Tumor Res., **8**, 151–157, 1973

Studies on lactate dehydrogenase pI isoenzymes in embryonal state and in malignancy

Morimichi FUKUDA, Satoshi KATAYAMA, Yoshiaki NAKANO, Ichiko YAMAGUCHI and Ichiro URUSHIZAKI

Department of Medicine, Cancer Research Institute, Sapporo Medical College, Sapporo, Japan

Isoelectric fractionation[1] is a technique used for the separation of amphoteric macromolecules such as proteins and nucleic acids. By the use of carrier ampholytes, Ampholine, and under the influence of an electric field, a stable pH gradient is established. Amphoteric macromolecules will focus at points in the system where the pH values correspond to their isoelectric points. The present report describes the analyses of the isoenzymes of lactate dehydrogenase (EC 1.1.1.27), especially on their changes in sera of the patients suffering from a variety of malignant neoplasmas and also their certain resemblance to the isozyme pattern of cord serum. Furthermore, changes in LDH pI isoenzymes of the rat liver tissues were investigated in 3′-Me-DAB hepatoma, embryonal state, hepatic regeneration after partial hepatectomy, normal liver at its postpartal period and in acute hepatic injury by carbon tetrachloride treatment.

MATERIALS AND METHODS

Fresh human sera were obtained from a variety of malignant tumor cases by venipuncture and were analysed immediately by electrofocusing or stored in frozen state at −30°C until used. Fresh tumor tissues were obtained at surgical operation or in necropsy shortly after death and tumor tissues were excised from non-tumor tissue and minced and homogenized in a small volume of cold saline to give a 10% w/v homogenate and centrifuged at 105,000×g for 60 minutes at 4°C by a Beckman Model F-2 ultracentrifuge. The resulting supernatants were used as crude enzyme preparations. Purified LDH preparations of rabbit and bovine H and M types were purchased form Sigma and also analysed by disc electrophoresis and by electrofocusing techniques. Gel electrofocusing was performed in a disc electrophoresis apparatus according to the method of Dale and Latner[2] with slight modifications[3]. Due to the difficulty to obtain precise pH values and enzyme activities in a gel focusing experiment, a conventional density gradient electrofocusing was carried out using LKB 8101 column

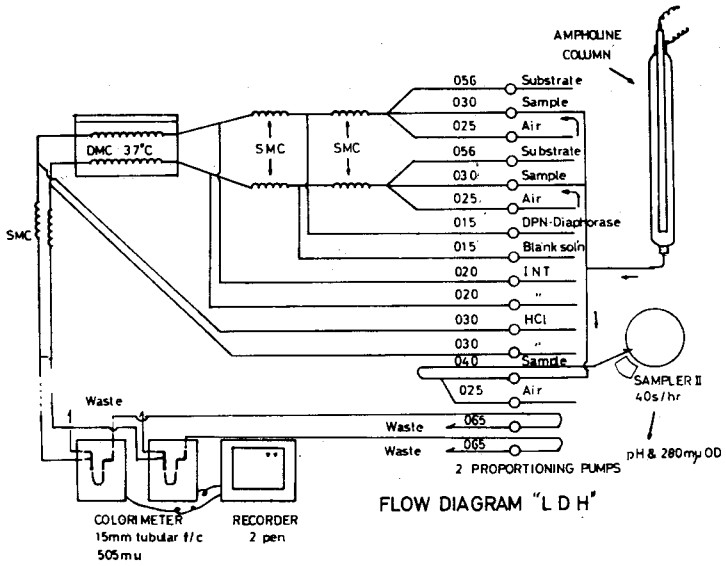

Fig. 1. Autoanalyzer flow diagram designed for automated determination of LDH pl isoenzymes.

of 110 ml capacity in parallel. Ampholine, pH range of 3 to 10 was purchased from LKB, Sweden and was used at a final concentration of 1% in a sucrose gradient. The conditions of separation were 800 v, 0.5 mA for 48 hours. Volumes of the samples applied were 1 ml in general and its enzyme activity was adjusted to approximately 1,000 mU per ml in cases of tissue extracts. Fig. 1 shows a manifold devised[3] for automated determination of LDH for the direct analysis of the eluants from the column and for the simultaneous fractionation of the eluants by Sampler II to be used for pH and UV absorption determinations. The former is essentially a modification of the method of Hochella and Weinhouse[4] and gave a good linearity up to 100 mU per ml LDH activity.

