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EFFECTS OF THIAMINE DEFICIENCY ON CEREBRAL AND VISCERAL PROTEIN SYNTHESIS*

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Abstract—The purpose of this study was to determine the effects of diet-induced thiamine deficiency on tissue protein synthesis. [^{14}C]valine incorporation into total protein of cortex, brainstem, cerebellum and subcortical structures of thiamine-deficient (TD) rats with neurological dysfunction was significantly depressed ($P < 0.001$) by 52, 49, 50 and 52 per cent as compared to pair-fed (PFC) control values. [^{14}C]valine incorporation into heart, kidney, pancreas and liver protein of TD rats was also depressed ($P < 0.001$) by 49, 53, 65, and 46 per cent respectively. Incorporation rates were only slightly (10 per cent) (yet significantly, $P < 0.05$) impaired in the brains of partly fasted PFC animals as compared to controls fed *ad lib.* In the viscera, only PFC heart incorporation rates were significantly ($P < 0.05$) reduced (20 per cent) as compared to rats fed *ad lib.* When neurologic dysfunction and anorexia of TD rats were reversed by three daily injections of 500 μg thiamine, [^{14}C]valine incorporation into protein of all tissues returned to normal except for a residual 8 per cent impairment in the cerebral cortex. Severe thiamine deficiency causes hypothermia. In TD rats with neurologic dysfunction whose body temperature had been restored to the normal value of 38°, valine incorporation rates remained significantly ($P < 0.02$) less than control values, but the initial (hypothermic) depression in incorporation was reversed by 56–73 per cent in various brain areas and by 29, 40, 18 and 35 per cent in heart, kidney, pancreas and liver respectively. Thiamine deficiency had no effect on the specific activity of valine precursor pools in any of the tissues studied. Thus, the observed inhibition in net protein synthesis *in vivo* in diet-induced thiamine deficiency seems to involve at least three components: (1) hypothermia to an important degree, (2) decreased food assimilation/utilization to a minor degree, and (3) probably an effect of thiamine deficiency *per se*.

Thiamine deficiency is commonly found in chronic alcoholics [1] and in its severe form may be associated with cardiac and neurological dysfunctions [1, 2]. Biochemically, it has been shown to induce depression of the hexose monophosphate shunt [3, 4] and of cerebral and visceral DNA synthesis [5, 6], to alter levels of amino acids in rat brain and liver [7, 8], and to impair labeled serine incorporation *in vitro* into hepatic microsomal protein [9]. Diet-induced thiamine deficiency in the rat causes anorexia, as well as severe body weight loss and reduced organ weight [5, 6]. The latter findings, which are commonly associated with deficits in protein accrual, as well as the reported alterations in amino acid levels and microsomal protein labeling *in vitro* prompted us to examine the effects of diet-induced thiamine deficiency on cerebral and visceral protein synthesis *in vivo*.

MATERIALS AND METHODS

Female Sprague–Dawley rats weighing 60–80 g were purchased from Harlan Industries, Inc., Indianapolis, IN, in sets of three littermates each. One rat of each set received a thiamine-deficient (TD) diet (Teklab Mills, Madison, WI) *ad lib.*; the second rat (PFC pair-fed control) was pair fed an identical but thiamine-replete diet, and the third (C, *ad lib.* control), received *ad lib.* the same thiamine-replete diet. Water was supplied *ad lib.* Rats feeding on the thiamine-deficient diet showed evidence of neurological dysfunction (ataxia, incoordination, convulsions) after 4–5 weeks. These signs were reversed within 6 hr of a single i.p. injection of 500 μg thiamine. In the present study, reversal of neurological signs was accomplished by three injections of 500 μg thiamine, one each, over a 3-day period.

Since thiamine deficiency is accompanied by a progressive drop in body temperature, a group of thiamine-deficient rats with neurologic dysfunction was placed in an incubator and body temperatures were normalized to 38°C for 1 hr prior to [^{14}C]valine injection and for the subsequent 1 hr until sacrifice by decapitation. Only about 40 per cent of the TD rats treated in this manner survived to be tested.

