Hepatocellular Lipoid Changes in Pantothenic Acid Deficiency

ZOLTON T. WIRTSCHAFTER, M.D.* AND JOHN R. WALSH, M.D.†

PANTOTHENIC acid is known to be essential in the intermediary metabolism of carbohydrate, fat and protein. Hence, one would expect pantothenic acid deficiency to involve the liver, and to cause lipid alteration. Yet such authoritative workers in the field as Popper and Schaffner[1] have declared "The best known deficiencies, the avitaminoses, have little effect on the liver. Hepatic changes, if any, are those of a non-specific reaction to disorders elsewhere in the body." In experimentally produced pantothenic acid deficiency in man, results of liver function tests (bromsulfalein and serum bilirubin) were normal.[2] The observations of Morgan and Lewis[3] further support the opinion that deprivation of pantothenic acid fails to cause fatty livers in rats. Cambridge and Copping[4] also reported an absence of lipid in the livers of pantothenic acid-deficient rats.

Yet other investigations give support to the known importance of pantothenic acid in hepatic function. Reports that the administration of pantothenic acid resulted in improved liver function are numerous.[5-9] Further, in human liver disease, a low serum pantothenic acid level and diminished urinary excretion of pantothenic acid has been observed.[5,6] Ueshima et al.[6] expressed the opinion that a metabolic disturbance of pantothenic acid may exist which produces an impairment and alteration of certain definite liver functions, particularly alterations in the metabolism of alpha-keto acids, cholesterol and diminution in hippuric acid synthesis.

From The Research Laboratory, V. A. Hospital, and the University of Oregon Medical School, Portland, Oregon.

* Associate Chief of Staff; † Chief of Medical Service, V. A. Hospital, Portland, Oregon.

The occurrence of fatty livers in pantothenic acid deficiency has been reported in dogs.[10,11] Jurgens and Pfaltz[12] showed the effect of pantothenic acid deficiency on growth in rats, and reported diffuse fatty livers. Thus, there exists, first of all, a probably therapeutic role for pantothenic acid in liver disease. Then there exists contradictory evidence as to the effect of pantothenic acid deficiency. Finally, there is a question of the technic used to evaluate the liver lipid if present, as some report[10-12] and, if absent, as others report.[3,4] Hence the present work was undertaken. A definite factor in our study, as in a previous one involving carbon tetrachloride and transaminase,[13] was the evident superiority of oil red O staining in clarification of lipid changes. Sections stained with hematoxylin and eosin cannot be relied upon to interpret the presence or absence of lipid globules in hepatic tissue.

MATERIALS AND METHODS

Animals: Mature rats of the Long-Evans strain (weight 150 to 250 gm.) were used in this experiment. The experiment was designed to provide groups of animals of comparable sex and age (Table 1).

Diet: The normal animals were on the Rockland Rat Diet (complete), which consists of cane molasses, soy bean meal, fish meal, condensed buttermilk, gluten meal, wheat germ oil, oats, wheat bran, wheat flour middlings, yellow corn, hulled barley, hulled oats, wheat, milk powder, alfalfa leaf meal, A & D Feeding Oil, steamed bone meal, salt, irradiated yeast, linseed oil meal, corn oil meal and calcium carbonate, fortified with minerals and vitamins.

The experimental animals were on this diet until they were placed on the pantothenic acid-deficient diet (see Appendix). This diet is the vitamin B complex test diet which is supplemented with all of the vitamin diet fortification mixture, with the exception of calcium pantothenate, and was procured

