Succinic and $NaDH_2$ Dehydrogenase in Livers of Vitamin B_6-Deficient Rats.* (28833)

SAMUEL W. FRENCH AND M. ELIZABETH MARSH (Introduced by H. D. Moon)
Department of Pathology, University of California Medical Center, San Francisco

Pyridoxal phosphate serves as the coenzyme for a great variety of enzymes catalyzing reactions of amino acids, *i.e.*: transamination(1), decarboxylation, dehydration, deamination and desulfhydration(2). Although Vit. B_6-deficiency does not impair maintenance of synthesis of body proteins in general(3), the synthesis of specific enzymes may be affected. This study is a part of a series to determine the effect of various experimental conditions on over-all activity and distribution of succinic dehydrogenase (SD) and nicotinamide adenine dinucleotide dehydrogenase ($NADH_2D$) in liver.

Materials and method. Fifty-four weanling male rats of Wistar CNF strain were maintained on a diet of the following percentage composition: vitamin free casein 24-30, sucrose 24-38, corn starch 28, Crisco 6, corn oil 2, non-nutrient fiber 2, and salt mixture Hawk-Oser 4. Each kilogram of diet contained the following vitamins in milligrams: thiamine HCL 3, riboflavin 5, para-amino benzoic acid 3, nicotinic acid 20, calcium pantothenate 25, folic acid 2, biotin 0.1, B_{12} 0.02, inositol 100, menadione 5, E 500 choline dihydrogen citrate 2000. Each kilogram of diet contained 20,000 units of Vit. A and 2000 of D. The control diet contained 3 mg/kg of pyridoxine HCL.

The 54 animals were treated as follows: biopsies were made of the livers of 24 rats (8 Vit. B_6-deficient, 8 control with limited intake and 8 control fed *ad libitum*), when the experimental animals first became acutely deficient. The experimental rats were then maintained in a state of chronic deficiency for 16 to 32 weeks by administration of small amounts (5-20 μg) of pyridoxine as required. Just prior to sacrifice, the entire livers were removed under ether anesthesia, examined grossly and sampled again. Livers of the other 30 rats (10 Vit. B_6-deficient, 10 control with limited intake, and 10 control fed *ad libitum*) were sampled only when the experimental animals were acutely deficient. Oxytetracycline (10 g/l) was added to their drinking water to prevent intercurrent infection.

The liver was homogenized by hand in a frozen mortar and a 1:50 dilution was made with a M/15 phosphate buffer of pH 7.4. The $NADH_2D$ activity was measured spectrophotometrically with ferricyanide as the hydrogen acceptor in a final dilution of 1:15,000(4). The Beckman spectrophotometer was equipped with a water jacket to maintain a constant temperature in the cell. The temperature of the cuvette was measured at the end of the assay and the results corrected to 27°C by the method of Slater (5).

SD activity was assayed by the method of Neufeld *et al*(6) in a final dilution of 1:570. The activity was corrected for the reduction obtained in a control solution without succinate although the method does not lend itself to an accurate control measurement since the reaction rate is not linear.

Enzyme activity was determined on the basis of wet weight and of protein content of the tissue. Protein was determined with the Folin phenol reagent by the method of Lowry *et al*(7). All measurements were carried out in duplicate or in triplicate.

Histochemical determinations of SD and $NADH_2D$ were made by the method of French(8) with the following modifications: the liver sections were cut 8 to 10 μ thick in a deep freeze at a temperature between —20° and —10°C. Sections were transferred to frozen coverslips, dried at room temperature, placed in a substrate solution, and incubated aerobically, 4 minutes for $NADH_2D$ and 6 for SD, in a Dubnoff metabolic shaker at 37°C. Sections of liver were also stained for fat with Sudan IV. Zenker-fixed liver

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sections were stained with hematoxylin and eosin.