Analytical techniques. The method of Dunlop *et al.* [10] was used to study [^{14}C]valine (New England Nuclear Corp., Boston, MA) incorporation into total organ protein. [^{14}C]valine (500 mM, 10 μmoles/g of rat) was injected subcutaneously (s.c.) at a dose of 5 μCi/m-mole/100 g of rat; the purpose of this very high dose was to equalize the specific activity of endogenous valine pools. Rats were sacrificed by decapitation at 0.5, 1.0, 2.0 and 3.0 hr after injection. Whole brain, heart, kidney, pancreas and liver were removed and frozen immediately on dry ice. Prior to protein extraction, the brain was dissected into four areas—cortex, brainstem, cerebellum, and subcortical structures—as previously

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described [5]. The extraction involved an initial tissue homogenization in 3 per cent sulfosalicylic acid followed by sequential extractions in 5% trichloroacetic acid (TCA) (cold and at 90°), methanol, chloroform–methanol (1:1), and ether. The resulting dry powder was weighed and dissolved in Unisol–Unisol Complement (Isolab; Akron, OH), and dissolved protein counted in a Packard TriCarb scintillation counter. [^{14}C]valine incorporation into tissue protein is expressed as dis./min/mg of protein ± S.E. The specific activities of tissue endogenous valine pools were determined using a split stream fraction collector on a Beckman model 119 amino acid analyzer. The statistical significance between TD and PFC values was determined by Student's paired *t*-test. The unpaired Student's *t*-test was used to compare values between nonpaired groups.

RESULTS

Brain protein synthesis in symptomatic rats. Figure 1 illustrates the effect of severe thiamine deficiency, i.e. the presence of neurological signs (hereafter referred to as "symptomatic" rats), on incorporation of [^{14}C]valine into brain protein. ^{14}C-incorporation rates were linear up to 3 hr post-injection of [^{14}C]valine in all four brain areas studied. Thiamine-deficient (TD) values were significantly ($P < 0.001$) depressed as compared to pair-fed control (PFC) and to *ad lib.* controls (C) at all time intervals in each of the four brain areas. Inhibition of [^{14}C]valine incorporation into cortical protein averaged 52 per cent (Fig. 1A); inhibition was 49 per cent in the brainstem (Fig. 1B), 50 per cent in the cerebellum (Fig. 1C), and 52 per cent in subcortical structures (Fig. 1D). There was no significant difference between PFC and C values at 0.5 hr, but subsequently there was a slight (about 10 per cent) depression of [^{14}C]valine incorporation into PFC cortex, cerebellum and subcortical structure protein. At 1 hr, cortical PFC valine levels were significantly ($P < 0.05$) reduced by about 6 per cent. By 2 hr, cortical and subcortical values were down by 10 per cent ($P < 0.05$) and at 3 hr all three PFC protein specific activities averaged 10 per cent less than in the *ad lib.* controls ($P < 0.05$). Thus, food deprivation played a small yet significant role in the observed changes in apparent protein synthesis. Incorporation rates were comparable in all four brain areas studied, for each control or experimental group.

Visceral protein synthesis in symptomatic rats. [^{14}C]valine incorporation into protein of heart, kidney, pancreas and liver of TD rats was also significantly ($P < 0.001$) depressed compared to PFC and C values (Fig. 2, panels A–D). Heart incorporation rates (Fig. 2A) were linear over the entire 3-hr post-injection period and showed an overall average of 49 per cent depression in the TD rats. Kidney ^{14}C-levels increased in a linear manner to 3 and 2 hr for TD and PFC-C rats respectively (Fig. 2B). Average inhibition of incorporation during the linear phase was 53 per cent in the TD group. Liver incorporation rates (Fig. 2C) were linear to 2 hr for the TD rats, to almost 2 hr for PFC, and to about 1.5 hr for the C group. Inhibition of TD incorporation at 0.5 and 1.0 hr post-injection was 46 per cent. Protein incorporation rates in the pancreas (Fig. 2D) were linear in the TD group for 3 hr while linearity in PFC and C rats was apparent to about 2 hr. [^{14}C]valine incorporation into pancreas protein over 2 hr was inhibited by about 65 per cent when TD and

