Heptulose Phosphate Formation in Thiamine Deficiency and Other Physiological States.* (28475)

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Recently a marked depression of activities of enzymes involved in conversion of nucleoside and sugar phosphate to heptulose phosphates has been observed in rat tissues made thiamine-poor by dietary lack of Vit. B_1(1). The following are questions for further study. Can the enzyme depression be produced by other means, as, for example, by oral feeding to mice of the antimetabolite, pyrithiamine hydrobromide? It so, does thiamine administration later restore the enzyme activities to normal in such vitamin-deficient rats and mice? What is the influence of growth and development? What events follow pancreatic diabetes or adrenalectomy? Is there any difference in the behavior of enzymes responsible for 2-ketoheptose phosphate formation *vs* those controlling the synthesis of 3-ketoheptose phosphate? Experiments designed to provide answers to these questions are described here.

Experimental. Materials. The chemicals and their suppliers were respectively: glucose-6-phosphate, Ba salt; fructose-6-phosphate, Ba salt; glucose-1-phosphate, K salt; adenosine, guanosine, inosine, thiamine HCl (Nutritional Biochemicals Corp.); pyrithiamine hydrobromide (Mann Research Lab., Inc.).

All of the other reagents of analytical grade were purchased from the usual commercial sources.

Dietary thiamine deficiency in the rat. Male rats weighing approximately 200 g were maintained on a commercial purified thiamine-free diet and were fed *ad libitum*. After 6 weeks, paralytic symptoms of severe Vit. B_1 deficiency developed. Half of the animals were sacrificed, and the other half were injected intraperitoneally with thiamine HCl (1.0 mg/0.5 ml of saline solution) 3 times a week for 2 weeks. Normal rats were maintained on Purina Chow. The animals were sacrificed and the tissues removed, and a 10% homogenate of each tissue was prepared and centrifuged at 18000 × g for 30 minutes. The supernatant was used for assay of the system which produces heptulose phosphate from sugar phosphate and nucleosides. The properties of the enzymes involved and their measurement have been described for nucleosides(1,2) and sugar phosphate(1, 3).

Fig. 1 indicates that thiamine deficiency in the rat resulted in a marked reduction to 5 to 50% of the level in control animals in

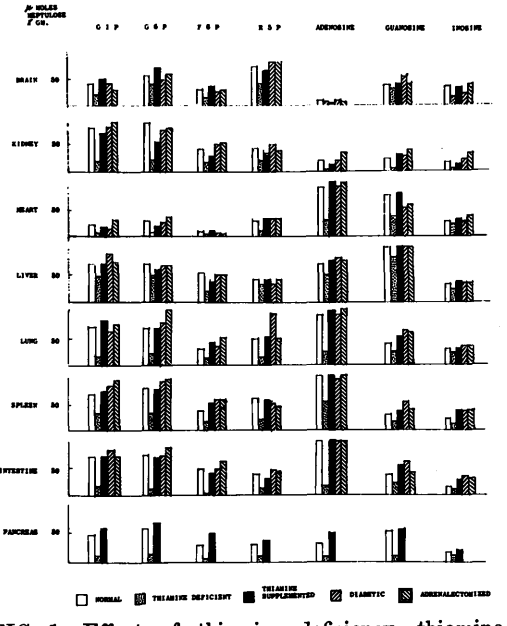

FIG. 1. Effect of thiamine deficiency, thiamine supplementation, diabetes and adrenalectomy on capacity of rat tissues to form heptulose phosphate from sugar phosphates and nucleosides. Ordinate represents heptulose phosphate formed per gram of fresh tissue. (Same in Fig. 2 and 3.)

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Abbreviations: 3-KHP, 3-ketoheptose phosphate; 2-KHP, 2-ketoheptose phosphate; G6P, glucose-6-phosphate.

the capacity of the tissue to convert a number of sugar phosphates to heptulose phosphate in the lung, spleen, intestine and pancreas. On the other hand, a reduced effect is observed in liver enzyme as noted previously(1).

Various effects on the nucleoside to heptulose phosphate transformation are evident. In thiamine deficient tissue, the enzyme activity was slightly decreased in liver and brain (10 to 30%), extensively reduced in intestine, lung and spleen (50 to 80%) and it almost completely disappeared from the heart.

A subsequent increase of enzyme activity to normal level was achieved after the animal's recovery from avitaminosis.

Effect of adrenalectomy and diabetes on heptulose phosphate formation. Diabetic rats were produced by injecting subcutaneously 1 ml of 2% (freshly crystallized) alloxan solution. After 12 days, rats with blood glucose greater than 500 mg % were sacrificed. Adrenalectomy was kindly performed by Dr. M. Hayashi on rats. These were given 1% NaCl in their drinking water for 5 days post-operatively and then sacrificed.

