Intestinal Metabolism in Thiamine Deficiency[1] (37642)

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Thiamine deficiency is a common finding in selected chronic alcoholic patients (1) who often exhibit anorexia, weight loss, and malabsorption of a variety of substances (D-xylose, fat, folic acid, thiamine, vitamin B_{12}) (2–6). The role of thiamine deprivation *per se* in these complex clinical disorders is uncertain. It is of interest, however, that diet-induced pure thiamine deficiency in rats is accompanied by anorexia, delayed gastric emptying (7–9), and failure of the thiamine-deficient rats to gain weight comparably to their pair-fed controls whose weighed daily dietary intake is identical (10). Similar findings have been reported when thiamine deficiency was induced with thiamine antagonists (7, 11). In addition, studies performed in experimental animals some 30 years ago have suggested that both thiamine and group B vitamin depletion may impair the absorption of glucose and galactose (8, 12, 13). More recently, decreased amino acid absorption from the intestine has also been reported in thiamine deficiency (14).

The biochemical basis of the low thiamine-induced anorexia, weight loss, and presumed malabsorption is unclear. It is known, however, that thiamine pyrophosphate is a coenzyme for three important enzymes: transketolase (EC 2.2.1.1), pyruvate decarboxylase (EC 4.1.1.1), and α-ketoglutarate decarboxylase (EC 1.2.4.2). Prior studies have clearly shown that thiamine deficiency may induce a significant depression of the activity of these enzymes, especially the former two, in various tissues (13, 15–18). In the intestine, both pyruvate decarboxylase and transketolase activity were found to be depressed but a better temporal relationship to anorexia was found with a decrease in the transketolase (7). Theoretically a depression in the activity of pyruvate decarboxylase and α-ketoglutarate decarboxylase, enzymes vital to the operation of the citric acid cycle, could result in decreased synthesis of ATP in the gut. This in turn could interfere with active transport of various nutrients via the intestine. Decreased transketolase activity, on the other hand, could impair overall hexose monophosphate shunt activity since this enzyme is believed to be rate limiting for this process (19). Such malfunction might interfere with the synthesis of nucleic acids and reduced triphosphopyridine nucleotide (NADPH). Neither ATP stores nor hexose monophosphate shunt activity have been previously measured in thiamine-deficient gut. Accordingly, it was our purpose in this study to correlate thiamine deficiency-induced changes in gut transketolase and pyruvate decarboxylase activity with overall hexose monophosphate shunt activity and ATP production as assessed from net available intestinal ATP stores.

Methods. Animal model of thiamine deficiency. Thiamine deficiency was induced by dietary thiamine deprivation, a procedure previously described in detail (10). In brief, paired female littermate rats of the Sprague–Dawley strain, 60–80 g in weight, were placed singly in metabolic cages. One member of each pair (thiamine-deficient, TD) was provided daily with 20 g of a purified pelleted thiamine-deficient diet replete with other nutrients (Nutritional Biochemical Co., Cleveland, Ohio). The other animal (pair-fed control, PFC) initially received 20 g of an iden-

[1] This research was supported by a grant from the National Institutes of Health, No. 5RO1 MH-19200-03, and V. A. Research funds.

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tical diet supplemented with thiamine and subsequently received weighed amounts equivalent to the measured food consumption of the TD rat in the previous 24 hr. A separate group of female rats of comparable weight which had received regular laboratory chow (Ralston Purina Co., St. Louis, Mo.) *ad libitum* was also studied; these animals served as normal controls (NC). All animals had free access to water.

The TD rats developed gross neurologic signs of thiamine deficiency at 4–6 wk. The PFC littermates remained neurologically normal. Both the TD and PFC rats failed to gain weight normally (10); in effect the PFC rats were partially starved since their caloric intake was progressively restricted in accord with the spontaneous progressive decrease in food consumption exhibited by the TD rats. The mean body weight of these two groups of animals at the time of onset of neurologic signs was: TD, 86 ± 2 g SE, $n = 109$; and PFC, 123 ± 2 g, n = 109. The mean body weight of the normally fed control rats at the time of study was 186 ± 1 g, $n = 131$. A similar weight curve has been shown previously in thiamine-deficient and pair-fed rats given the same diets (10).