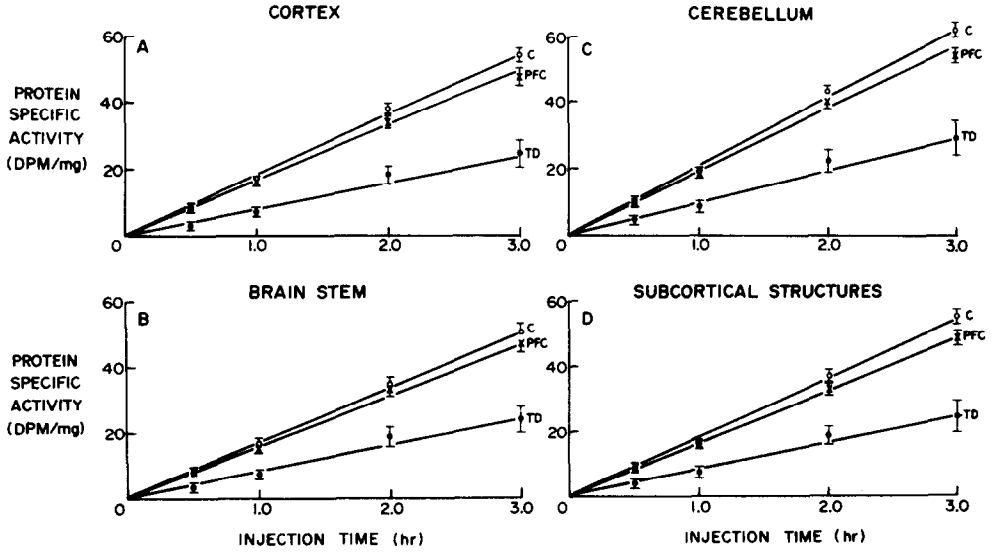

Fig. 1. Effect of thiamine deficiency on brain protein synthesis. The vertical axis represents the specific activity (dis./min/mg of protein) of extracted protein in thiamine-deficient (TD), pair-fed control (PFC), and in *ad lib.* control (C) littermates. TD rats were tested at the symptomatic (ataxic) stage. The horizontal axis represents the time period (hr) between an s.c. injection of [^{14}C]valine (500 mM; 5 μCi/m-mole/100 g of rat) and sacrifice. All TD values are significantly ($P < 0.001$) less than PFC and C. Vertical lines represent standard errors for means of 10, 9, 9 and 5 sets of three rats for times 0.5, 1.0, 2.0 and 3.0 hr respectively.

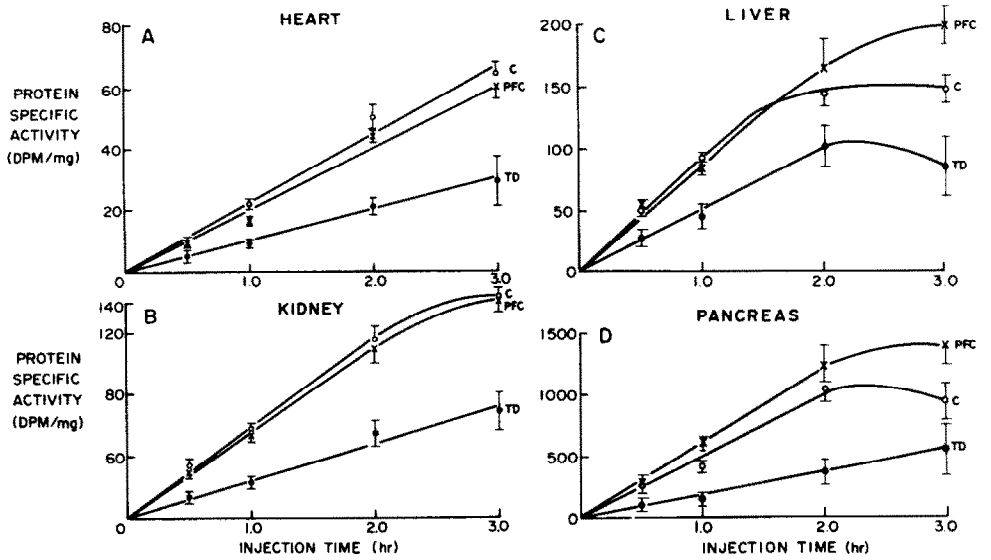

Fig. 2. Effect of thiamine deficiency on visceral protein synthesis. The vertical axis represents the specific activity (dis./min/mg of protein) of extracted protein in thiamine-deficient (TD), pair-fed control (PFC) and *ad lib.* control (C) littermates. TD rats were symptomatic (ataxic) when tested. The horizontal axis represents the time period (hr) between s.c. injection of [^{14}C]valine (500 mM; 5 μCi/m-mole/100 g of rat) and sacrifice. All TD values are significantly ($P < 0.001$) less than PFC and C. Vertical lines are standard errors for means of 10, 9, 9 and 5 sets of three rats for times 0.5, 1.0, 2.0 and 3.0 hr respectively.