TABLE I
Effect of Pantothenic Acid Deficiency on Weight

Long-Evans Rat	Sex	Age (days)	Duration of Pantothenic Acid-Deficient Diet	Weight Gain (gm.)	Liver Weight (gm.)	Adrenal Weight (gm.)	Kidney Weight (gm.)	Spleen Weight (gm.)
			Group 1					
60xW3	F	66	Control	10	4.850	0.062	1.054	0.508
60xBH4	F	66	1 week	7	6.490	0.066	1.248	0.576
60 B98	F	66	1 week	12	6.230	0.068	1.414	0.610
			Group 2					
60xW6	M	74	Control	37	9.870	0.048	1.794	1.244
60 B99	M	74	2 weeks	40	9.038	0.034	1.748	1.272
60xW5	M	74	2 weeks	34	9.370	0.038	1.668	0.904
			Group 3					
60xTH28	M	67	Control	109	10.650	0.024	2.198	0.784
60xTH33	M	67	3 weeks	65	6.251	0.050	1.570	0.372
60xTH27	M	67	3 weeks	40	7.275	0.046	1.492	0.302
			Group 4					
60xGH47	M	74	Control	138	14.128	0.082	2.260	0.714
60xTH46	M	74	4 weeks	75	9.000	0.062	1.832	0.560
60xBH45	M	74	4 weeks	101	12.216	0.070	1.816	0.496
60 B72	M	58	4 weeks	21	5.310	0.030	1.074	0.412
60 B74	M	73	6 weeks	70	6.320	0.024	1.221	0.310

from the Nutritional Biochemicals Corporation, Cleveland, Ohio.

Environment: The experimental animals were housed in an air-conditioned room in which the temperature was maintained at 73° to 75°F., and the relative humidity at 45 to 50 per cent.

Autopsy: Animals were autopsied serially at one, two, three, four and six weeks. All autopsy procedures were performed with rats under ether anesthesia, and a detailed inspection was made of all viscera. Particular attention was focused upon the liver. All organs were removed and weighed on a Roller-Smith torsion balance, except the liver, which was weighed on an analytical balance. Sections of the liver were directly immersed in 10 per cent neutral formalin for fixation. Frozen microscopic sections were stained with oil red O, as described by Lillie.[14]

RESULTS

Growth Rate

The effect of pantothenic acid deficiency on growth rate is seen in Table I. The weight of these animals was unaffected until the third week of the deficiency. In the third and fourth week, there was a marked difference in weight gain in comparison to the control animals. These findings are similar to those of Barboriah et al.[15] The weights of the liver and spleen, and to a lesser extent the kidneys, are reduced in the three and four week experimental animals.

Lipid Alterations

In normal rat liver cells, fat globules are not visible[13] (Fig. 1). In contrast, rats on a pantothenic acid-deficient diet display prominent lipid globules in hepatic cells within one or two weeks (Fig. 2). With the duration of a deficient diet increased to three to six weeks, there is a progressive increase in the amount of well circumscribed lipid aggregates in the central zone of the hepatic lobule, and in the mid-zonal and periportal areas (Fig. 3). Note

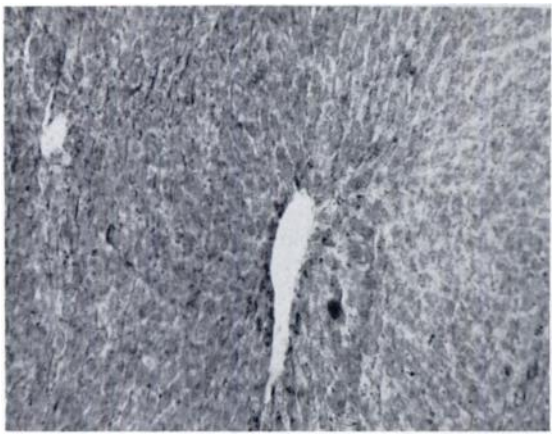

FIG. 1. Fat droplets are seldom visible in normal rat liver cells. Original magnification × 100, oil red O stain.

FIG. 2. Liver section from rat on a pantothenic acid-deficient diet for two weeks demonstrates dispersed, nonconfluent fat droplets in hepatic cells. Original magnification × 37, oil red O stain.