Metabolic determinations. For enzyme, ATP, and pentose cycle studies, rats were lightly anesthetized with ether, the abdomen opened, and the appropriate intestinal segment was opened along the avascular antimesenteric border. The mucosal surface was then cleaned with saline and gently blotted dry with cotton gauze. The mucosa was scraped off the proximal 12.5 cm of small intestine and assayed unfrozen for enzyme activity and protein concentration or frozen quickly (within 5 sec) in liquid nitrogen for immediate ATP determinations. Transketolase and ATP were also measured in segments of whole intestine (duodenum and ileum) obtained from 5 each TD, PFC, and NC rats. Transketolase activity was measured by the procedure of Dreyfus and Moniz (20), pyruvate decarboxylase by the method of Dreyfus and Hauser (21), ATP by luciferin-luciferase luminescence (22), and protein by the technique of Lowry *et al.* (23).

The pentose cycle in mucosal samples was determined as described by Katz *et al.* in 1966 (24). Mucosal scrapings were taken from the proximal 12.5 cm of the small intestine, weighed, and immediately homogenized in 3.1 ml of cold Krebs-Ringer phosphate buffer at pH 7.4. The homogenate was kept at 4° until used. The assay was carried out in 25-ml Erlenmeyer flasks to which were added 1.0 ml of the mucosal homogenate and 2.1 ml of the Krebs-Ringer phosphate buffer containing 2.5 μmoles of glucose labeled with ^{14}C in the 1 or 6 position. The flasks were capped with stoppers to each of which was affixed a polyethylene cup containing 0.5 ml of hydroxide of Hyamine to collect evolved CO_2. The flasks were gassed with 95% O_2 and 5% CO_2 for 30 sec, then incubated at 37° in a shaker bath (120 strokes/min). 1 *N* HCl was added to stop the reaction after 1 hr of incubation. The flasks were shaken for an additional 2 hr after which they were placed on ice for 10 min. The Hyamine cup was immersed in toluene scintillation fluor and samples were counted in a Packard Tri-Carb counter. Sample activity was considered to represent the $^{14}CO_2$ derived from the metabolism of ^{14}C-glucose. Each mucosal sample was incubated with [1-^{14}C]glucose, [6-^{14}C]glucose, or without glucose as a blank. Controls were run with reaction mixtures to which buffer was added in place of mucosal homogenate. ^{14}C yields from the control samples were consistently 5% or less of those in which homogenate was used. Glucose utilization was determined by comparing final glucose concentrations in the homogenate samples to glucose levels in the control samples. Glucose was assayed by the glucose oxidase method (Worthington Glucostat Special Kits). Blank absorbance was less than 1% of the test values. Total glucose utilization was maintained at 30% or less and was linear throughout the 60-min incubation. Specific yields of $^{14}CO_2$ were expressed as the fraction of utilized glucose converted to CO_2 (G_1 for [1-^{14}C]glucose and G_6 for [6-^{14}C]glucose). G_1 and G_6 yields were linear during the 60-min incubation provided total glucose utilization was less than 40%. Pentose cycle activity (PC) was calculated (24) as follows: $PC = S/(3 - 2S)$, where $S = (G_1 - G_6)/$

TABLE I. Effects of Thiamine Deficiency on Intestinal Mucosal Metabolism in the Rat.

	Thiamine deficient[a] (TD)	Pair-fed control (PFC)	*Ad libitum* control (NC)	% Decrease (TD vs PFC)	p (TD vs PFC)
Pyruvate decarboxylase[b]	37.3 ± 5.1 (8)	85.0 ± 14.1 (8)	71.0 ± 14.1 (9)	56.1	<0.05
Transketolase[c]	0.697 ± 0.229 (5)	10.35 ± 0.52 (5)	12.49 ± 1.30 (5)	93.3	<0.05
ATP[d]	0.232 ± 0.018 (7)	0.241 ± 0.007 (7)	0.261 ± 0.021 (7)	3.7	NS[e]

[a] TD rats exhibited overt neurological signs at the time of study.
[b] nmoles of pyruvate decarboxylated/mg intestinal mucosal protein/45 min, mean ± SE. The numbers in parentheses indicate the number of animals studied in each group.
[c] μmoles of sedoheptulose elaborated/g intestinal mucosal protein/30 min, mean ± SE.
[d] μmoles/g mucosa, mean ± SE.
[e] NS signifies no significant difference from PFC.