PFC values are compared. The relative rates of [^{14}C]valine incorporation into protein in all groups were pancreas > liver > kidney > heart > brain.

The specific activities of endogenous valine pools, determined by split stream amino acid analysis, were comparable in TD and PFC tissue samples. In brain samples, the valine specific activities ranged from 6.1 dis./min/nmole for subcortical structures to 6.95 for cortex. Visceral pool specific activities ranged from 6.5 dis./min/nmole for kidney to 8.9 for heart. These values never varied more than about 6 per cent from TD to PFC for tissue from a given organ.

PFC incorporation rates were consistently slightly less than *ad lib.* control values in heart and kidney ($P < 0.05$ only for heart). Liver PFC and C rates were essentially the same during the linear period, while in the pancreas PFC values were higher than those for *ad lib.* controls.

Reversal of thiamine deficiency. A series of experiments was done in which symptomatic TD rats were injected i.p. with 500 μg thiamine on appearance of neurological signs and once daily for 2 days thereafter. Neurological signs generally disappeared within 6 hr of the initial injection and food intake increased to normal within 2 days. Pair feeding in controls was continued until sacrifice of all animals. Figure 3 illustrates the effect of thiamine repletion on the previously depressed [^{14}C]valine incorporation into cerebral protein. After the thiamine injections, the cortical [^{14}C]valine incorporation rate remained depressed but only by 8 per cent ($P < 0.05$), as compared to a 52 per cent decrease during the symptomatic stage. The other three brain areas showed a complete reversal of initial depressed TD values to levels that were not significantly ($P > 0.05$) different from their PFC control. Visceral [^{14}C]valine incorporation (Fig. 4) was also completely reversed by thiamine repletion. The heart incorporation rate increased to a level significantly ($P > 0.01$) greater than control values, while the kidneys, liver and pancreas levels were no different from controls. PFC values were no longer less than in *ad lib.* controls, indicating a reversal of the partial fasting effect.

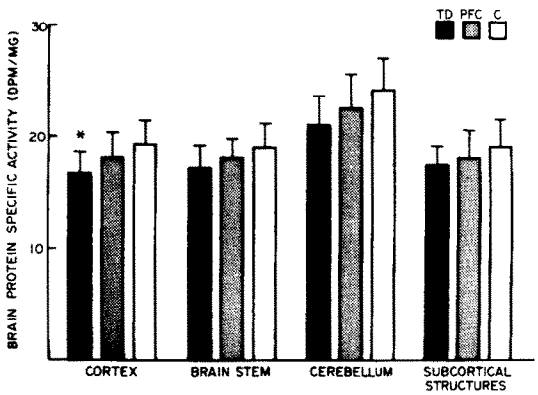

Fig. 3. Effect of reversal of thiamine deficiency by parenteral thiamine administration on regional cerebral protein synthesis. The vertical axis represents the specific activity (dis./min/mg of protein) of cerebral protein in thiamine-repleted TD rats (TD), pair-fed control (PFC), and *ad lib.* control (C) littermates. TD rats were injected i.p. with 500 μg thiamine on appearance of ataxia and once daily for 2 days thereafter. [^{14}C]valine incorporation for a 1 hr time period is indicated by the vertical bars (solid for TD, dotted for PFC, and white for C). The [^{14}C]valine (500 mM; 5 μCi/m-mole/100 g of rat) was injected s.c. The asterisk indicates statistical significance ($P < 0.05$) from PFC and the vertical lines represent standard errors for 8 sets of rats.

