A ROLE FOR THIAMINE IN THE REGULATION OF FATTY ACID AND CHOLESTEROL BIOSYNTHESIS IN CULTURED CELLS OF NEURAL ORIGIN[1]

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Abstract—Cultured glial (C-6) and neuronal (neuroblastoma) cells were utilized to define the role of thiamine in the regulation of fatty acid and cholesterol biosynthesis. Glial cells subjected to thiamine deficiency exhibited rates of fatty acid synthesis that were only 13% of the rates in thiamine-supplemented cells. The decrease in fatty acid synthetic rate was accompanied by a comparable decrease in the activities of fatty acid synthetase and acetyl-CoA carboxylase, the two critical enzymes in the pathway. Immunochemical techniques demonstrated that the decrease in *activity* of fatty acid synthetase reflected a decrease in enzyme *content* and that this change in content was caused by a decrease in enzyme *synthesis*. The disturbance of fatty acid synthesis was exquisitely sensitive to thiamine—i.e. marked improvement was evident within hours of replenishment with only 0.01 μg/ml of thiamine. Total recovery occurred in 1–2 days. Thiamine-deficient glia also exhibited reduced rates of cholesterol biosynthesis, i.e. 60% of the rates in thiamine-supplemented cells. This effect was accompanied by a comparable reduction in activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting step in cholesterol biosynthesis. Unlike the glial cells, the neuronal cells exhibited either no or only a slight reduction in lipid synthesis under similar conditions of thiamine deficiency.

The data have important implications for the genesis of the neuropathology in states of altered thiamine homeostasis and for the mechanisms of regulation of lipid synthesis.

OUR INTEREST in undertaking this investigation of a potential role for thiamine in the regulation of fatty acid and/or cholesterol biosynthesis was stimulated by two well-established disturbances associated with thiamine deficiency. The first relates to the neuropathologic, and the second, to the metabolic consequences of deficiency of this vitamin.

The occurrence of necrotic lesions in brain stem and diencephalon in Wernicke's encephalopathy (VICTOR *et al.*, 1971), related to thiamine deficiency, and in Leigh's disease (FRIEDE, 1975), related to a disturbance of thiamine metabolism, emphasizes the importance of thiamine for the maintenance of cellular integrity in the nervous system. The metabolic basis for the disturbance of cellular integrity has remained obscure. Thus, although the activities of the three thiamine-dependent enzymes, pyruvate decarboxylase, α-ketoglutarate decarboxylase and transketolase, are depressed in brain of thiamine-deficient animals (DREYFUS, 1965; HOLOWACH *et al.*, 1968; MCCANDLESS & SCHENKER, 1968; MCCANDLESS *et al.*, 1976), levels of ATP (HOLOWACH *et al.*, 1968) and flux through the pentose phosphate cycle (MCCANDLESS *et al.*, 1976) are *not* depressed concomitantly. In view of the importance of fatty acids and sterols in cellular membranes, a role for a disturbance of the synthesis of these lipids in thiamine deficiency is important to investigate. No previous study of the effects of thiamine deficiency on lipid synthesis in brain is available, and the data relative to effects in liver have been inconsistent (WILLIAMS & ANDERSON, 1959; MILLER *et al.*, 1965; ITOKAWA *et al.*, 1973; GUBLER *et al.*, 1974) and not accompanied by studies of the critical enzymes.

An important metabolic consequence of thiamine deficiency, diminished activity of pyruvate decarboxylase, would be expected to lead to diminished levels of acetyl-CoA. Since many of our previous observations (see VOLPE & VAGELOS, 1976, for review) are compatible with the hypothesis that the synthesis of the important lipogenic enzyme, fatty acid synthetase, is regulated by intermediates of carbohydrate metabolism at the triose phosphate step or beyond, including perhaps acetyl-CoA, thiamine deficiency appeared to be an excellent model to further test this hypothesis.

Because of the topographic and cellular specificity of the pathology in thiamine deficiency (DREYFUS & VICTOR, 1961; COLLINS, 1967) and the feasibility of

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producing thiamine deficiency in cultured C-6 glial and neuroblastoma cells (SCHWARTZ *et al.*, 1975; SCHWARTZ & MCCANDLESS, 1976), we elected to study these cultured cells of neural origin with the following objectives: (1) to determine whether thiamine deficiency and replenishment have major effects on the regulation of fatty acid and cholesterol biosynthesis, (2) to determine the enzymatic loci of these effects, (3) to define the mechanism causing changes in the activity of fatty acid synthetase, and (4) to compare the quantitative aspects of the effects in the glial vs the neuronal cells.