(1 — G_6) and PC = the fraction of glucose utilized via the pentose cycle.

Statistical analysis utilized the paired t test or the Wilcoxon matched pairs signed rank test for more than 5 pairs of rats. Nonpaired rats were compared by the Mann-Whitney U Test (25).

Results. The effects of diet-induced thiamine deficiency on intestinal mucosal transketolase and pyruvate decarboxylase activity as well as the ATP concentration are shown in Table I. Thiamine deficiency induced a decline in mucosal pyruvate decarboxylase activity of 56% as compared to pair-fed controls while transketolase activity dropped by 93% ($p < 0.05$). Whole gut duodenum segments showed a transketolase drop of 70% (359 ± 68 SE for PFC to 107 ± 39 SE μM of sedoheptulose elaborated/g tissue protein/45 min for TD animals) while ileum transketolase was depressed over 80% (197 ± 17 SE for PFC to 37 ± 1.8 SE μM of sedoheptulose elaborated/g tissue protein/45 min for TD rats). Mucosal ATP was not significantly affected by thiamine deficiency. Whole duodenum and ileum ATP levels were comparable in normally fed, pair-fed and thiamine-deficient rats ($p > 0.05$). The normally fed rat duodenal and ileal values were 1.52 ± 0.06 SE and 1.39 ± 0.12 SE μmoles/g intestinal wet weight, respectively. These values for whole intestinal ATP are in close agreement with those recently obtained by Carter and Isselbacher in normal rats (26).

The effects of thiamine deficiency on the pentose cycle contribution to glucose metabolism (PC) by the intestinal mucosa are shown in Figs. 1–4. It can be seen in Fig. 1 that, during the first wk of thiamine deficiency, the PC activity rose, but thereafter it fell to about 53, 31, and 24% of normally-fed control levels at 2 and 4 wk and during the symptomatic stage, respectively. The PC values at these three periods differed significantly ($p < 0.05$ or less) from the NC group but rose rapidly to an almost normal level following the administration of 50 μg thiamine/day for 3–7 days. This dose of thiamine uniformly reversed the neurologic deficit and in the past has been shown to increase the activity of various thiamine-dependent

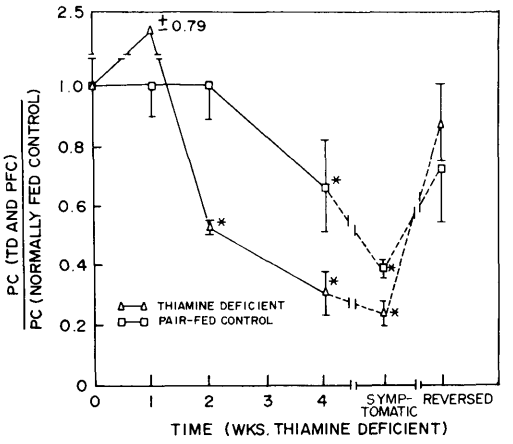

FIG. 1. The effect of thiamine-deficient and pair-fed control diets on intestinal mucosal pentose cycle activity. The vertical axis represents the ratio of pentose cycle (PC) activity in the intestinal mucosa of thiamine-deficient (TD) and pair-fed control (PFC) rats to that in the mucosa of normally fed controls. PC activity was calculated as described in the Methods. Normally-fed control PC activity was 0.1712 ± 0.014 (n = 13). Numbers of pairs of TD and PFC animals studied at 1 wk, 2 wk, 4 wk, symptomatic, and reversed stages, were 4, 4, 7, 7, and 5, respectively. SE of the mean is shown by the vertical bars except for the TD rats at 1 wk where SE is indicated by the number in the figure. Symptomatic animals received thiamine-deficient diets for 5.9 ± 0.4 wk. Animals were reversed with thiamine (50 μg ip daily) until all neurological signs disappeared (5.0 ± 1.1 days). Significant differences ($p < 0.05$ or less) for the TD and PFC animals, as compared to normally fed rats, are indicated by asterisks next to each mean value.