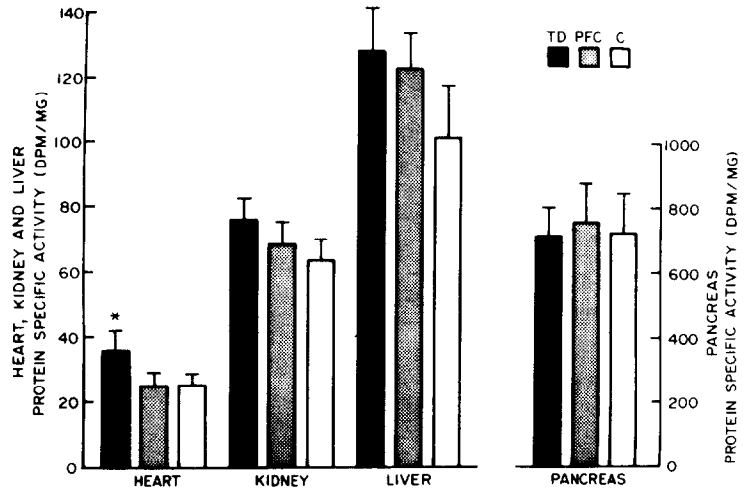

Fig. 4. Effect of reversal of thiamine deficiency by parenteral thiamine administration on visceral protein synthesis. The left vertical axis represents the specific activity (dis/min/mg of protein) of heart, kidney and liver protein and the right axis indicates the specific activity of pancreas protein in thiamine-deficient (TD), pair-fed control (PFC), and *ad lib.* control littermates. TD rats were injected i.p. with 500 μg thiamine on appearance of ataxia and once daily for 2 days thereafter [^{14}C]valine incorporation for a 1-hr time period is indicated by the vertical bars (solid for TD, dotted for PFC, and white for C). The [^{14}C]valine (500 mM; 5 μCi/m-mole/100 g of rat) was injected s.c. The asterisk indicates statistical significance ($P < 0.01$) from PFC and the vertical lines represent standard errors for 8 sets of rats.

Effect of hypothermia in thiamine-deficient rats on [^{14}C]*valine incorporation.* Diet-induced thiamine deficiency causes a progressive drop in body temperature. As Fig. 5 indicates, body temperature falls significantly below normal levels by day 20 of the dietary regimen. After about 30 days, a precipitous drop occurs until a mean of 32° is reached at the onset of neurological signs. At this point (about 38 days on the thiamine-deficient diet), brain thiamine levels average 15 per cent of normal values [11]. A single i.p. injection of 500 μg thiamine reverses this hypothermia to about 35° within 7 hr and to a normal 38° within 24 hr. In order to determine the contribution of this hypothermia to the observed alteration of [^{14}C]valine incorporation *in vivo* into tissue protein, a series of experiments was carried out in which symptomatic rats were warmed to 38° for 1 hr prior to and following injections of [^{14}C]valine. By normalizing body temperature, a partial reversal of the previous decreased valine

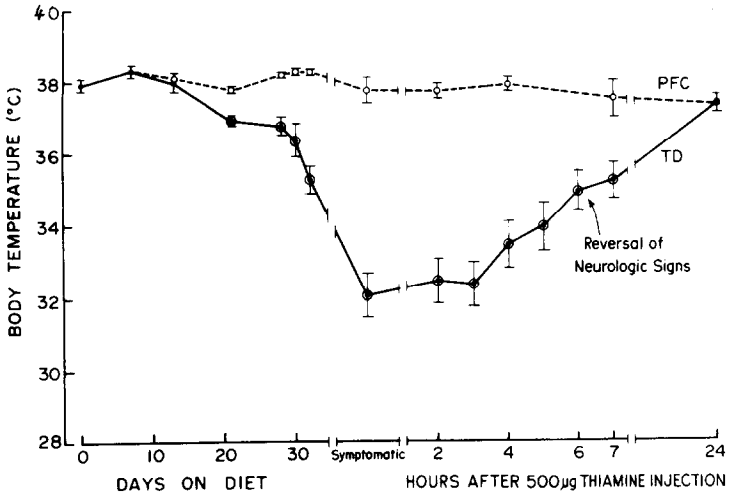

Fig. 5. Effect of thiamine deficiency on body temperature. The vertical axis represents body temperature (°) as taken by a 2-cm rectal probe connected to a thermocouple. The horizontal axis indicates the time during which rats had been on a thiamine-deficient diet until appearance of ataxia (symptomatic stage) and for 24 hr after a single i.p. injection of 500 μg thiamine. The solid line represents body temperature for rats on the thiamine-deficient diet (TD) and the dashed line indicates temperatures of pair-fed controls (PFC) on a thiamine-containing diet. Vertical lines indicate standard errors of ten rats for each point. Key: ⊙ symbols refer to values which are statistically significantly ($P < 0.05$) below control results.

