[Chem. Pharm. Bull. 18(5)1050—1052(1970)]

UDC 577.15.04 : 547.556.33.09 : 615.277.4.034.

Effect of Basic Cupric Acetate on the Biochemical Changes in the Liver of the Rat Fed Carcinogenic Aminoazo Dye. II.[1] Activity and Isozyme Pattern of Lactate Dehydrogenase

Yasuhiro Yamane, Kazuo Sakai, Miyoko Hayashi, Midori Matsuzaki,[2a] and Akira Hanaki[2b]

Faculty of Pharmaceutical Sciences, University of Chiba[2a] and National Institute of Radiological Sciences[2b]

(Received December 16, 1969)

We described in the preceding paper[1] that the administration of basic cupric acetate, which was shown to protect or delay the hepatic carcinogenesis in the rat fed 4-dimethylaminoazobenzene(DAB), stimulated remarkably the activity of azo-bond reduction system in the liver homogenates. This information led us to speculate that a role of copper might be concerned with the detoxication of the carcinogen, by which the contact of the target with the carcinogen was hindered. The activities of other enzymes or enzyme systems are expected to be varied by the administration of cupric acetate with the carcinogenic dye.

Lactate dehydrogenase(LDH) in the liver of rat was shown to be affected by the administration of the carcinogenic aminoazo dye, but not changed by the noncarcinogenic dye.[3] The LDH isozyme pattern of lung was changed by the inhalation of beryllium which induced pulmonary carcinogenesis in the rat.[4]

In this paper, we described the changes of the activity and the isozyme pattern of LDH in the liver of the rat fed DAB with and without basic cupric acetate.

Experimental

Animals——Female Wistar rats weighing from 100 to 150 g at the start were used and devided into 4 groups consisted of 40 rats respectively. One group of rats received 0.09% DAB(i), the second 0.5% basic cupric acetate hexahydrate(ii) and the third 0.09% DAB and 0.5% basic cupric acetate hexahydrate(iii) in maize diet. The fourth group received mazie diet alone(iv) and served as a control. Those experimental diets were given five days a week, and CE-2 (Nippon Clea Ltd.) was given for two days; on Tuesdays and Wednesdays.

Preparation of Liver Homogenates and the Supernatants——The rats in each group were killed by decapitation and the livers were immediately perfused with chilled 1.15% KCl, rapid excised, weighed, and homogenized below 4° in 1.15% KCl with a teflon homogenizer of Potter–Elvehjem type. 10% liver homogenates thus prepared were centrifuged at 7000 g for 30 min at 0°. This supernatant was used for assay of the enzyme activity.

Assay of Total Activity of LDH——The activity of LDH, that is the sum of all the activities of LDH isozymes, were measured by decrease of NADH according to a modified Hill's method.[5]

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \rightleftarrows \text{L-lactate} + \text{NAD}^+$$

The reaction mixtures contain 0.2 ml of the sample solution diluted the above supernatant and 6 ml of the solution (1 ml of 0.25M lithium pyruvate, 25 ml of 0.1M phosphate buffer, pH 7.2, and 4 ml of distilled water). The reaction was initiated by adding a definite amount of NADH corresponding to $E_{340\,m\mu}$ 0.600—0.700.

1) Y. Yamane, K. Sakai, I. Uchiyama, M. Tabata, N. Taga, and A. Hanaki, *Chem. Pharm. Bull.* (Tokyo) **17**, 2488 (1969).
2) Location: *a*) *Yayoi, Chiba*; *b*) *Anagawa, Chiba.*
3) H.L. Johnson and R.F. Kampschmidt, *Proc. Soc. Exptl. Biol. Med.*, **120**, 557 (1965).
4) A.L. Reeves, *Cancer Res.*, **27**, 1895 (1967).
5) B.R. Hill and C. Levi, *Cancer Res.*, **14**, 513 (1954).