RESULTS

In a gel focusing experiment, bovine H and M type enzymes, which showed a single activity band corresponding to LDH 1 and 5 positions by disc electrophoresis, separated into 2 and at least 6 discrete bands, respectively. Gel focusing patterns of normal human sera stained for LDH showed the presence of 7 activity bands in the region of pH 4 to 7. Sera of various carcinoma cases with elevated SLDH activity, on the other hand, invariably showed an increased number of activity zones in the neutral and alkaline pH regions. Density gradient electrofocusing also confirmed

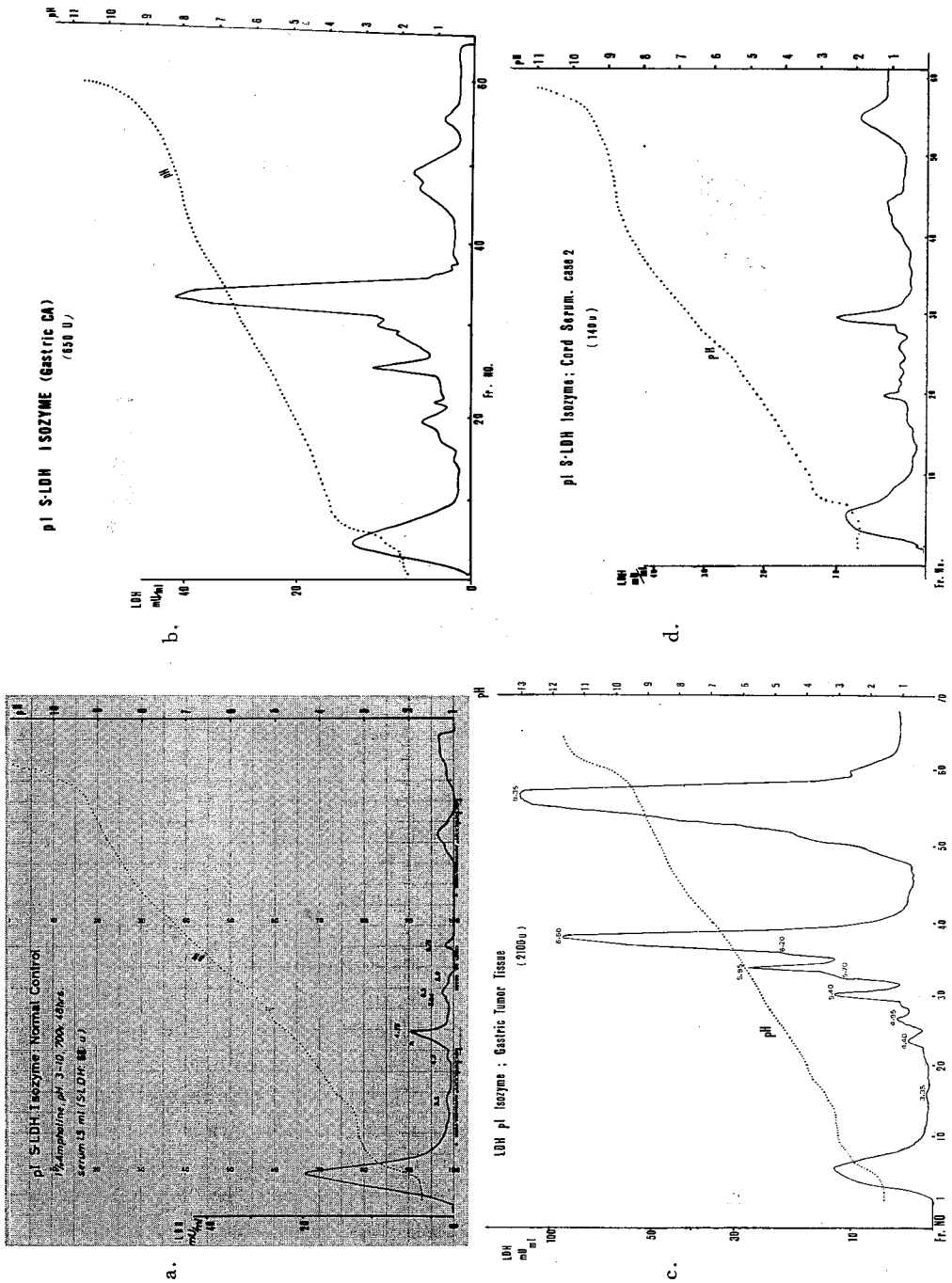

Fig. 2. LDH pI isoenzyme profiles. a. normal human serum., b. gastric carcinoma with elevated SLDH., c. carcinoma tissue extract of case b., d. cord serum.

the above findings. Fig. 2 shows various LDH pI isoenzyme patterns by this procedure. Normal human serum exhibited rather low LDH peaks having different pI values between pH 3.5 to 6.75. A few peaks closely adjacent to each electrode solution were non-enzyme peaks which also appeared in the column without enzyme specimens. As is shown in Fig. 2-b, in gastric carcinoma, a number of LDH peaks with different pI values could be demonstrated in the range of pH 4.7 to 7, the highest peak corresponding to pI 6.5. A close similarity in pI isoenzyme profiles between serum and tumor tissue extract was observed as shown in Fig. 2-c, and the same was true in cases with primary hepatoma, gastric carcinoma, uterine carcinoma and several other malignancies with elevated SLDH.

