (1996) reported the
cisplatin induced inhibition of variety of dehydrogenases (isocitrate dehydrogenase, ƒÀ-hydroxybu-
tyrate dehydrogenase, glutamate dehydrogenase, malate dehydrogenase, succinate dehydrogenase
and lactate dehydrogenase) in liver and kidney of rats, and suggested that the inhibition of dehydro-
genase activity may be involved as a mechanism behind cisplatin-induced toxicities. Stefanini

* Corresponding author.
* * Present address: Assam University, P.O. Box 63, Silchar-788 001, Assam.

260•@ Surya B. Prasad and Anirudha Giri Cytologia 64

(1985) has discussed the changes in various enzymes in patients with malignancies and suggested that enzymes, isozymes and enzyme variants could be very useful in the detection and evaluation of biomarkers in the malignancies. Enzymes involved in the synthesis of nucleic acid (thymidine ki-nase, uridine kinase), of essential amino acids (phosphoserine phosphatase), or in glycolysis (hex-okinase, aldolase) have been reported to be elevated in malignant tissue (Yesher 1978).
Lactate dehydrogenase (LDH, EC 1.1.1.27), the terminal enzyme in the anaerobic glycolysis, catalyses a reversible reaction of pyruvate to lactate. Increased activity of LDH has been reported in testicular cancer (Lippert and Javadpour 1981), cancerous breast (Stefanini 1985) and pulmonary tissue (Yesher 1978). Isoenzyme or isozyme determinations of the particular enzyme are becoming widely used means of detecting specific tissue specificity/disturbances. LDH exists in five isozyme forms, controlled by two genes which synthesize unique peptides (Cahn et al. 1962, Markert 1963). A single peptide combines with itself forming a tetramer representing the native LDH molecule. The two LDH tetramers, thus formed, are the electrophoretic extremes, i.e. LDH-1 moves farthest toward the anode during electrophoresis (most anodic) and LDH-5 is the extreme cathodic enzyme. These enzyme forms of LDH have been denoted as H4 and M4 where H represents the peptides characteristic of heart muscles and M for the skeletal muscles (Appella and Markert 1961, Cahn et al. 1962). The intermediate isoenzymes LDH-2, LDH-3 and LDH-4 are hybrids formed by random association of H and M subunits into tetramers. Thus, LDH-2, -3 and -4 would contain H3M, H2M2 and HM3 subunits respectively. The accepted nomenclature for LDH isoenzymes is LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5. Rajadhyaksha et al. (1986) reported that LDH isozyme distri-butions were different in the thymocytes of normal and leukemic mice. The thymocytes from leukemic animals displayed very low values of H : M ratio and they suggested that it may serve as a sensitive marker parameter to probe leukemic transformation in murine systems.
The biochemical events that accompany cisplatin-induced growth inhibition and/or cytotoxici-ty, besides binding of the drug to nucleic acids, are largely unknown. Seeing the importance of LDH in malignancy, the present studies were undertaken to investigate the LDH activity and analyse its isozyme patterns in the tissues of normal, tumor bearing and cisplatin treated tumorous mice.

Materials and methods

Chemicals
All the biochemicals used in the assay of LDH, polyacrylamide gel electrophoresis and detec-
tion of LDH isozymes were purchased from Sigma Chemicals Co., U.S.A. Other chemicals utilized in the experiments were of analytical grade. Cisplatin, obtained from Prof. C. L. Litterst of NIH, U.S.A. as a gift, was thoroughly mixed in 0.89% NaCl in darkness 10-15 min before use. Glass double distilled water was always used in the preparation of various solutions.

Tumor maintenance

Ascites Dalton's lymphoma is being maintained in vivo in 10-12 weeks old inbred Swiss albi-
no mice by serial intraperitoneal (i.p.) transplantations of 1 X 107 tumor cells per animal (0.25 ml vol. in phosphate buffered saline, PBS, 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.4). Tumor transplanted hosts survive for 22-24 days.