The mixtures were shaken mechanically at 37° for 30 min, and $E_{340\ m\mu}$ was measured at the start and at the end. From the difference of those two $E_{340\ m\mu}$ values, an activity unit was calculated as follows:

$$\text{LDH activity} = E_{340\ m\mu} \times 1000/\text{mg liver tissue}/30\ \text{min}$$

Separation of LDH Isozyme[6]——LDH is constituted from five isozymes. The isozymes were separated by electrophoresis on an agar gel plate in veronal buffer at pH 8.4 in this experiment. The separation was conducted in the cold room at 5—10° for about 90 min with a constant current 55 mA and resultant voltage 110—120 V. The spot of each isozyme was detected by staining LDH with nitrobluetetrazolium.

Results

Total Activity of LDH

The variation in the total activity of LDH in each group was shown in Fig. 1. In groups (ii) and (iii) a fairly high level of the activity was continued during the entire experiments. While, in group (i), the activity, though showed a comparable

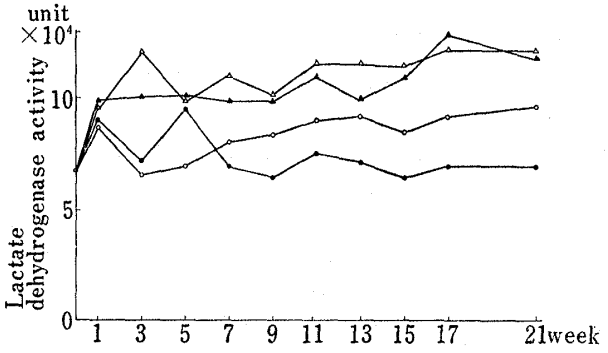

Fig. 1. Total Activity of Lactate Dehydrogenase in the Liver of the Rats Fed Basic Cupric Acetate with and without DAB

(i) DAB: —●— (ii) copper: —△—
(iii) DAB and copper: —▲—
(iv) maize alone: —○—
The measurement of the activity is described in the experimental part.