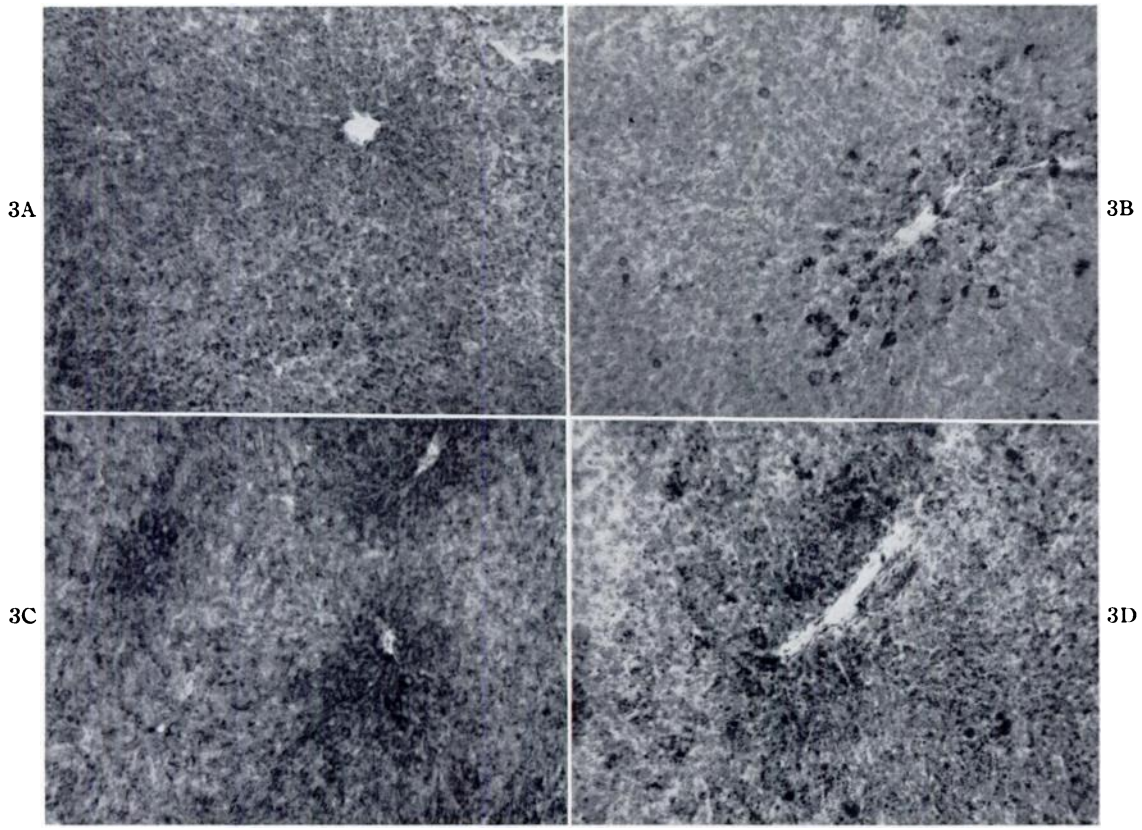

FIG. 3. Liver section from rat on a pantothenic acid-deficient diet for four weeks demonstrates a marked increase of fat droplets in the centrolobular and mid zonal areas (A), and in the periportal areas (B) Liver section from rat on Pantothenic acid-deficient diet for six weeks demonstrates a marked increase of fat droplets in the centrolobular areas (C) and in the periportal area (D). Original magnification × 100, oil red O stain.

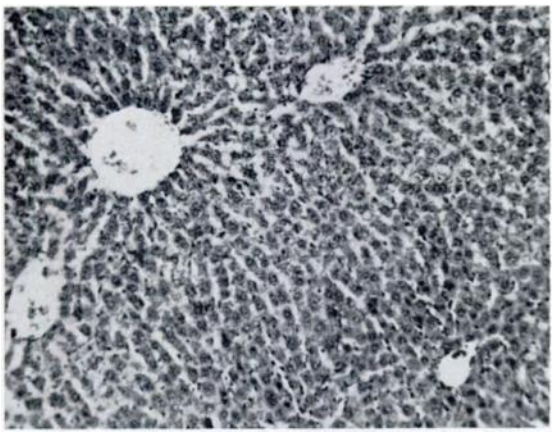

FIG. 4. Hematoxylin and eosin liver section from the same animal described in Figure 3A and 3B does not demonstrate presence of marked disease shown in the oil red O section. Original magnification × 100.

that these observations were obtained by the oil red O method, yet microscopic liver sections of the same animal as described in Figure 3, stained with hematoxylin and eosin, showed minimal change (Fig. 4). Thus the oil red O technic clearly showed unequivocal hepatocellular alterations that were not reflected by hematoxylin and eosin staining.