Cisplatin treatment and LDH assay
Therapeutic dose of cisplatin against malignant tumors has been established to be 8-10 mg/kg body weight (Rosenberg 1985). In present studies single dose of cisplatin (8 mg/kg body wt, i.p.) was administered to four tumor bearing mice on the 10th day post-tumor transplantation which is the log phase of tumor growth. We have noted that with the regression of the Dalton's lymphoma

1999•@ Effect of Cisplatin on the Lactate Dehydrogenase Activity and its Isozyme Pattern 261•@

very little ascites was recoverable from the hosts after 6-7 days of cisplatin treatment (Prasad et al. 1998), so the treatment schedule for 1 to 4 days only was used. After 1, 2, 3, and 4 days of cisplatin treatment (i.e. on 11, 12, 13 and 14th day after tumor transplantation) liver, kidney, blood and tumor were collected. The same tissues were collected from the control tumor-bearing hosts also which had been injected with the same volume of 0.89% NaCl. Sera were separated from the blood sam-ples. Ascites tumor was centrifuged (2500 rpm for 10 min, at 4°C) to separate the tumor cell pellet and ascites tumor sypernatant. Liver, kidney and blood serum were also collected from the normal animals without tumor, treated with 0.89% NaCl only. The cisplatin treatment and enzyme assay was repeated independently four times.
LDH activity was measured in the tissues using Sigma diagnostics enzyme determination kit following the method of Cabaud and Wroblewski (1958). Briefly, to 1 ml of supplied pyruvate sub-strate containing NADH (1 mg/ml), 0.1 ml of six fold diluted serum or tumor supernatant or tissue homogenate (10% in PBS, in case of tumor cells, liver and kidney) was added. It was mixed gently and incubated for 30 min at 37°C. Then, 1 ml of "Sigma colour reagent" (2,4-dinitrophenylhy-drazine, 20 mg/dl in 1N HC1) was added, mixed by swirling and allowed to stand at room tempera-ture. After 20 min, 10 ml of 0.4 N NaOH solution was added and mixed properly. After 10 min the absorbance was read at 525 nm in the spectrophotometer. The change in absorbance (activity) in various treated groups was expressed as a percentage of control. Values given are the average of samples from 4 separate determinations.

Determination of LDH isozyme patterns
The homogenates were centrifuged at 8,000 g for 20 min at 4°C. The supernatant was used for determination of the isoenzyme pattems. LDH isozymes were separated by electrophoresis on verti-cal disc polyacrylamide gels following the method of Davis (1964). 50 ktl of the tissue sample was mixed with equal volume of glycerol and two drops of the marker dye (bromophenol blue, 0.01%), and it was loaded on the top of the polymerized gel in the tube with the help of a syringe. After loading of the samples, the electrode buffer (0.05 M Tris-glycine, pH 8.3) was added gently in the tubes. The electrophoresis was carried at 4°C with the current of 1.5 mA/tube for 15 min, and con-tinued later with 3 mA/tube till the marker dye reached to the bottom of the tubes. The gels were carefully removed and stained for LDH following the method of Rosalki (1974). The staining solu-tion contained 2.5 ml of 1 M Tris-HC1 buffer, pH 8.3, 0.5 ml of 1N lithium lactate, 80 mg of nicoti-namide adenine dinucleotide (NAD), 1.2 mg of phenazine methosulphate (PMS), 0.8 mg of p-ni-troblue tetrazolium chloride (NBT) and 47 ml of water. The gels in the test tubes were completely immersed in the staining solution and incubated for 15 min at 37°C. Violet colour LDH bands ap-peared after incubation. The gels were then rinsed once with fixative (7% acetic acid), stored in fix-ative and photographed.

Results

LDH activity
Serum LDH activity in tumor-bearing animals was found to be more than two fold higher than that of normal animals. Cisplatin treatment of the tumor bearing hosts for 1 to 4 days resulted in further significant increase in the serum enzyme activity (Fig. 1A). In the liver of tumor-bearing hosts also LDH activity increased by about 40% of the normal animals (Fig. 1B), whereas, in the kidney of normal and tumor-bearing mice no significant variation in the enzymatic activity (Fig. 1 C) was noted. Following cisplatin treatment, an overall decrease in the activity was noted in liver and kidney, although, on day 2 of treatment slight increase in the enzymatic activity was also ob-served. (Fig. 1B, C). In the ascites tumor supernatants of 1-2 days cisplatin-treated hosts, LDH ac-tivity was noted to be more than 2-fold higher than that of control (untreated tumorous hosts). How-