One important point to be stressed is the fact that the very acidic fraction of LDH usually underwent some inactivation due possibly to the effect of low pH exposure which inevitably occurs during separation.

Although there appears some diversity of the pI isoenzyme patterns among various different malignant tumors, a rather unique change was the appearance of higher enzyme activity in the range around pH 6.5. A very interesting finding was the occurrence of this type of enzyme peak in cord serum. Fig. 2-d shows a characteristic pattern obtained from human cord serum having only 140 mU per ml LDH activity. And furthermore, this peak was lacking in the corresponding maternal serum obtained at the time of delivery. Similar observations were reported by Meade[5] and by others using other electrophoretic means, therefore these findings were also substantiated by electrofocusing.

To confirm LDH pI isoenzyme changes observed in a human experiment, animal experiments using rat liver tumors were made. The results are shown in Fig. 3. Analysis of the normal liver extract prepared from untreated rat of Wistar strain showed that the major LDH activity resides in the extreme alkaline region of pH 8.6 to 10. Multiple peaks with lesser activities having pIs ranging from pH 4 to 8 were demonstrated and general patterns were the same irrespective of rat strains used. Fig. 2-b and-c show pI isoenzyme patterns of 3′-Me-DAB induced hepatoma and of fetal rat liver, the former was produced in normal adult rat of Wistar strain by feeding the animals over 29 weeks on a special diet containing 0.06% 3′-Me-DAB as a carcinogen. Presence of a marked deviation from the normal rat liver pattern was evident and a marked decrease in activity in alkaline pH region with concomitant increase of the activity around pH 6.4 was observed in both cases. Interestingly, the fetal isoenzyme pattern noted in the late stage of gestation tended to return to the adult pattern shortly after the delivery. In the adult liver, also, a seemingly similar