The rate of the enzymatic synthesis of heptulose phosphate in rat tissue was not influenced by adrenalectomy or alloxan diabetes when compared to the control group of normal rats illustrated in Fig. 1.

In this connection, Vincent(4) found no difference *in vivo* in rate of formation of co-carboxylase after administration of thiamine S^{35} to both normal and alloxan diabetic rats, and Ochoa and Rossiter(5) reported the same amount of co-carboxylase content of boiled extracts of livers of adrenalectomized and normal rats and no difference in the ability of liver slices from these animals to phosphorylate thiamine *in vitro*.

Effect of growth. The newborn rat possesses the systems found in the adult for conversion of nucleoside and sugar phosphate to heptulose phosphate (Fig. 2). Thus, growth resulted in a decreased amount of enzyme activity in heart tissue with the sugar phosphate substrate. On the other hand, a slight increase of enzyme activity towards adenosine was manifested in several tissues (kidney, lung, liver and spleen) in relation to growth. However, liver tissue exhibited a 200% increase in activity towards guanosine in animals 9 days old.

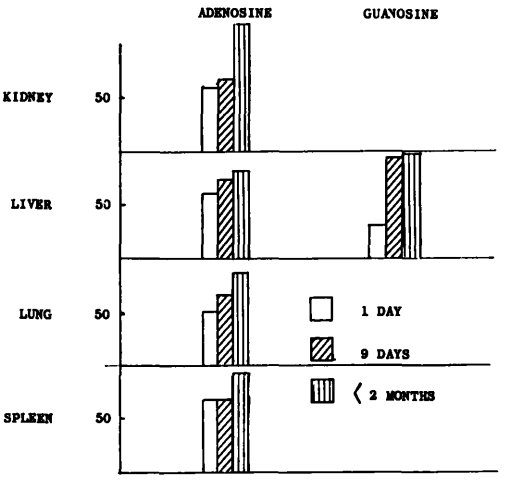


FIG. 2. Effect of age on the capacity of rat tissues to convert nucleoside to heptulose phosphate. Groups of different ages are identifiable by shading of bars.

Pyrithiamine-induced thiamine deficiency in mice. Three-month-old male white Swiss mice were fed orally with pyrithiamine hydrobromide solution 2 mg/day/mouse divided into 3 doses and given 4 hours apart. The sign of thiamine deficiency, paralysis, was generally evident after 5-7 days. Half of the animals were sacrificed at the time and the other half were injected intraperitoneally with thiamine HCl 1.0 mg/0.5 ml of saline solution, 3 times a week for 2 weeks. Then the animals were sacrificed and tissue supernatants were prepared as before. Normal mice were kept on Purina Chow.

In normal mouse tissue the level of enzyme activity of heptulose phosphate synthesis from either sugar phosphate or nucleoside was approximately the same as in normal rat tissue. The effect of thiamine deficiency on heptulose formation, however, was less marked in mice than it was in rats. The enzyme showed no uniform reduction in activity. However, a substantial decrease in activity was noticeable in all tissues against the substrate adenosine (Fig. 3). Addition of co-carboxylase *in vitro* to kidney, liver or lung did not stimulate enzyme activity significantly.

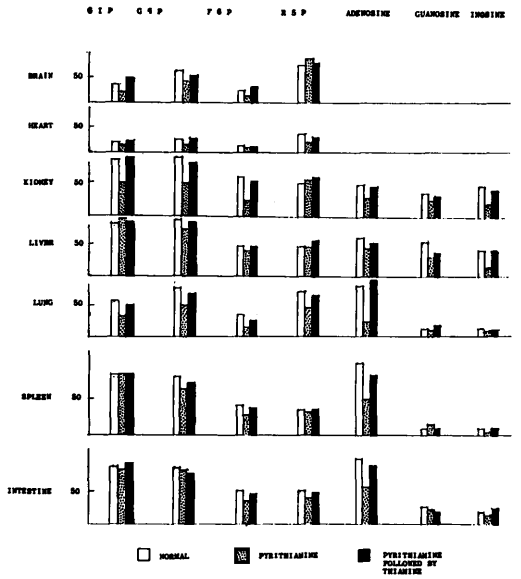


FIG. 3. Effect of pyrithiamine and pyrithiamine plus thiamine on ability of mouse tissues to form heptulose phosphate from sugar phosphates and nucleosides.

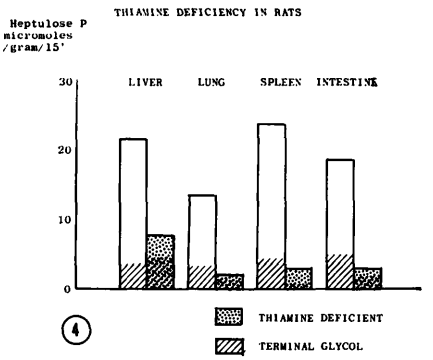
Comparison of capacity to form 3-keto and 2-ketoheptose phosphates. A method(6) was successfully devised for measuring 3KHP which possesses a terminal glycol linkage. This can be split after periodate oxidation to liberate measurable formaldehyde(7). This method was successfully employed to study the amount of formation of 2KHP and 3KHP and, in the present experiments, was applied to well-dialyzed tissue supernatant preparations.