262•@ Surya B. Prasad and Anirudha Giri Cytologia 64

A B

C D

E

Fig. 1. Bar charts showing the changes in lactate dehydrogenase (LDH) activity in various tissues of
Dalton's lymphoma (DL)-bearing mice treated with or without cisplatin. A) Serum; note more than two
fold increase in the enzymatic activity in the tumor bearing hosts than that of normal animals. Cisplatin treatment results further increase of serum LDH. B) Liver; as compared to normal mice higher LDH ac-tivity was observed in tumor bearing hosts. Note an overall decrease in LDH activity following cisplatin treatment. C) Kidney; no significant variation in the enzymatic activity was noted between the normal and tumor bearing mice. Following cisplatin treatment, overall decreased LDH activity was observed, ex-cept on the day 2 of treatment. D) Ascites supernatant; contrary to DL cells, enzymatic activity increased in the ascites supernatant following cisplatin treatment of the DL. Note the presence of about 2 fold in-creased activity on the day 1-2 of cisplatin treatment. E) Tumor (DL) cells; note the steady decrease in LDH activity following cisplatin treatment. N=normal mice; TB =Tumor bearing mice (control); TB cisplatin treated—Tumor bearing mice treated with cisplatin (8 mg/kg body wt); TS =Ascites super-natant; Numbers 1, 2, 3 and 4 denote the number of days after cisplatin treatment. Statistical analysis: student's t-test, n=4, *=p<0.05; **=p<0.02; ***=p<0.01; ****=p<0.001; compared to respec-tive untreated control TB, TS or DL.

1999•@ Effect of Cisplatin on the Lactate Dehydrogenase Activity and its Isozyme Pattern 263•@

ever, following 3-4 days of the treatment the enzyme activity in the ascites supernatant decreased (Fig. 1D). DL cells on the other hand, showed the decrease in LDH activity following 1-4 days of cisplatin treatment (Fig. 1E).

LDH isozyme analysis

Serum: LDH isozyme patterns showed that in serum all the five isozymes of LDH (i.e. LDH-1, 2, 3, 4, 5) were present in varying intensities. LDH-5 was noted to be maximally intense while LDH-1 was the least (Fig. 2A). In the serum of the normal animals, LDH-2 and 3 seem to be more prominently expressed than that of tumor-bearing hosts. Following cisplatin treatment of the tumor-

Fig. 2. Lactate dehydrogenase (LDH) isozyme patterns in various tissues of mice. The lanes I, II, III,
IV, V and VI represent the gels from the tissues of normal mice without tomor or treatment, tumor bear-
ing control, and 1, 2, 3, 4 days of cisplatin treated tumorous mice respectively. A) Serum, Note the pres-ence of all the five isozymes with the predominance of LDH-5 and its increased activity after cisplatin treatment. Note the presence of new isozyme variant, named LDH-T (arrow head), in the serum of tumor bearing hosts. B) Kidney, also showing the presence of all the five isozymes with poor LDH-5 isozyme and relatively higher activity of LDH-2 and -3 isozymes. C) Liver, showing the predominance of LDH-5. LDH-4 and -3 were other other isozymes noticeable which disappeared after cisplatin treatment. Ref.-C shows the reference sample showing all isozyme bands. D) Ascites supernatant, high activity of LDH-5 and very weak activity of LDH-2, -3 and -4 is notable. Also note the appearance of very darkly stained diffused LDH-5 isozyme in cathodic region in 1 and 2 days cisplatin treated group which may suggest the presence of increased activity of LDH-5 and/or the appearance of the additional isozyme(s) variant. Ref.-D shows the teference sample showing all isozyme bands. E) Dalton's lymphoma (DL) cells, show-ing the presence of very darkly stained only LDH-5 isozyme. Note the increased activity after cisplatin treatment. 1-2 faint isozyme variants were also observed in the cathodic side of LDH-5. Ref.-E shows the reference sample showing all isozyme bands.