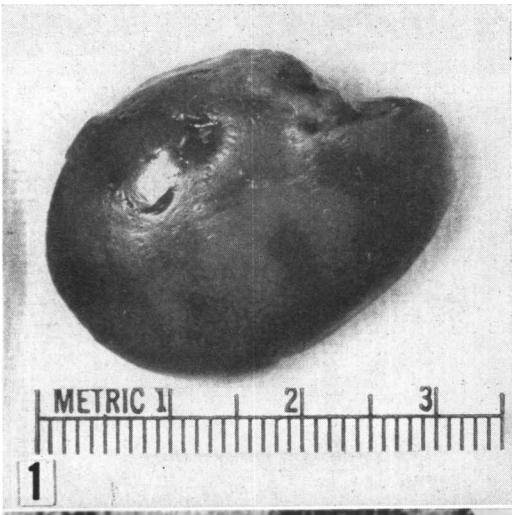
Results. Histochemical changes. In 10 of the 11 rats with fatty metamorphosis, the fat was present in only a portion of the liver (Fig. 1). The marked reduction in staining for SD activity in 9 of the deficient animals was observed only in the fatty portions of the liver (Fig. 2). In the majority of the control rats this centrolobular reduction was slight. Staining for $NADH_2D$ demonstrated no effect of Vit. B_6-deficiency on the activity of this enzyme.

Enzyme assays. Since Vit. B_6-deficiency causes fatty metamorphosis of the liver(9) the changes in enzyme activity were correlated with the presence or absence of fatty metamorphosis. When the enzyme activities of the fatty and non-fatty portions of the livers were compared, SD activity was found to be 22% lower in the fatty portion and $NADH_2D$ activity 29% lower. However, when the activities of both enzymes were expressed as units of protein they were slightly, but not significantly, lower in the fatty portion of the liver.

To assure uniformity in the samples, only the results obtained from non-fatty portions of the deficient livers were included in the data summarized and all data were expressed in terms of units of protein (Table I). Neither food restriction nor a deficiency of Vit. B_6 influenced the SD activity, as determined by this method. Food restriction alone did result in a significant reduction of $NADH_2D$ activity, but a deficiency of Vit. B_6 produced a further significant reduction.

Discussion. Although our chemical assays agree with Tulpule and Patwardhan's(10) finding that Vit. B_6-deficiency does not influence the SD activity of the whole liver, both our histochemical studies and our homogenate assays reveal diminished SD activity in the fatty portions of the deficient livers. Although the co-existence of fat and SD would seem to indicate that the fat is responsible for the decreased SD activity, SD activity may actually be independent of fatty change. For example, both fat and SD activity may increase centrally in the livers of chronic alcoholics (8).

Vit. B_6-deficiency caused a significant reduction in $NADH_2D$ measured by homogenate assay and calculated in terms of units



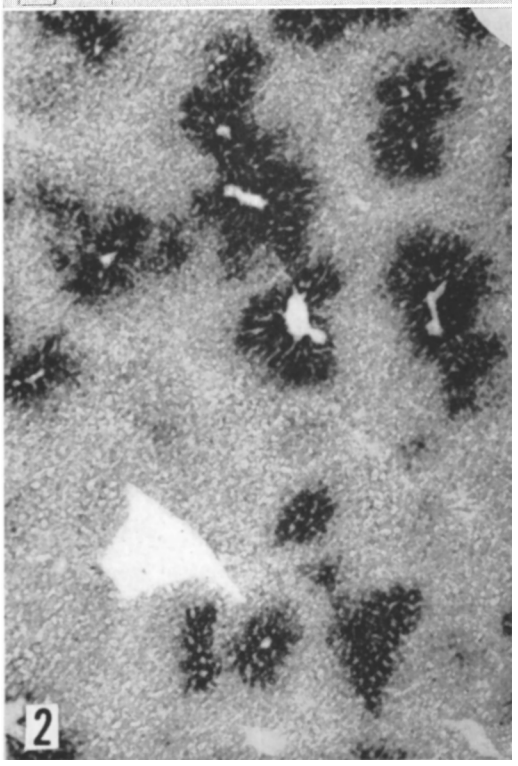


FIG. 1. A fatty liver from a pyridoxine-deficient rat showing pale focal areas that represent fat deposition.