In normal rat tissue (Fig. 4) about 20% of the heptulose phosphate formed from G6P contains terminal glycol linkages and is presumed to be 3KHP. In the deficient animals, the rate of decrease in formation of 2KHP was greater than of 3KHP in tissues like liver, spleen and intestine.

The amount of 3KHP formation by normal mice tissue was 30 to 45% of the total heptulose phosphate synthesized. A depressed heptulose phosphate enzyme system was also indicated in thiamine-deficient mice. The extent of the decrease in the 3KHP system was greater in spleen and intestine than in liver, lung and kidney (Fig. 5).

Discussion. Disorders in carbohydrate biochemistry associated with beri beri in man (8) and polyneuritis in birds(9) have been attributed to the disappearance from tissues of thiamine pyrophosphate which is an essential prosthetic group of a number of enzymes(10,11). Investigation of the metabolic changes during thiamine deficiency has centered on reduction of thiamine pyrophosphate from the tissue and on the *in vitro* reactivation of some enzymes which possess thiamine pyrophosphate as a prosthetic group, like pyruvic dehydrogenase α-keto glutaric dehydrogenase(10) and transketolase(11).

The present study indicates that the rate of metabolism of nucleoside and sugar phosphate to heptulose by animal tissue was distinctly influenced by the amount of thiamine pyrophosphate in the animal tissue. This amount, in turn, is dependent on the dietary source. The greater the limitations of this factor, the greater was the severity in reduction of heptulose formation as exhibited in



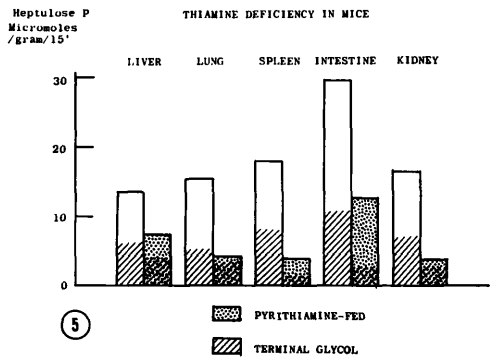


FIG. 4. Relative formation of 3-KHP and of 2-KHP by thiamine-deficient rat tissues. Diagonal lines indicate μmoles of heptulose P possessing a terminal glycol linkage.

FIG. 5. Relative formation of 3-KHP and of 2-KHP by thiamine-deficient mouse tissues. See Fig. 4 legend.

thiamine-deficient rat tissue of lung, spleen, intestine and pancreas. This low activity can be restored to normal by administering thiamine HCl to the deficient animal.

Vit. B_1-deficient mice were successfully produced by feeding pyrithiamine hydrobromide. The effect on heptulose phosphate production from sugar phosphate was less pronounced in the deficient mouse than in the rat. The tissue enzyme showed no uniform reduction in activity. However, a substantial decrease in activity was noticeable in all tissues with the nucleoside, adenosine, as substrate, but not guanosine and inosine. A restoration of enzyme activity to the normal level was accomplished after administration of thiamine to the deficient mice.

The failure of the *in vitro* addition of thiamine pyrophosphate to restore the nucleoside activity in Vit. B_1-deficient mouse tissue may signify that thiamine pyrophosphate has a double function both as co-enzyme and as a prerequisite for apoenzyme synthesis as well (compare with(1)). Similarly, Takeichi *et al.*(12) reported a decrease of pyruvate apo oxidase activity in Vit. B_1 deficient rats.

Summary. In the rat the systems responsible for heptulose phosphate formation from sugar phosphate and nucleoside were completely developed at birth with the exception of low activity in the liver towards nucleoside. The thiamine-deficient adult rat exhibited a reduction of 50 to 95% of enzyme activity in heptulose phosphate formation in lung, spleen, intestine and pancreas. Subsequently, a supplement of thiamine HCl to the deficient animal restored the enzyme level to normal. The effect on heptulose phosphate formation was less extensive in mice made Vit. B_1 deficient by feeding pyrithiamine hydrobromide than it was in rats, but there was a marked decrease in activity towards adenosine. Neither the absence of insulin nor of adrenocorticoid hormone had any effect on heptulose phosphate formation from sugar phosphate and nucleoside. In normal rat and mouse tissues 20 to 45% of heptulose phosphate was due to 3-ketoheptose phosphate, the formation of which was also depressed in Vit. B_1 deficient tissue.

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