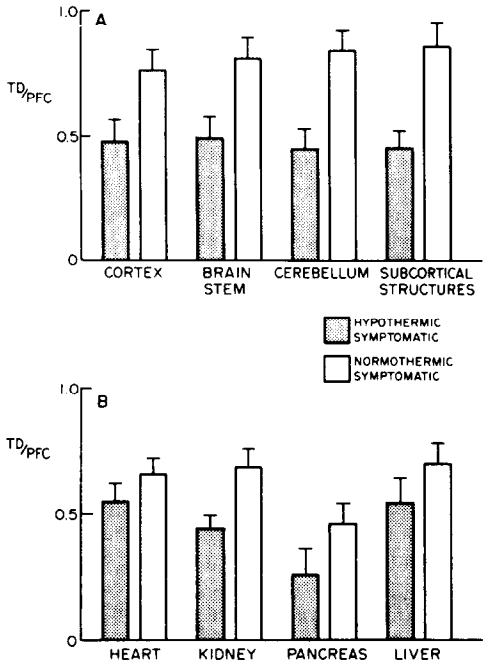

Fig. 6. Effect of normalizing body temperature on brain and visceral protein synthesis in thiamine deficiency. The vertical axis represents the ratio of the specific activity of isolated protein in TD brain and viscera to that found in the corresponding pair-fed control rats. On appearance of ataxia, TD rats were placed in an incubator and their body temperature was raised to 38° for 1 hr prior to injection of [^{14}C]valine and for 1 hr post-injection to sacrifice. These rats are labeled normothermic symptomatic (white bars). For reference, TD/PFC ratios are included for hypothermic symptomatic rats (dotted bars). [^{14}C]valine (500 mM; 5 μCi/m-mole/100 g of rat) was injected s.c. The vertical lines represent standard errors for 6 sets of rats (normothermic) and 9 sets (hypothermic). All TD values were significantly less than PFC values ($P < 0.05$).

incorporation occurred; Fig. 6 indicates these results in cerebral and visceral tissue. These values are expressed as ratios of TD to PFC [^{14}C]valine incorporation rates. In order to illustrate the reversal of depressed [^{14}C]valine incorporation in normothermic TD rats, values for hypothermic TD rats are included. The increase in [^{14}C]valine incorporation caused by normalizing body temperature was most marked in brain (Fig. 6A) tissue where 56, 61, 68 and 73 per cent of the initial (i.e. hypothermic) depressed incorporation rates were reversed in cortex, brainstem, cerebellum and subcortical structures respectively. Visceral reversal (Fig. 6B) was much less marked, with a 29, 40, 18 and 35 per cent reversal of the initial (hypothermic) depressed [^{14}C]valine incorporation rates in heart, kidney, pancreas and liver respectively. All of the observed 1-hr [^{14}C]valine incorporation rates in temperature-normalized symptomatic rats were significantly ($P < 0.01$) higher than those in their hypothermic counterparts. Thus, a significant fraction of the observed depression in apparent protein synthesis in severe thiamine deficiency is due to concomitant hypothermia. In spite of the reversals induced by normalizing body temperature, [^{14}C]valine incorporation remained significantly depressed in all tissues studied; cortex, brainstem, cerebellum and subcortical incorporation (Fig. 6A) were reduced by 23, 19, 16 and 14 per cent, respectively, in normothermic TD rats ($P < 0.05$). In the viscera of normothermic TD rats (Fig. 6B), heart, kidney, pancreas and liver values remained reduced by 35, 32, 53 and 30 per cent respectively ($P < 0.001$).

DISCUSSION

These studies indicate that diet-induced thiamine deficiency causes a marked abnormality in cerebral and visceral protein metabolism. The method of Dunlop *et al.* [10], utilizing administration of large doses of labeled valine and its subsequent incorporation into protein, was chosen for our determinations of net protein synthesis *in vivo* for two reasons. First, by injecting large doses of labeled valine, variations in endogenous pools of this compound should not affect estimations of protein synthesis. Thiamine deficiency has been shown to induce alterations in endogenous stores of amino acids [7, 8], yet amino acid analysis of acid-soluble filtered fractions of our samples indicated that specific activities of valine precursor pools in all tissues examined were comparable for TD and control rats. Second, this method required only a single subcutaneous injection of labeled valine followed by a short time interval until sacrifice, an important factor in view of the large number of animals required and their very short survival time after onset of neurological signs.