enzymes in most tissues (10, 15–17, 27). The pair-fed PC activity fell by 33% from normally-fed levels after 4 wk of pair-feeding and reached a level of 44% of NC values at time corresponding to the symptomatic stage. A comparison of the PC activity in thiamine-deficient and pair-fed thiamine-replete controls is given in Fig. 2. It can be seen that after 2 wk of thiamine deficiency PC activity fell to about 60% of the pair-fed values and rose to above the pair-fed level after administration of thiamine. The consistent increase in PC activity at 1 wk of thiamine deficiency is not understood at present. Thiamine deficiency may also alter glucose metabolism by pathways other than the pentose cycle, as shown in Figs. 3 and 4. The ratio of glucose utilization to $^{14}CO_2$ from C_1 and C_6 labelled glucose was 5.3 ± 0.67 (SE) for normally-fed control rats. Little change in this ratio was found in pair-fed controls (Fig. 3). However, thiamine deficiency caused a gradual increase in the G_1/G_6 ratio which was about 5 times greater than pair-fed control after 4 wk of thiamine deficiency. It fell slightly with the development of neurologic signs and reverted essentially to normal after thiamine administration (Fig. 3). Figure 4 illustrates the mechanism for the G_1/G_6 ratio increase in thiamine-deficient intestinal mucosa. During the first 4 wk on the thiamine-deficient diet there was a 90% fall in C_6-glucose utilization as compared to a 71% decrease in C_1-glucose metabolism. Clearly, this accounts for the increase in the G_1/G_6 ratio. After reversal of thiamine deficiency there was increase in both C_1 and C_6 glucose utilization resulting in a near normal G_1/G_6 ratio.

Discussion. The purpose of this study was to detect metabolic alterations in intestinal mucosa induced by severe dietary thiamine deficiency. Specific metabolic parameters examined were the activities of two thiamine-dependent enzymes, transketolase and pyru-

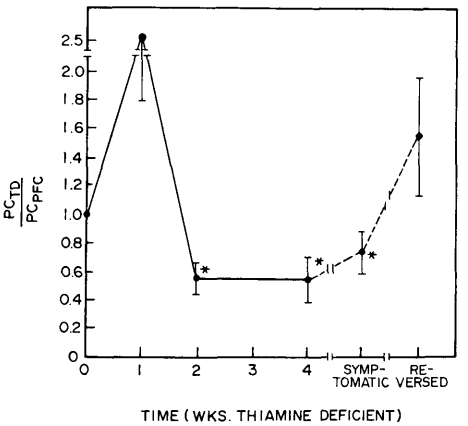

FIG. 2. The comparison of pentose cycle activity in intestinal mucosa of thiamine-deficient rats to that of pair-fed control animals. The vertical axis represents the ratio of PC activity in thiamine-deficient (TD) rats to that in their pair-fed (PFC) controls. Time scale for symptomatic and reversed stages and the number of pairs of rats studied at each time are given in the legend to Fig. 1. SE is shown by the vertical bars. Statistically significant differences ($p < 0.05$) are indicated by asterisks next to each mean value.

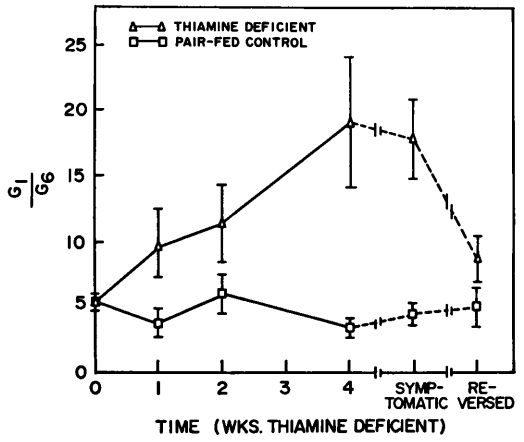

FIG. 3. The effect of thiamine-deficiency and pair-feeding on the G_1/G_6 ratio. The vertical axis depicts the ratio of G_1/G_6. G_1 and G_6 represent the fraction of utilized glucose converted to $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose, respectively. Numbers of animals studied are as given in Fig. 1. SE is shown by vertical bars. TD refers to thiamine-deficient and PFC to pair-fed control groups. Time scale for symptomatic and reversed stages and the number of pairs of rats studied at each time are given in the legend to Fig. 1.