COMMENTS

This study, by means of the oil red O fat stain method indicating lipid globules in hepatic

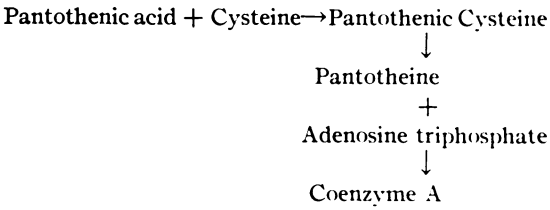

FIGURE 5.

tissues, has demonstrated the intense effects of pantothenic acid deprivation. The progressive increase in centrolobular, periportal and midzonal fat establishes the relationship of the hepatocellular damage to this vitamin deficiency. It is valuable to recall that essentially all pantothenic acid is present in tissues as coenzyme A (Fig. 5), known for its central position in intermediary metabolism.

A summary of the synthesis of coenzyme A may be expressed as follows: Coenzyme A combines with acetate to form acetyl coenzyme A, which in this active form participates in several metabolic processes (Fig. 6). One major function of coenzyme A is acetylation, forming acetylcholine, acetylated amino acids, hyaluronic acid, acetysulfanilamide, acetoacetic acid and others. Another is its participation in the Krebs citric acid cycle, in which acetyl coenzyme combines with oxalacetic acid to form citric acid. This cycle funnels the oxidative activity for carbohydrates, fats and

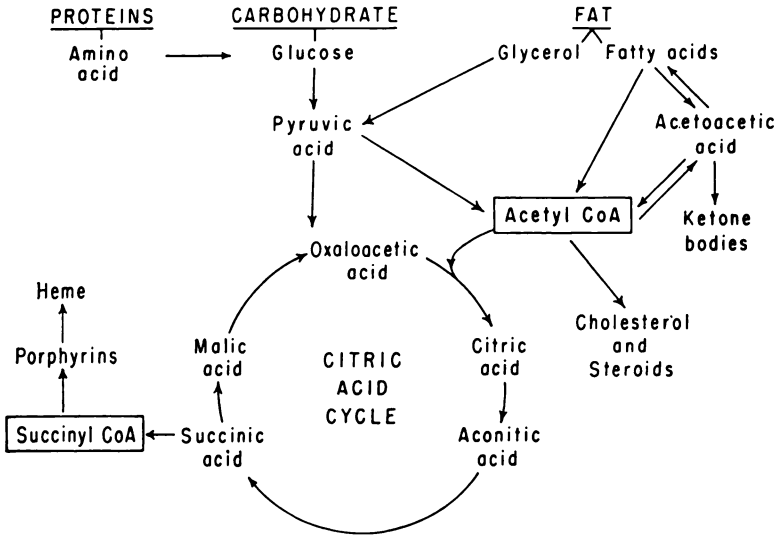

FIG. 6. Metabolic relationships of coenzyme A.

many amino acids. In fat metabolism, acetyl coenzyme A is formed by the beta-oxidation of fatty acids, and aceto-acetic acid. In the formation of heme, the prosthetic group of hemoglobin, the coenzyme A derivative of succinic acid, succinyl coenzyme A, combines with glycine to form alpha-amino beta-ketoadipic acid, which is the precursor of delta-amino-levulinic acid. The latter is converted into porphobilinogen, then sequentially through the porphyrins to heme.

The complicated chemical relationships exhibited in these combinations give evidence of the role pantothenic acid deficiency might play in a wide variety of metabolic disorders. This morphologic study of hepatocellular changes occurring during the course of pantothenic acid deficiency confirms in one mode, one particular kind of evidence. It shows that the pantothenic acid-deficient rat displays a hepatic lesion characterized by centrolobular, periportal and mid-zonal fat accumulation.

SUMMARY

Mature rats on a pantothenic acid-deficient diet showed hepatic changes characterized by progressive increase in lipid globules in the central, periportal and mid-zonal areas. Morphological studies employing oil red O technic clearly established the relationship of hepatocellular damage to the deprivation of pantothenic acid.

ACKNOWLEDGMENT

Mr. Dean C. Altman, head of the Illustrations Department of the Veterans Administration Hospital, prepared the photomicrographs.