It is appreciated that neither this method nor others are fully exempt from some criticism. The first concern with this technique relates to the potential effects of very large doses of precursor amino acids on the protein synthesis apparatus. This subject is addressed in some detail by Dunlop *et al.* [10] and an extensive discussion of it is not within the scope of the present paper. Briefly, however, this group found no evidence of inhibition of protein synthesis, even when 15 μmoles valine/g (one-third more than used in our studies) was administered. This was apparent from comparisons of [^{14}C]valine incorporation rates at doses ranging from about 2 to 15 μmoles. In addition, in our studies, both experimental and control rats received the same weight-adjusted dose of labeled valine. Second, we did not determine the possible role of protein degradation on the observed decreased [^{14}C]valine accumulation in protein during thiamine deficiency *in vivo*. It was not possible to follow the disappearance of the ^{14}C-label from protein in TD rats, due both to the long periods of linear incorporation (more than 3 hr) and the short survival times of symptomatic rats. However, at the time when the rats in this study were tested for protein synthesis activity, they were about 70-days-old. In adult rats such as these, nonregenerating tissue net protein levels remain relatively constant [10, 12], implying that synthesis and degradation are approximately equal. Thus, although we cannot dissociate the possible roles of decreased synthesis vs increased degradation in this study, the decrease in [^{14}C]valine incorporation into tissue protein of TD rats clearly represents a highly significant depression in net

protein synthesis (i.e. the balance between synthesis and degradation) induced by thiamine deprivation. This is further supported by the work of Chakrabarti and Pandit [9] in which liver microsomal protein synthesis *in vitro* was depressed in severely thiamine-deficient rats by about 45 per cent. Our determinations indicated a 46 per cent depression *in vivo* in livers of symptomatic rats.

The apparent depression in protein synthesis observed in our experiments was severe in all tissues examined. The four brain areas studied exhibited reductions in [^{14}C]valine incorporation of about 50 per cent each, while heart, kidney and liver values were depressed 46–53 per cent, and TD pancreas incorporation was reduced to 35 per cent of PFC values. Alterations such as these in protein biosynthetic mechanisms would normally be expected to be reflected in altered growth patterns and deficient protein accrual. It has been a consistent finding [5, 6] that TD rats show a significantly depressed weight-growth pattern when compared to carefully pair-fed controls. At least part of this weight reduction could be due to a reduced protein accrual. This is partially substantiated by previous studies in this laboratory [6] that showed that total protein content and tissue concentration of protein are reduced in the TD symptomatic heart. Effects on other organ protein levels are less definitive. Brain net protein concentrations remain unchanged as do those for kidney, pancreas and liver. The results obtained previously [5, 6] by assaying tissue homogenates using the Lowry method, as described in Ref. 13, were comparable to those obtained in the present study in which total protein was extracted and weighed. These observations are somewhat analogous to those previously reported [5, 6] concerning the effects of thiamine deficiency on brain and visceral DNA synthesis in that thiamine deficiency severely inhibits DNA synthesis *in vivo* yet does not significantly reduce tissue DNA levels except in the liver. This pattern can be best interpreted as a selective inhibition of synthesis of specific pool(s) of protein, the inhibition of which does not significantly reduce net total levels of protein. Alternatively, a general inhibition of protein synthesis at a late point in thiamine deficiency may have been too brief to reduce significantly total protein levels prior to the onset of severe neurological signs and sacrifice.

