transport as well as its incorporation into protein as a function of protein synthesis. Bovine insulin (0.1 unit/ml) significantly increased the intracellular accumulation of proline when protein synthesis was maximally reduced by addition of puromycin to the tissue. It is concluded that the stimulation of amino acid transport into muscle cells by insulin is independent of the increase in protein synthesis effected by the hormone.

We are indebted to Dr. M. A. Root, Eli Lilly & Co., for the gift of insulin.

1. Fritz, G. R., Knobil, E., *Fed. Proc.*, 1964, v23, 409.

2. ———, *Nature*, 1963, v200, 682.

3. Carlin, H., Hechter, O., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 127.

4. Akedo, H., Christensen, H. N., *J. Biol. Chem.*, 1962, v237, 118.

5. Manchester, K. L., Young, F. G., *Vitamins and Hormones*, 1961, v19, 95.

6. Kostyo, J. L., Knobil, E., *Endocrinology*, 1959, v65, 525.

7. Peckham, W. D., Knobil, E., *Biochim. Biophys. Acta*, 1962, v63, 207.

8. Fritz, G. R., Knobil, E., *ibid.*, 1963, v78, 773.

9. Kipnis, D. M., Reiss, E., Helmreich, E., *ibid.*, 1961, v51, 519.

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Transketolase Activity in Experimental Thiamine Deficiency and Hepatic Necrosis.* (29395)

JAMES FENNELLY, OSCAR FRANK, HERMAN BAKER AND CARROLL M. LEEVY
Division of Hepatic Metabolism and Nutrition, Department of Medicine, Seton Hall College of Medicine, Jersey City, N. J.

Measurement of red blood cell transketolase activity has been widely used to evaluate thiamine status(1). However, there is often no relationship between enzyme and vitamin levels in thiamine deficiency. Absent correlation is most marked in the presence of liver disease(2). The reason for this lack of agreement is unknown since no data are available on the effects of hepatic injury on circulating and tissue transketolase and thiamine levels. We investigated the influence of experimental liver necroses with and without dietary-induced thiamine deficiency on these parameters.

Materials and methods. One hundred and twenty weanling Sprague-Dawley rats were fed a complete diet containing all of the B-complex vitamins. After 10 days on this regimen, one-half of the animals were switched to a thiamine deficient diet for 21 days; litter mate controls were maintained on the complete diet. After 21 days on a thiamine deficient diet, animals were given 200 μg of thiamine HCl intramuscularly each day for 5 to 21 days. Acute liver injury was induced in thiamine deficient and repleted rats by a single oral dose of 0.2 ml of carbon tetrachloride (CCl_4) per 100 g rat weight. At 48 hour intervals from the start of the thiamine deficient diet, representative animals were anesthetized and 5 to 6 ml of blood drawn from the aorta and collected in citrated and dry tubes. The liver was then removed, a section obtained for histology, and the remainder flushed with saline and rapidly frozen. Transketolase activity was measured in red blood cell homogenates, serum, and 10% liver homogenates, using the method of Brin *et al*, with and without *in vitro* addition of thiamine pyrophosphate(3). For this purpose, red blood cells were washed twice with saline; particular care was taken in washing because of the known hemolytic tendency of red cells from CCl_4-intoxicated animals. Results were expressed in micrograms of ribose utilized per milliliter per hour. Thiamine levels were determined in whole blood and liver with a protozoan, *Ochromonas danica* (4). Tissue, enzyme, and vitamin levels were

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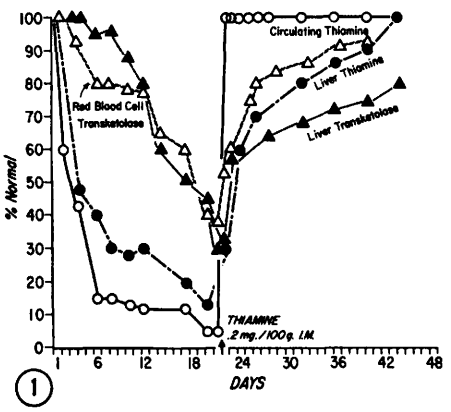

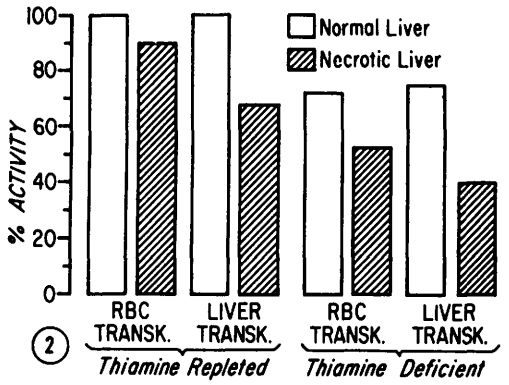

FIG. 1. Influence of thiamine depletion and repletion on thiamine and transketolase levels in Sprague-Dawley rats.

FIG. 2. Influence of hepatic necrosis on red blood cell and liver transketolase in thiamine-repleted and deficient Sprague-Dawley rats.

related to dry weight and nitrogen content as measured by the Dumas method(5).

Results. Histological studies of liver revealed no abnormalities in the uncomplicated thiamine deficient animals or normal controls. Specimens obtained 24 hours after giving CCl_4 exhibited centrilobular necrosis and inflammation which occupied one-third of the hepatic lobule; sections obtained 48 hours after CCl_4 showed numerous mitoses with fat and less necrosis or inflammation. Those obtained at 120 hours after CCl_4 were normal (6). The thiamine content of normal rat blood was 322 ± 69 mμg per ml, and of liver 47.4 ± 10.6 mμg per mg dry weight. Red blood cells from control animals utilized 881 ± 68 μg of ribose per ml per hour, serum utilized 222 ± 33 μg of ribose per ml per hour, and the 10% liver homogenate 1025 ± 143 μg of ribose per ml per hour.