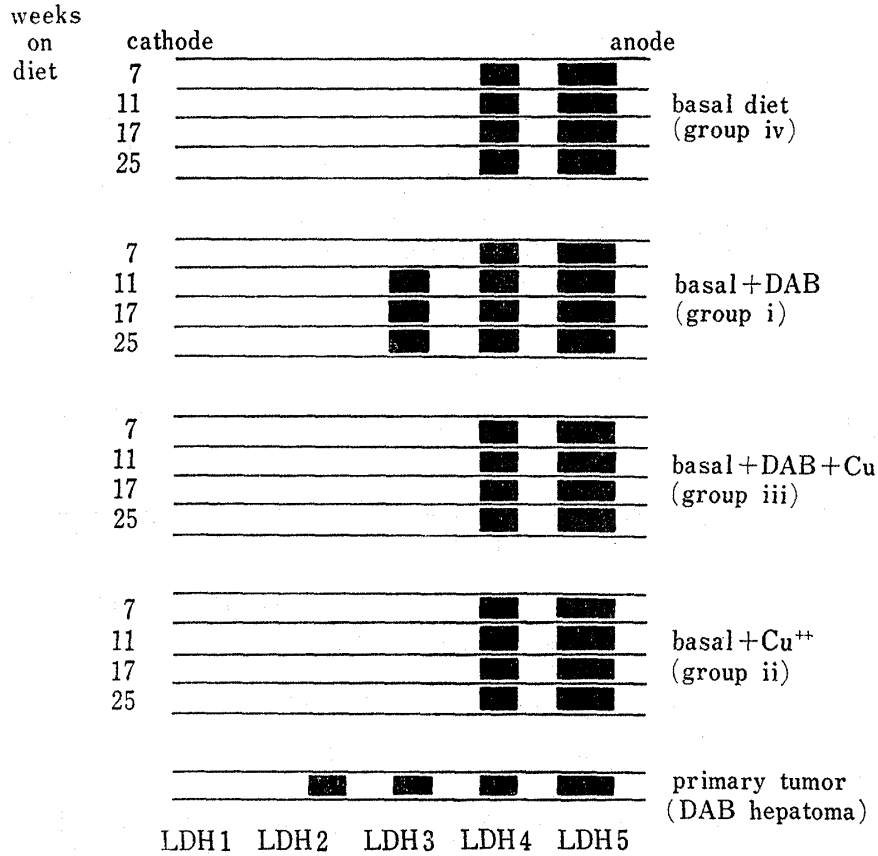

Fig. 2. Diagram of Lactate Dehydrogenase Isozyme Patterns of Livers of Rats Fed DAB and/or Basic Cupric Acetate for Varying Times Separated by Agar Gel Electrotrophoresis

isozymes number from the cathode toward the anode of agar gel plate (LDH 1→LDH 5)

6) M. Yoshida, *Seibutsu-Butsurikagaku*, **11**, 69 (1966).

level to the control in the initial stage, was dropped after 6 weeks from the start. Considering that the LDH activity did not decrease in the group of rats fed cupric acetate with the dyes, a role of copper might be to recover the LDH activity that was reduced by the administration of DAB.

Isozyme Pattern of LDH

The main component of LDH isozyme in the rat liver was LDH_5 fraction. When 2% homogenate was applied to the electrophoretic separation, only LDH_5 component was detected on the plate. If 10% homogenate was subjected to the separation, LDH_4 fraction, as well as LDH_5 fraction, was appeared. The administration of cupric acetate with and without DAB did not change the isozyme pattern observed in the control, while the administration of DAB alone was appeared to affect the isozyme pattern. As shown in Fig. 2, LDH_3 fraction was newly found in group(i) after 11 weeks from the start, and this pattern seemed to resemble that of the primary tumor induced by DAB. From the above facts, it was found that the administration of cupric acetate repressed the change of the LDH isozyme pattern in the course of carcinogenesis in the liver of the rat fed DAB.

Discussion

The administration of DAB, though reduced the total activity of LDH, gave rise to the appearance of a new LDH isozyme, LDH_3 fraction, which was never detected in the homogenate of the control. The fact indicates that the administration of DAB might mainly repress LDH_5 fraction of LDH isozyme, and that thereby the total activity is reduced. The appearance of aerobic fraction, LDH_3 might be related to the induction of hepatic carcinoma by feeding DAB, because another aerobic fraction, LDH_2, was appeared in primary carcinoma. Thus, the effect of feeding DAB might be related to the repression of anaerobic fraction of LDH, and the feeding of copper might recover the activity and isozyme pattern of this enzyme.

The above facts were added to support of the results of the previous report which indicated that the administration of basic cupric acetate stimulates remarkably of azo-reduction and protects the hepatic carcinogenesis in the rat fed DAB.

[Chem. Pharm. Bull. 18(5)1052—1055(1970)]

UDC 547.963.3.07 : 547.857.04

Synthesis of Adenosine 2′-, and 3′-Phosphate 5′-Pyrophosphates and Their Esters[1)]

YASUHIRO HOSOKAWA, YASUHIKO YOTSUI, OSAMU NAGASE, and MASAO SHIMIZU

Research Laboratories, Daiichi Seiyaku Co., Ltd.[2)]

(Received January 13, 1970)

Adenosine 2′- (IVa) and 3′-phosphate 5′-pyrophosphate (Va) are the known compounds, of which the former was obtained from the enzymatic hydrolyzate by Kaplan, *et al.*[3)] and the

1) This report represents Part XXV of "Investigations on Pantothenic Acid and Its Related Compounds"–Chemical Studies (12); *a*) Part XXIV: M. Shimizu, O. Nagase, Y. Hosokawa, H. Tagawa, and Y. Yotsui, *Chem. Pharm. Bull.* (Tokyo), **18**, 838 (1970).
2) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo, 132, Japan.*
3) N.O. Kaplan, S.P. Colowick, E.F. Neufeld, and M.M. Ciotti, *J. Biol. Chem.*, **205**, 17 (1953).