An important cause of the depressed [^{14}C]valine incorporation into tissue protein in diet-induced thiamine deficiency is hypothermia, since, as indicated in Fig. 6, a substantial portion (18–73 per cent) of the observed inhibition can be reversed by elevating the body temperature of symptomatic rats to normal values. This situation is not without precedent. Chlorpromazine (CPZ) is known to reduce body temperature and to depress incorporation of amino acids into brain protein; however, no such abnormality was apparent in normothermic CPZ-treated animals or in microsomal systems exposed *in vitro* to this drug [14, 15]. It is interesting, however, that CPZ-induced hypothermia did not alter amino acid incorporation into heart protein [15], implying the existence of tissue differences in protein synthesis control mechanisms. It is tempting to draw a parallel between this observation and those noted in our thiamine deficiency studies, in that normalizing body temperatures in TD rats causes a much more dramatic reversal of the depressed valine incorporation (56–73 per cent) into brain tissue protein than in the four visceral organs studied (18–40 per cent). Only a 29 per cent reversal was seen in normothermic thiamine-deficient heart.

It is not possible from our experiments to determine the contribution of thiamine deficiency alone (independent of hypothermia) to the observed depression in [^{14}C]valine incorporation into tissue protein. The symptomatic rats that were placed in an incubator to normalize their body temperature had a very short survival time. Thus, the maximum time that the body temperatures of the rats could remain at 38° was for 2 hr—1 hr prior to isotope injection and 1 hr past the injection time. This time interval, while sufficient to yield a significant increase of the initial depressed [^{14}C]valine incorporation fell short of a complete reversal especially in the viscera. By contrast, administration of thiamine, which reversed both the neurologic dysfunction and the hypothermia, essentially restored protein synthesis to normal. It is possible, however, that a longer normalization of body temperature alone may have resulted in complete normalization of rat protein synthesis as well.

Elucidation of the specific mechanism(s) involved in the inhibition of [^{14}C]valine incorporation into tissue protein in symptomatic thiamine-deficient rats requires further studies. Thus, while the phenomenon of hypothermic block of protein synthesis, shown in our studies, has been documented in other mammalian systems [10, 14, 15], the mechanism by which this occurs *in vivo* has not been established. It also appears from our studies, when comparing PFC and controls fed *ad lib.* (Fig. 1, panels A–D), that decreased food intake or utilization may contribute to a minor extent to the depressed [^{14}C]valine incorporation in TD rats. This would be consistent with decreased weight gain in TD rats as compared to pair-fed controls [5, 6]. Thus, in the diet-induced thiamine deficiency state, at least three components of inhibition of net protein synthesis in brain and other viscera may be involved: (1) hypothermia to an important degree, (2) decreased food assimilation/utilization by thiamine-deficient rats to a minor degree, and (3) some as yet undetermined effect on the thiamine deficiency *per se*.

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REFERENCES

1. G. B. Phillips, M. Victor, R. D. Adams and G. S. Davidson, *J. clin. Invest.* **31**, 859 (1952).
2. M. Akbarian, N. A. Yankapaulos and W. H. Abelman, *Am. J. Med.* **41**, 197 (1966).
3. G. I. Henderson, D. W. McCandless and S. Schenker, *Proc. Soc. exp. Biol. Med.* **144**, 596 (1973).
4. S. J. Wolf, M. Brin and C. S. Davidson, *J. clin. Invest.* **37**, 1476 (1968).
5. G. I. Henderson and S. Schenker, *J. Lab. clin. Med.* **86**, 77 (1975).

6. G. I. Henderson, M. Dillon and S. Schenker, *Biochem. Pharmac.* **25,** 2275 (1976).
7. M. K. Gaitonde and R. W. K. Nixey, *J. Neurochem.* **22,** 53 (1974).
8. J. Holowach, F. Kauffman, M. G. Ikossi, C. Thomas and D. B. McDougal, Jr., *J. Neurochem.* **15,** 621 (1968).
9. C. H. Chakrabarti and V. I. Pandit, *J. Vitamin.* **15,** 211 (1969).
10. D. S. Dunlop, W. van Elden and A. Lajtha, *J. Neurochem.* **24,** 337 (1975).
11. D. W. McCandless and S. Schenker, *J. clin. Invest.* **47,** 2268 (1968).
12. S. Roberts, C. E. Zomzely and S. C. Bondy, in *Protein Metabolism of the Nervous System* (Ed. A. Lajtha), p. 3. Plenum Press, New York (1970).
13. E. F. Hartree, *Analyt. Biochem.* **48,** 422 (1972).
14. L. Shuster and R. Hannam, *J. biol. Chem.* **239,** 3401 (1964).
15. P. L. Mason and H. J. Rogers, *J. Pharm. Pharmac.* **23,** 299 (1971).