In normal rats, the thiamine deficient diet caused a 40% reduction in blood thiamine within 48 hours followed by an equivalent depression of liver thiamine within 96 hours. During this period there was no significant change in the red blood cell, plasma, or liver transketolase. A 40% decrease in liver enzyme levels occurred in 14 days, in plasma enzyme in 16 days, and red blood cell enzymes in 17 days after initiating the diet (Fig. 1). Liver necrosis in thiamine repleted animals was associated with a 3-fold increase in serum transketolase activity, 32% reduction in liver transketolase, and 10% reduction in red blood cell transketolase. Hepatic necrosis in animals maintained on a thiamine deficient diet for 14 days caused a 58% further reduction in liver transketolase, and a 45% further decrease in red blood cell transketolase (Fig. 2).

Addition of thiamine pyrophosphate to the deficient red blood cell or liver transketolase test system caused restoration of depressed enzyme activity to 85 to 100% of control values during the first 14 days of thiamine deficiency; after this period the effect became progressively less. When liver injury was superimposed on thiamine deficiency, *in vitro* addition of thiamine pyrophosphate was less effective; it caused increase of enzyme activity to 60 to 80% of control values. Thiamine therapy in animals with a normal liver caused a rapid return of circulating vitamin levels with a slower restoration of liver thiamine, red blood cell transketolase, and liver transketolase (Fig. 1). Despite *in vivo* administration of thiamine, transketolase activity in both red blood cells and liver remained low as long as active evidence of hepatic necrosis was present.

Discussion. In experimental thiamine deficiency, circulating thiamine provides the most sensitive index to vitamin depletion followed by tissue thiamine, red blood cell transketolase, liver transketolase and serum transketolase, in that order. A significant increase in serum enzymes occurs with liver injury; it is, therefore, necessary adequately to wash red cells and remove all serum to obtain accurate red cell transketolase measurements.

If time factors are considered, transketolase levels are related to thiamine content in the presence of a normal liver. In contrast, there is no correlation between enzyme and vitamin levels in the presence of experimental liver necrosis. This is due to the fact hepatic injury accentuates the disparity between rate of reduction of thiamine and transketolase levels during vitamin depletion and impedes or prevents the restoration of tissue thiamine during replacement therapy. Interference with conversion of free thiamine to its metabolically active coenzyme and to synthesis of its apoenzyme is contributory since, in the presence of active hepatic necroses, there is incomplete response to both *in vitro* and *in vivo* addition of thiamine pyrophosphate.

Summary. Transketolase activity in rat liver and red blood cells is decreased at a significantly slower rate than circulating or tissue thiamine in dietary-induced thiamine deficiency; superimposition of carbon tetrachloride-induced liver necrosis on thiamine deficiency increases the disparity between alterations of vitamin and enzyme levels. Replacement therapy in thiamine deficient animals with a normal liver only slowly restores tissue thiamine and transketolase; in the presence of active liver necrosis low tissue vitamin and enzyme levels persist despite administration of thiamine.

1. Wolfe, S. J., Brin, M., Davidson, C. S., *J. Clin. Invest.*, 1958, v37, 1476.

2. Fennelly, J., Baker, H., Frank, O., Leevy, C. M., *Clin. Research*, 1963, v11, 182.

3. Brin, M., *Ann. N. Y. Acad. Sci.*, 1962, v98, 528.

4. Baker, H., Frank, O., Fennelly, J., Leevy, C. M., *Am. J. Clin. Nutr.*, 1964, v14, 197.

5. Steyermark, A., *Quantitative Organic Microanalysis*, Steyermark, A., ed., Academic Press, 1961, 2 ed., p151.

6. Leevy, C. M., George, W., Deysine, M., Gnassi, A. M., *Exp. and Molecular Path.*, 1962, v1, 457.

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Myosin from Mice with Hereditary Muscular Dystrophy.* (29396)

H. OPPENHEIMER, K. BÁRÁNY AND A. T. MILHORAT
Institute for Muscle Disease, New York City

Myosin, one of the principal contractile proteins in muscle, is considerably reduced in quantity in various forms of muscular dystrophy and atrophy(1-5), but little is known about any qualitative changes of these proteins and their possible relation to the wasting process. This paper deals with the properties of myosin from mice with hereditary muscular dystrophy(6), particularly in terms of its macromolecular structure in solution.

Materials and methods. Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, supplied dystrophic mice and control litter mates of strain 129. They were sacrificed when 2-3 months old. Myosin was extracted repeatedly from their hind legs by the procedure of Hasselbach and Schneider(7), and it was freed of actomyosin and other impurities by at least 2 cycles of fractional precipitation. Sarcoplasmic proteins were obtained by extraction with 0.1 μ K-phosphate buffer, pH 7.5, in the cold for 2 hours. They were further fractionated in an LKB paper electrophoresis instrument(8) in 0.1 μ K-phosphate buffer, pH 6.4, at 1°-2°C for 16 hours at 5 volts/cm. Quantitative determination of muscle protein fractions (sarcoplasm, myosin, actin, and stroma), which were obtained by differential extraction(7), was done by Nesslerization(9) or the Biuret method (10). Actin was prepared from rabbit skeletal muscle by the procedure of Mommaerts (11). ATPase activity of myosin was assayed at 25°C for 3 minutes in media containing 0.25 mg myosin/ml, 30 mM KCl, 20 mM histidine buffer, pH 7.0, and either 10 mM Ca Cl_2, 5 mM ATP or 1 mM Mg SO_4, 1mM ATP. The latter medium was also used to assay activation of myosin ATPase

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