vate decarboxylase, and two biochemical events which could be affected by their dysfunction, pentose cycle activity and ATP generation, respectively. As indicated in Table I, the severely thiamine-depleted rats showed markedly diminished activities of both transketolase and pyruvate decarboxylase in the intestinal mucosa. The degree of transketolase depression observed in this study for whole gut agrees well with the data obtained previously for whole intestine in rats sacrificed after somewhat shorter periods of thiamine deficiency (11). By contrast, the mucosal pyruvate decarboxylase and transketolase depression in our study were more substantial than those reported by Bai *et al.* (7). Since these depressions are a function of the degree and duration of thiamine deficiency (7), it is likely that this discrepancy is due to our animals being sacrificed at a more advanced stage of thiamine deprivation. The uniform depression of both of these enzymes in thiamine-deficient gut follows the pattern previously described by us in the heart, liver, and kidneys (15–17).

Transketolase is acknowledged as the rate-limiting enzyme for the pentose cycle (19), and it has been suggested that this pathway may be significantly inhibited in thiamine-depleted intestinal mucosa, conceivably accounting for the anorexia and weight loss observed in thiamine-deficient rats (7). The activity of the pentose cycle in thiamine-deficient intestinal mucosa has not been measured previously. Our data (Figs. 1–4) indicate that the pentose cycle is indeed significantly inhibited in the gut mucosa of thiamine-deficient rats and that the degree of depression is striking as compared both to the normally-fed and asymptomatic pair-fed controls (Figs. 1 and 2). With resolution of the neurologic deficit by the administration of thiamine, the pentose cycle reached control levels, consistent with a specific causal role of thiamine. This appears to be the first documentation of decreased pentose cycle activity in any tissue studied in thiamine deficiency. It is of interest, however, that pair-feeding alone (which implies semi-starvation in this experimental design) also induced a

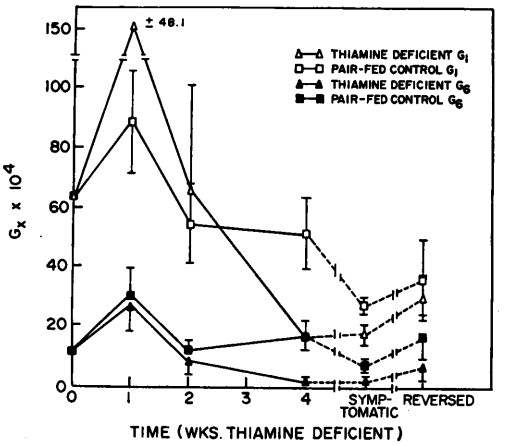

FIG. 4. The effect of thiamine-deficient and pair-fed diets on glucose utilization to CO_2 by rat intestinal mucosa. The vertical axis (G_x) represents the fraction of utilized [1-^{14}C]glucose or [6-^{14}C]glucose converted to $^{14}CO_2$ by intestinal mucosal homogenates. The upper pair of lines indicate G_1 utilization for thiamine-deficient and pair-fed animals, respectively. The lower two lines represent G_6 utilization values for these two groups of animals. SE is indicated by vertical bars. Time scale for symptomatic and reversed stages and the number of pairs of rats studied at each time are given in the legend to Fig. 1.

fall, albeit a later and lesser one, in pentose cycle measurements. This demonstrates again the importance of the pair-fed controls in interpreting data obtained in diet-induced thiamine deficiency (27).

The fall in pentose cycle activity in the pair-fed animals after 4 wk on the diet might suggest that they also developed a mild form of thiamine deficiency. However, previous studies in our laboratory have shown that these animals have a normal brain thiamine content and cerebral and hepatic transketolase and pyruvate decarboxylase activities which are similar to or higher than those found in normally-fed rats (10, 17). Our present data also indicate that intestinal mucosal pyruvate decarboxylase activity and C_1/C_6 glucose utilization in the pair-fed rats corresponded to those of normally-fed animals. Other studies of these pair-fed rats have shown that they may exhibit a small decrease in erythrocyte, renal, and cardiac transketolase activity and that pyruvate decarboxylase activity in the heart and kidneys may also be diminished (15, 16). However, the erythrocyte thiamine pyrophosphate effect (increase in transketolase activity after *in vitro* addition of thiamine) in the pair-fed animals is comparable to normal values and administration of excess thiamine parenterally during the fourth and fifth weeks of the diet does not alter the decreased blood transketolase activity (16). In addition, a previous study in our laboratory showed that rats chronically fed the high sucrose pair-fed diet *ad libitum* or the standard lab chow *ad libitum* had no difference in mucosal pentose cycle activity. These composite data therefore suggest that the decrease in the intestinal mucosal pentose cycle in the pair-fed rats is related to their semi-starvation rather than to a mild thiamine deficiency or to a difference in composition of the diet. The increase in the pentose cycle in the pair-fed controls for the thiamine-deficient reversed rats is most likely due to the increased food provision for these control animals at this stage of the experiment.