FIG. 2. Succinic dehydrogenase activity in a fatty portion of liver of a B_6-deficient rat. Marked diminution of enzyme activity in centrolobular zones is indicated by light staining in these areas (× 25).

TABLE I. Effect of B_6-Deficiency on Liver Protein, SD and $NADH_2D$.

Dietary regimen	No. rats	Protein, %	SD, units/mg protein	$NADH_2D$, M/kg protein/hr
B_6-deficient*	18	20.1 ± .38†	10.2 ± .55	25.5 ± .77‡
Paired wt control	18	19.7 ± .49	10.9 ± .31	29.3 ±1.21§
Ad libitum control	18	19.7 ± .35	10.1 ± .38	33.4 ± .98

* Data from non-fatty portions only. † S.E. of mean. ‡ $P<.02$ when compared with two control group. § $P<.02$ when compared with *ad libitum* group.

per liver protein. However, there was no reduction in staining for $NADH_2D$ by the histochemical method employed. This discrepancy may be because the histochemical method is not satisfactory for quantifying small differences in enzyme concentrations.

The cause of the reduction in liver $NADH_2D$ in B_6-deficiency is not immediately apparent. Burch *et al*(11), who demonstrated the reduction of this enzyme in a child who had died of kwashiorkor and in dying riboflavin-deficient rats, found it to be held very firmly by liver tissue and regarded it as a crucial flavin enzyme. Their results may indicate that a rather profound alteration in metabolism is the cause. Wainio and co-workers(12) reported that DPNH-cytochrome C reductase falls in protein deficient rats; however, Beaton *et al*(3) could find no evidence that B_6-deficiency impairs maintenance or synthesis of tissue protein in the albino rat.

Summary. In histochemical studies fatty change in the livers of Vit. B_6-deficient rats was associated with reduction in SD activity in the centrolobular portions. In homogenate assays of non-fatty portions of liver, Vit. B_6-deficiency had no effect on the SD activity. The deficiency significantly reduced the $NADH_2D$ activity, however, even after the nonspecific effect of undernutrition and fatty change had been eliminated.

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1. Marsh, M. E., Greenberg, L. D., Rinehart, J. F., *J. Nutr.*, 1955, v56, 115.

2. Snell, E. E., *Physiol. Rev.*, 1953, v33, 509.

3. Beaton, J. R., Beare, J. L., Beaton, E. F., Caldwell, E. F., Ozawa, G., McHenry, E. W., *J. Biol. Chem.*, 1954, v207, 385.

4. Burch, H. B., Lowry, O. H., Padilla, A. M., Combs, A. M., *ibid.*, 1956, v223, 29.

5. Slater, E. C., *Biochem. J.*, 1950, v46, 484.

6. Neufeld, H. A., Scott, C. R., Stotz, E., *J. Biol. Chem.*, 1954, v210, 869.

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *ibid.*, 1951, v193, 265.

8. French, S. W., *A.M.A. Arch. Path.*, 1960, v69, 303.

9. Rinehart, J. F., Greenberg, L. D., *Am. J. Clin. Nutr.*, 1956, v4, 318.

10. Tulpule, P. G., Patwardhan, V. N., *Arch. Biochem.*, 1952, v39, 450.

11. Burch, H. B., Arroyave, G., Schwartz, R., Padilla, A. M., Behar, M., Viteri, F., Scrimshaw, N. S., *J. Clin. Invest.*, 1957, v36, 1579.

12. Wainio, W. W., Eichel, B., Eichel, H. J., Person, P., Estes, F. L., Allison, J. B., *J. Nutr.*, 1953, v49, 465.

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Cell Culture Studies on Antiviral Agents: I. Action of Cytosine Arabinoside and Some Comparisons with 5-Iodo-2-Deoxyuridine.* (28834)

D. A. BUTHALA (Introduced by R. O. Stafford)
The Upjohn Co., Kalamazoo, Mich.

An understanding of some of the mechanisms of cell-virus interaction has encouraged investigators searching for chemotherapeutic antiviral drugs. Recently herpes keratitis of of humans and rabbits has been successfully treated with 5-iodo-2-deoxyuridine (IUDR)