APPENDIX

The pantothenic acid-deficient diet is composed of the following: Vitamin test casein (hot alcohol extracted, 18 per cent; sucrose, 68 per cent; corn oil, 10 per cent; and U.S.P. Salt Mixture No. 2,* 4 per cent. This diet is supplemented by the vitamin diet fortification mixture which is composed of the following: (these figures are given in grams per 100 pounds diet) vitamin A concentrate (200,000 units per gm.), 4.5; vitamin D concentrate (400,000 units per gm.), 0.25; alpha tocopherol, 5; ascorbic acid, 45; inositol, 5; choline chloride, 75; menadione, 2.25; *p*-aminobenzoic acid, 5; niacin, 4.5; riboflavin, 1; pyridoxine hydrochloride, 1; thiamine hydrochloride, 1; (the following figures are given in milligrams per 100 pounds diet) biotin, 20; folic acid, 90; and vitamin B_{12}, 1.35.

* U.S.P. Salt Mixture No. 2 is composed of 13.58 per cent calcium biphosphate, 32.50 per cent calcium lactate, 2.97 per cent ferric citrate, 13.70 per cent magnesium sulfate, 23.98 per cent potassium phosphate (Dib.), 8.72 per cent sodium biphosphate, 4.35 per cent sodium chloride.

REFERENCES

1. Popper, H. and Schaffner, F. Nutritional hepatic injury. *Arch. Int. Med.*, 94: 785, 1954
2. Hodges, R. E., Bean, W. B., Oneson, M. A. and Bleiler, R. Human pantothenic acid deficiency produced by omega-methyl pantothenic acid. *J. Clin. Invest.*, 38: 1421, 1959.
3. Morgan, A. F. and Lewis, E. M. The modification of choline deficiency by simultaneous pantothenic acid deficiency. *J. Biol. Chem.*, 200: 839, 1953.
4. Cambridge, C. and Copping, A. Effect of pantothenic acid deficiency on certain organs of the rat. *Brit. J. Nutrition*, 2: 67, 1957.
5. Ueshima, T., Shigeta, Y., Wada, M., Oji, K and Yoshida, T. Studies on the metabolism of Pantothenic acid in liver damage. I. Urinary excretion of pantothenic acid in patients with various liver diseases and its correlation with liver function. *J. Vitamin., Osaka*, 2: 299, 1956.
6. Ueshima, T., Unno, H., Shigeta, Y., Wada, M., Oji, K. and Yoshida, T. Studies on the metabolism of pantothenic acid in liver damage. II. Pantothenic acid treatment in patients with liver disease. *J. Vitamin., Kyoto*, 4: 149, 1958.
7. Annoni, G. Primi esperimenti clinici con la pantetina. *J. Farmaco ed Sc., Pavia*, 10: 772, 1955.
8. Annoni, G. and Lucchelli, P. L'azione dell acido pantotenico sulla funzionalita epatica. *Acta vitamino.*, 4: 60, 1950.
9. Banche, M. and Dapri, L. Acido pantotenico ed hepatopatie (nota preventiva). *Minerva med.*, 2: 1271, 1950.
10. Schaefer, K., McKibbin, J. and Elvehjem, C. Pantothenic acid deficiency in dogs. *J. Biol. Chem.*, 143: 321, 1942.
11. Silber, R. Studies of pantothenic acid deficiency in dogs. *J Nutrition*, 27: 425, 1944.
12. Jurgens, R. and Pfaltz, H. Entzundliche

Erkrankungen der Respirationsorgane bei Ratten infolge von Pantothensauremangel. *Internat. Ztschr. Vitaminforsch.*, 14: 243, 1944.

13. WIRTSCHAFTER, Z. T. and TSUJIMURA, J. K. Serum glutamic oxalacetic transaminase. Specificity of values in hepatocellular injury. *Arch. Environmental Health*, 2: 16, 1961.

14. LILLIE, R. D. Various oil soluble dyes as fat stains in supersaturated isopropanol technic. *Stain Technol.*, 19: 55, 1944.

15. BARBORIAH, J. J., KREHL, W. A. and COWGILL, G. R. Effect of short term Pantothenic acid deficiency in the growing rat. *J. Nutrition*, 64: 251, 1958.