264•@ Surya B. Prasad and Anirudha Giri Cytologia 64

bearing hosts, LDH-5 activity (intensity) was increased. The important notable feature was the pres-ence of one extra isozyme band, named here as LDH-T, near the cathodic position in the serum of tumor bearing hosts treated with or without cisplatin (Fig. 2A).
Kidney: In the kidney also all the five isozymes were observed with LDH-2 and 3 having more intense staining and hence reflecting comparatively higher activity. Following cisplatin treatment, no significant variation in the intensities of isozymes was observed (Fig. 2B).
Liver: In the liver, only three isozymes-3, 4 and 5 were observed of which LDH-5 is noted to
be the predominant form. Interestingly, after cisplatin treatment LDH-3 and 4 intensities (activity) decreased and by the 4th day of treatment, LDH-5 appeared to be the only isozyme form present in liver (Fig. 2C VI).
Ascites supernatant: Dalton's lymphoma ascites tumor collected on the day 4 of cisplatin treat-ment was very viscous, thus, the cells and supernatants collected only upto 3 days of treatment have been used for LDH isozyme analysis. In the ascites supernatant (Fig. 2D) LDH-5 was seen as the most active isozyme form. LDH-2, -3 and -4 were very faintly present. On the day 1 and 2 of cis-platin treatment the LDH-5 intensity increased very dense towards cathode region of the gel which suggests the presence of increased activity of LDH-5 and/or the appearance of the additional isozyme band (Fig. 2D III, IV). The LDH-4 isozyme activity also increased after cisplatin treat-
ment.

Tumor cells: In the Dalton's lymphoma cells LDH-5 was the only isozyme form observed. The activity of the isozyme increased after cisplatin treatment (Fig. 2E IV). The additional 1-2 faint isozyme bands were also noted near LDH-5 towards cathode. (Fig. 2E).

Discussion

Enzymatic changes may reflect the overall changes in metabolism that occur in malignancy (Stefanini 1985). In present study, as compared to normal animals, more than two fold increase in serum LDH activity has been observed in Dalton's lymphoma-bearing mice (Fig. 1A) which may suggest the release of LDH in the tissues of tumor-bearing hosts. In general a higher rate of glycol-ysis has been reported in tumor cells (Warburg 1956, Yesher 1978), and it has been suggested that the rapid turnover of malignant cells should release ecto- and endo-enzymes into the blood stream (Stefanini 1985). The higher LDH activity in tumor-bearing mice was observed in liver also, the main site of glycolysis, (Fig. 113) which in turn support the earlier findings. It has been suggested that the enzymatic/metabolic changes in biochemical processes aiding cancerous cells over normal surrounding tissue may be related to the aggressiveness of the tumor, as in the case of J3-hex-osaminidase activity in ovarian cancer and of glucose-6-phosphate dehydrogenase in carcinoma of the prostate (Zampella et al. 1982). Cisplatin treatment of the tumor bearing hosts resulted in an overall decrease in LDH activity in liver, kidney and tumor cells (Fig. 1B, C, E) which may indicate decreased synthesis and/or increased leakage of the enzyme from the cells due to injury. The sec-ond possibility may be more implicated here, since, simultaneously a sustained increase of LDH level/activity in the serum of cisplatin-treated mice was also noted (Fig. 1A). Enzymes getting into the blood after cell necrosis of certain organs have been used to indicate the degree of tissue dam-age. The effect of some pesticides (copper sulphate, paraquat and methidation) on fish causing tis-sue necrosis has been demonstrated by increased levels of LDH in blood sera (Asztalos and Nemc-sok 1985). Following cisplatin treatment, the decrease in LDH activity in tumor cells was noted (Fig. 1E) with the parallel elevation of LDH in ascites supernatant which was very high on the day 1-2, but slightly lower on the day 3-4 of treatment (Fig. 1D). This may fairly indicate cell injury and/or altered membrane permeability of DL cells. It has been reported that cisplatin treatment brings about definite changes in the arrangement of surface membrane ruffles/blebs of DL cells with the appearance of membrane vesicles, alongwith a decrease in ascites supernatant protein con-