The physiologic importance of the depressed pentose cycle activity in thiamine-deficient intestinal mucosa as regards the function of the intestine is uncertain. Prior studies in the rat have indicated that the pentose cycle may account for about 5–13% of total glucose utilization (28), presumably primarily for the synthesis of nucleic acids and NADPH. In this study, pentose cycle contribution to intestinal glucose metabolism of normal and experimental animals was much smaller. This may be due to the much smaller animals presently studied or more probably to our different assay method which measures directly all glucose utilized, resulting in greater glucose flux. The small contribution of the pentose cycle to overall intestinal mucosal metabolism in this study suggests that its decrease in thiamine deficiency is not physiologically significant. However, the possible importance of this pathway to some critical metabolic sequence in the intestinal mucosa cannot be ascertained, and the question of its functional importance remains open.

Despite the substantial decrease in intestinal pyruvate decarboxylase activity noted (which theoretically could limit the entry of pyruvate into the Krebs cycle and thereby decrease ATP synthesis), the gut ATP levels in the thiamine-deficient animals remained normal (Table I). In previous studies with the thiamine-deficient rat heart, liver, liver, and renal medulla, a similar degree of impairment of pyruvate decarboxylase activity caused a small but significant decrease in tissue ATP (15–17). There are a number of possible reasons for the normal ATP stores in thiamine-depleted gut. First, the intestine, like the renal cortex (29), may utilize considerable fat for fuel. This would replenish the Krebs cycle via acetyl CoA, bypassing the thiamine-dependent block in pyruvate oxidation. Second, the methodologically optimal ATP data given here pertain to whole gut. Our studies also suggest that the ATP concentration in thiamine-deficient gut mucosa is normal. However, because of difficulties in rapidly freezing viable intestinal mucosa, reliable measurements of ATP in this tissue are difficult to obtain. Thus, the concentration of ATP in the intestinal mucosa, or in a critical absorptive component of it, actually could be depressed in thiamine deficiency. Finally, net levels of ATP do not pro-

vide data concerning rates of energy synthesis or utilization. Accordingly, although present data suggest that the stores of available energy in the form of ATP are normal in thiamine-depleted small intestine, further studies in this area are required. Such investigations will require advances in currently available methodology.

Analysis of the differentially labeled glucose experiments also gives additional information about the relative depression of the pentose and Krebs cycles in gut mucosa in thiamine deficiency. The data show that both the C_1- and C_6-glucose utilization decreased in severe thiamine deficiency (Fig. 4), but that the depression was greater for the C_6-glucose as compared to C_1-glucose. Accordingly, the increase in the G_1/G_6 ratio seen with thiamine deficiency (Fig. 3) is primarily explained by a disproportionate decrease in the utilization of C_6-glucose possibly resulting from impaired pyruvate decarboxylation which could control glucose flux via the Krebs cycle (24). These data constitute further evidence (in addition to measurement of pyruvate decarboxylase activity) that the operation of the Krebs cycle, at least in the intestinal mucosa, is depressed in severe thiamine deficiency.

Summary. Severe thiamine deficiency in rats was found to depress intestinal mucosal pyruvate decarboxylase and transketolase activities by 56 and 93% ($p < 0.05$), respectively. Whole gut transketolase activity in thiamine deficiency was also reduced by 70–80% ($p < 0.05$). Despite the major decline in intestinal pyruvate decarboxylase activity, ATP levels in both mucosa and whole gut of thiamine-deficient rats remained normal. By contrast, the decrease in mucosal transketolase activity in thiamine-deficient animals was accompanied by a 40% decline in pentose cycle activity ($p < 0.05$), which reverted to normal after administration of thiamine.

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Received June 14, 1973. P.S.E.B.M., 1973, Vol. 144.