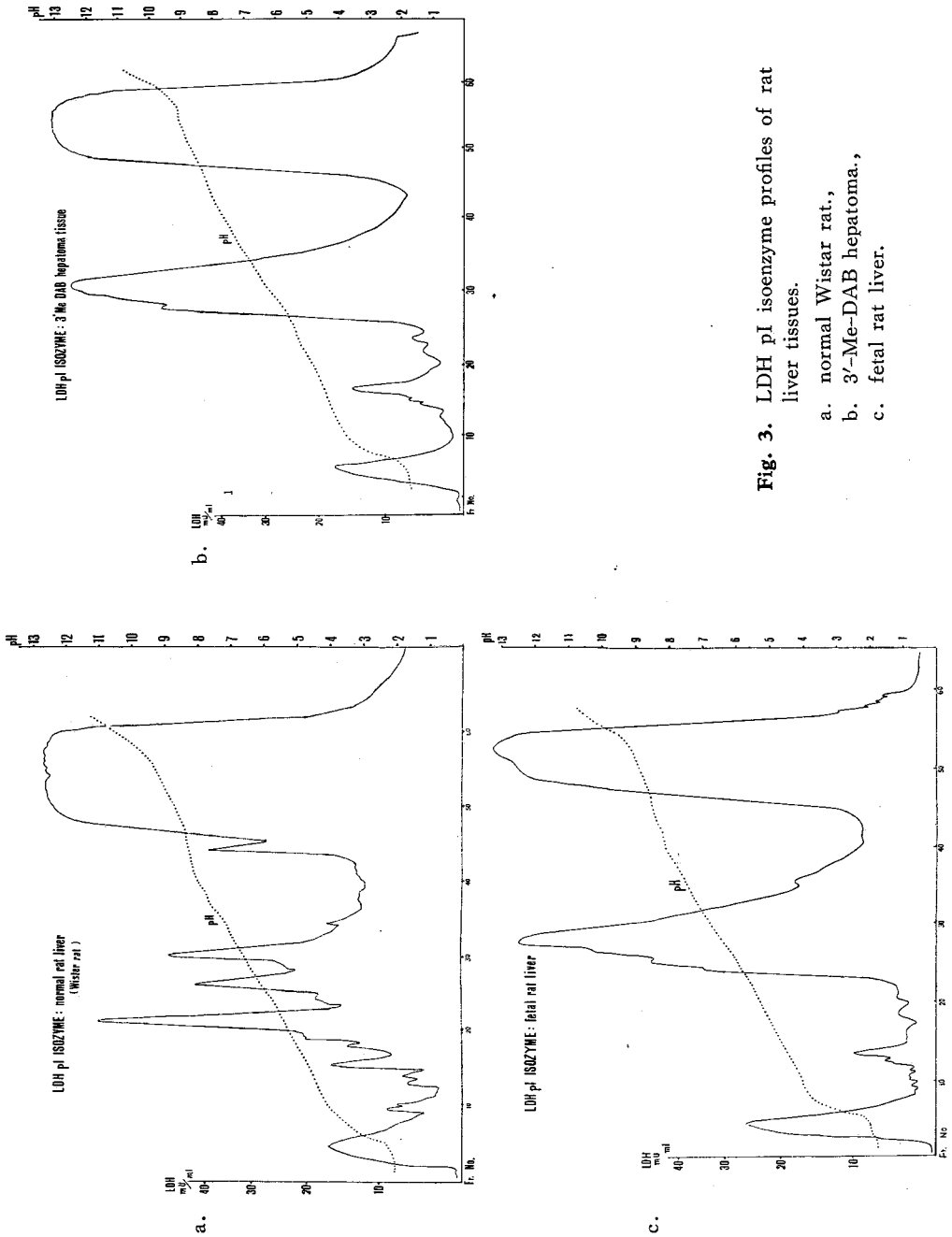

Fig. 3. LDH pI isoenzyme profiles of rat liver tissues.

a. normal Wistar rat.,
b. 3'-Me-DAB hepatoma.,
c. fetal rat liver.

change took place during the regeneration period after partial hepatectomy. In this case, too, return to the normal liver pattern was observed after 48 hours of operation whereas deviation from the normal pI isozyme pattern was certainly apparent after 24 hours in which time the mitotic indices were the highest.

DISCUSSIONS AND SUMMARY

The changes of several enzyme activities in malignant neoplasmas were well documented[6]. And also in certain enzymes both qualitative as well as quantitative deviations from the normal adult pattern to the one observed in a embryonal state were amply demonstrated[7]. As far as the enzyme lactate dehydrogenase is concerned, Pfleiderer, Starkweather[8], and others reported a marked elevation of LDH 3, 4 in malignant tumors, and also an elevation of LDH 3 and 4 in cord serum while a similar elevation in placental extract were reported by others.

In animal experiments, Johnson and Kampschmidt, Kline and Clayton, Schapira and Nechaud[9] reported an increase of LDH subfractions with a slightly higer mobility in experimentally induced hepatoma with carcinogens.

Rosado *et al.*[10] reported recently that the composition of H and M subunits differed according to the growth rates of the various minimal deviation hepatomas. The present results on human and rat LDH pI isoenzymes agree well with those of previous reports. However, the exact cause of the diversity of LDH pI isoenzymes is as yet largely unknown and analyses on changes in subunit levels are currently being carried out and will be reported shortly.

REFERENCES

1. VESTERBERG, O. and SVENSSON, H.: Isoelectric fractionation, analysis, and characterization of ampholytes in natural pH gradient. IV. Further studies on the resolving power in connection with separation of myoglobins. *Acta Chem. Scand.*, **20**, 820 (1966).
2. DALE, G. and Latner, A. L.: Isoelectrofocusing in polyacrylamide gels. *Lancet*, **I**, 847 (1968).
3. FUKUDA, M., KATAYAMA, S. and URUSHIZAKI, I.: Analytical studies on human serum protein subfractions. III. On LDH pI isoenzymes. *Seibutsu Butsuri Kagaku* (Physico-Chemical Biology), **15**, 246 (1971). (in Japanese)
4. HOCHELLA, N. J. and WEINHOUSE, S.: Automated assay of lactate dehydrogenase in urine. *Anal. Biochem.*, **13**, 322 (1965).
5. MEADE, B. W. and ROSALKI, S. B.: Lactic dehydrogenase isoenzymes in pregnancy. *Lancet*, **I**, 1407 (1962).
6. LATNER, A. L. and SKILLEN, A. W.: "Isoenzymes in biology and medicine". Academic Press, N. Y. (1968).

7. KNOX, W. E.: "Enzyme patterns in fetal, adult and neoplastic rat tissues". S, Karger Publ., Basel (1972).
8. STARKWEATHER, W. H. and SCHOCH, H. K.: Some observations on the lactate dehydrogenase of human neoplastic tissue. *Biochim. Biophys. Acta*, **62**, 440 (1962).
9. SCHAPIRA, F. and NECHAUD, B.: Presence anormale d'isozymes de la lactico-deshydrogenase, type coeur, dans des hepatomes experimentaux. *C. R. Soc. Biol.* (Paris), **162**, 86 (1968).
10. ROSADO, A., MORRIS, H. P. and WEINHOUSE, S.: Lactate dehydrogenase subunits in normal and neoplastic tissues of the rat. *Cancer Res.*, **29**, 1673 (1969).