1999•@ Effect of Cisplatin on the Lactate Dehydrogenase Activity and its Isozyme Pattern 265•@

tent by 3-4 days of cisplatin treatment (Prasad and Gin 1994). It has been observed that cisplatin treatment of cultured renal epithelial cells and renal cortical slices in vitro causes the leakage of LDH from the cells into the medium and it may indicate the cell injury (Gemba and Fukuishi 1991, Kim et al. 1997).
The LDH isozymes has been used as a diagnostic aid (Rambotti and Davis 1981, Rajadhyak-sha et al. 1986). Results from the present investigations showed that in the serum of normal and tumor bearing mice all the five LDH isozymes (or isoenzymes) were present with LDH-5 showing more intensity/activity than others. Also, serum LDH-2 and -3 appeared to be more expressed in normal animals than those of tumor-bearing hosts (Fig. 2A). On the other hand, in kidney, also showing all isozymes, LDH-5 was found to be the least active isozyme form whereas LDH-2 and -3 were noted to be relatively more active forms. Interestingly, in other three tissues, i.e. liver, tumor cells and ascites supernatant, distinct variations were notable in relation to number and nature of various isozymes. In the liver, only three isozymes, LDH-3, -4 and -5 were noticed with the LDH-5 being the main isozyme form. After cisplatin treatment, LDH-3 and -4 isozymes reduced markedly and by the 4th day of treatment LDH-5 was the only isozyme form noticeable in liver (Fig. 2C). In ascites supernatant also LDH-5 was observed to be the most prominant isozyme (Fig. 2D) while in tumor cells LDH-5 was the only isozyme form present (Fig. 2E). Thus, the comparative analysis of isozyme patterns very clearly showed the predominance of LDH-5 in the tumor. It has been report-ed that human malignant cells, both lymphoid (Rambotti and Davis 1981) and from other organs (Fleischner et al. 1981) possess LDH isozyme patterns distinguished by lower H : M ratios (i.e. pre-dominance of LDH-5) and present observation of the predominance of LDH-5 in DL cells is in this accordance. Tumor cells have higher glycolytic activity (Warburg 1956, Yesher 1978) and it is known that LDH 5 is highly active in anaerobic glycolysis (Dawson et al. 1964). Thus, the predomi-nance of LDH-5 may reflect the anaerobic metabolism of these tissues, since hypoxic conditions have been shown to exist in the tumors (Kallman 1972). In the serum of tumor bearing animals cis-platin treatment resulted the increased activity of LDH-5 (Fig. 2A III—VI). The increased expres-sion of LDH-5 in DL cells and serum following cisplatin treatment (Fig. 2E, A), therefore, suggests that cisplatin treatment may further increase the hypoxic condition in the tumor. This is supported from the observations of the significant decreased oxygen consumption by DL cells treated with cisplatin in vivo (Giri 1995). In the ascites supernatant also after cisplatin treatment for 1-2 days, the LDH-5 intensity appeared very dense towards cathode region of the gel which suggests the presence of increased release of LDH-5 from the tumor cells and/or the appearance of the addition-al isozyme bands. It has been postulated that anaplasia is accompanied by molecular adjustments, which precede morphologic changes in malignancy and may manifest themselves through changes in enzyme patterns, thus, there is predominance of LDH-1 in normal colonic mucosa and of LDH-5 in colonic cancer tissue (Carda-Abella et al. 1982).
Another important notable feature was the presence of additional band(s) near the LDH-5 isozyme towards cathode in the serum of tumor-bearing hosts (Fig. 2A) which is also seen faintly in tumor supernatant/tumor cells, but absent in kidney and liver. This may indicate the presence of new isozyme variant and it has been tentatively designated as LDH-T (Fig. 2A) for being present in tumorous condition. The exact physiological significance of this new isozyme variant in serum of tumor bearing hosts cannot be clearly understood at present, but it could be the consequence of a physiological adaptation of tumor cells to the general hypoxic condition. It may serve as an useful marker for Dalton's lymphoma in vivo. It has also been suggested that oncogenes may have a role in the production of variants by malignant cells (Stefanini 1985). LDH has been suggested to be a fair-ly sensitive marker for most solid tumors with the presence of some isozyme variants of LDH in the serum of these patients (Lippert et al. 1981).
Thus, the results from the present bichemical studies indicate that LDH activity is definitely affected in the tissues of tumor-bearing hosts and during tumor regression after cisplatin treatment.

266•@ Surya B. Prasad and Anirudha Giri Cytologia 64

It suggests that changes in LDH activity could be very useful parameter in malignancy and cisplatin-mediated chemotherapy. LDH isozyme patterns revealed the presence of tissue specificity of differ-ent isozymes, with only LDH-5 in tumor cells and appearance of some isozyme variants in the serum of tumor-bearing hosts.

Acknowledgements

We are grateful to the Head, Department of Zoology for providing laboratory facilities and to
the North-Eastern Hill University for financial support. We are thankful to the Head, Department of Biochemistry, North-Eastern Hill University for the photographic facility.

References

Aggarwal, S. K. 1993. A histochemical approach to the mechanism of action of cisplatin and its analogues. J. Histochem. Cytochem. 41: 1053-1073.

－ and Meara, D. J. 1966. Inhibition of dehydrogenase activity as a mechanism behind cisplatin-induced toxicities. In:
Collery, Ph., Corbella, J., Domingo, J. L., Etienne, J. C. and Llobet, J. M. (eds.). Metal Ions in Biology and Medi-
cine. John Libbey Eurotext, Paris, Vol. 4, pp. 113-115.
Appella, E. and Markert, C. L. 1961. Dissociation of lactate dehydrogenase into subunits with guanidine hydrochloride. Biochem. Biophys. Res. Commun. 6: 1-76,
Asztalos, B. and Nemcsok, J. 1985. Effect of pesticides on the LDH activity and isozyme pattern of carp (Cyprinus carpio L.) sera. Comp. Biochem. Physiol. 82C: 217-219.
Cabaud, P. G. and Wroblewski, F. 1958. Colorimetric measurement of lactic dehydrogenase activity of body fluids. Am. J. Clin. Pathol. 30: 234-240.
Cahn, R. D., Kaplan, N. 0., Levine, L. and Zwilling, L. E. 1962. Nature and development of lactic dehydrogenases. Science 136: 962-969.
Carda-Abella, P., Perez-Cuadrado, S., Lara-Barique, L., Gil-Grande, L. and Nunez-Puertas, A. 1982. LDH isozyme patterns in tumors, polyps, and uninvolved mucosa of human cancerous colon. Cancer 49: 80-93. Chu, G. 1994. Cellular responses to cisplatin. J. Biol. Chem. 269: 787-790.
Davis, B. J. 1964. Disc electrophoresis: II: Method and application to human serum proteins, Ann. N. Y. Acad. Sci., 121: 404-427.

Dawson, D. N., Goodfriend, T. L. and Kaplan, N. C. 1964. Lactic dehydrogenases: Functions of two types. Science 14: 929-933.
Fleischner, M., Wasserstrom, W. R., Schold, S. C., Schwartz, M. K. and Posner, J. B. 1981. Lactic dehydrogenase isozymes in the cerebral fluidof patients with systemic cancer. Cancer 47: 2654-2659.
Gemba, M. and Fukuishi, N. 1991. Amelioration by Ascorbic Acid of Cisplatin-Induced Injury in Cultured Renal Epithelial Cells. In: Koide, H., Endou, H. and Kurokawa (eds.) Cellular and Molecular Biology of the Kidney: Contributions
to Contributions to Nephrology. K. S. Karger, Basel, pp. 138-142.
Giri, A. 1995. Studies on the effect of cisplatin on malignant and normal cells: preliminary investigations on cisplatin com-bination chemotherapy, Ph.D. thesis, North-Eastern Hill University, Shillong, India, pp. 1-268.
－ ,Khynriam, D. and Prasad, S. B. 1998. Vitamin C mediated protection on cisplatin induced mutagenicity in mice. Mut.
Res. 421: 139-148.
Goldman, R. D., Kaplan, N. C. and Hall, T. C. 1964. Lactic dehydrogenase in human neoplastc tissues. Cancer Res. 24: 929-935.
Kallman, R. E 1972. The phenomenon of reoxygenation and its implications for fractionated radiotherapy. Radiology 105: 135-139.
Keller, K. A. and Aggarwal, S. K. 1983. Embryotoxicity of cisplatin in rats and mice. Toxicol. Appl. Pharmacol., 69: 245-266.
Kim, Y. K., Jung, J. S., Lee, S. H. and Kim, Y. W. 1997. Effects of antioxidants and Ca+ in cisplatin-induced cell injury in rabbit renal cortical slices. Toxicol. Appl. Pharmacol. 146: 261-269.
Kociba, R. J., Sleight, S. D. and Rosenberg, B. 1970. Inhibition of Dunning ascitic leukemia and Walker-256 carcinosarco-ma with cis-diamminedichloroplatinum (NSC-1 19875). Cancer chemother. Rep. 54: 325-328. Lippert, M. C. and Javadpour, N. 1981. Lactic dehydrogenase in the monitoring and prognosis of testicular cancer. Cancer 48: 2274-2278.
－ ,Papadopoulos, N. and Javadpour, N. 1981. Role of lactate ddehydrogenase isoenzymes in testicular cancer, Urology 18:
50-53.

1999•@ Effect of Cisplatin on the Lactate Dehydrogenase Activity and its Isozyme Pattern 267•@

Markert, C. L. 1963. Lactate dehydrogenase isozymes. Dissociation and recombination of subunits. Science 140: 1329-1330.
Pil, P. and Lippard, S. J. 1997. Cisplatin and Related Drugs. In: Bertino, J. R. (ed.). Encyclopedia of Cancer. Academic press, Inc. San Diego, vol. 1, pp. 392-410.
Pinto, A. L. and Lippard, S. J. 1985. Binding of the antitumor drug cis-diamminedichloroplatinum (II) (cisplatin) to DNA. Biochim. Biophys. Acta 780: 167-180.
Prasad, S. B. and Giri, A. 1994. Antitumor effect of cisplatin against murine ascites Dalton's lymphoma. Indian J. Exp. Biol. 32: 155-162.
－ ,Giri, A. and Khynriam, D. 1998. Studies on the chromosomes of murine Dalton's lymphoma cells and the effect of cis-platin on chromosomes of these cells and bone marrow cells in vivo. Cytologia 63: 405-413.

Prestayko, A. W., D'Aoust, J. C., Issell, B. F. and Crooke, S. T. 1979. Cisplatin (cis-diamminedichloroplatinum-II). Cancer Treat. Rev. 6: 17-39.
Rajadhyaksha, M. S., Joshi, D. S., Phondke, G. P., Mitra, R. and Sundaram, K. 1986. Lactic dehydrogenase isozyme distibu-tions in lymphocytes from normal and leukemic mice. Cancer Biochem. Biophys. 8: 167-171.
Rambotti and Davis, S. 1981. Lactic dehydrogenase in normal and leukemia lymphocyte subpopulations: Evidence for the presence of abnormal T cells and B cells in chronic lymphocytic leukemia. Blood 57: 324-327.
Roberts, J. J., Knox, R. J., Pera, M. F., Friedlos, F. and Lydall, D. A. 1988. The Role of Platinum-DNA Interactions in the Cellular Toxicity and Anti-tumor Effects of Platinum Compounds. In: Nicolini, M. (ed.) Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy. Martinus Nijhoff publishing, Boston, pp. 16-31.
Rosalki, S. B. 1974. Standardisation of isoenzyme assays with special reference to lactate dehydrogenase isoenzyme elec-trophoresis. Clin. Biochem. 7: 29-40.
Rosenberg, B. 1985. Fundamental studies with cisplatin. Cancer 55: 2303-2316.
Schneider, R. J., Seibert, K., Sharon, P., Claudia, L., Timothy, G., Burton, J. L. III, Valerie, M. and Young, C. W. 1980. Prog-nostic significance of serum lactate dehydrogenase in malignant lymphoma. Cancer 46: 139-143. Stefanini, M. 1985. Enzymes, isozymes and enzyme variants in the diagnosis of cancer: a short review. Cancer 55: 1931-1936.
Warburg, 0. 1956. Origin of cancer cells. Science 123: 309-314.
Yesher, A. 1978. Spectrum of lung cancer and ectopic hormones. Pathobiol. Annu. 13: 217-240.
Zamble, B. D. and Lippard, S. J. 1995. Cisplatin and DNA repair in cancer chemotherapy. Trends Biochem. Sci. 20: 435-439.
Zampella, E. J., Bradley, E. L. and Pretlow, T. D. II. 1982. Glucose-6-phosphate dehydrogenase: a possible indicator for pro-static carcinoma. Cancer 49: 384-387.