MATERIALS AND METHODS

Materials

Malonyl-CoA, acetyl-CoA, DL-3-hydroxy-3-methylglutaryl-CoA (P-L Biochemicals, Milwaukee, WI), NADPH, ATP, dithiothreitol, thiamine hydrochloride (Sigma, St. Louis, MO), and Dowex AG 1-X8 (200–400 mesh, formate) (BioRad Laboratories, Richmond, CA) were obtained from the designated sources. Sodium [2-^{14}C]acetate (specific radioactivity, 53.2–58.8 Ci/mol), ^3H_2O (specific radioactivity, 1.8 mCi/mmol), L-[4,5-^{3}H]leucine (specific radioactivity, 31.4–34.2 Ci/mmol), sodium [^{14}C]bicarbonate (specific radioactivity 9.6 Ci/mol), and DL-3-hydroxy-3-methyl[3-^{14}C]glutaryl-CoA (18.0–26.2 Ci/mol) were obtained from New England Nuclear (Boston, MA). Radioactive products were counted in a preblended scintillation fluid (3a70, Research Products International, Elk Grove Village, IL). All sera, antibiotics and other reagents for cell culture were obtained from Grand Island Biological Company (Grand Island, NY) and tissue culture flasks from Falcon (Oxnard, CA).

Methods

Cell culture. The cells used in this study, C-6 glial cells, cloned originally from a rat astrocytoma, and neuroblastoma cells (Neuro 2a), derived from a murine tumor, were obtained from the American Type Culture Collection 5 years ago and maintained in this laboratory since. During that time, utilizing cells derived either from intact monolayers or frozen samples, we have detected no significant changes in the qualitative or quantitative aspects of the regulation of fatty acid or cholesterol synthesis, as defined in previous reports (VOLPE & MARASA, 1975*a*, 1976*a*, 1976*b*, 1977, VOLPE & HENNESSY, 1977). The various methods of cell culture have been described (VOLPE & MARASA 1975*a*).

The culture medium utilized was modified from those described by HIGUCHI (1970), DONTA (1973) and BARLEY *et al.* (1972), as previously described (VOLPE & MARASA, 1976*a*). For the current studies this basic medium was prepared without thiamine. Thiamine was removed from fetal calf serum by dialysis for 3 days with 5 changes of 20 vol of phosphate-buffered saline (0.14 M-NaCl, 0.01 M-potassium phosphate, pH 7.4). In the experiments described culture flasks contained 10% (v/v) dialyzed fetal calf serum in medium that contained either no added thiamine ('thiamine-deficient') or added thiamine, 400 μg/ml ('thiamine-supplemented'), unless otherwise indicated. Culture medium was changed daily for the first 4 days of each experiment, every 48 h thereafter, but always 24 h prior to determinations.

For each experiment cells were derived from a single flask. Size of each inoculum of both C-6 glial and neuroblastoma cells was identical and based on cell number, determined on a Gentian violet-stained aliquot and counted in a hemocytometer. Inocula were adjusted to give a final concentration of 0.5×10^6/ml in either 25-cm^2 or 75-cm^2 flasks.

Fatty acid synthesis. Synthesis of fatty acids was evaluated by measuring the incorporation of radioactivity into these products from [2-^{14}C]acetate or ^3H_2O (VOLPE & MARASA, 1975*a*, 1977). Pulses with the radioactive precursors were performed in fresh medium, and cellular fatty acids were isolated by TLC.

Protein synthesis. Synthesis of total protein was evaluated by measuring the incorporation of radioactivity into total protein from L-[4,5-^{3}H]leucine (VOLPE & MARASA, 1976*b*). Protein was isolated by trichloroacetic acid precipitation.

Fatty acid synthetase, acetyl-CoA carboxylase assays. Cellular extracts utilized for assay of these enzymes were prepared as described (VOLPE & MARASA, 1975*a*). Fatty acid synthetase was determined by the spectrophotometric assay (MARTIN *et al.*, 1961). One unit of enzyme activity is defined as the amount required to catalyse the oxidation of 1 nmol of NADPH/min at 37°C.

Acetyl-CoA carboxylase was assayed by measuring the recovery of acid-stable radioactivity after incubation with [^{14}C]bicarbonate (MARTIN & VAGELOS, 1962), modified as described (VOLPE & MARASA, 1975*a*). One unit of enzyme activity is defined as the amount required to catalyse the fixation of 1 nmol of [^{14}C]bicarbonate/min at 37°C.

Immunological procedures. The immunochemical techniques utilized antibody prepared against homogeneous rat liver fatty acid synthetase (VOLPE *et al.*, 1973), which has been shown to be immunologically identical to the enzyme of the glial cells (VOLPE & MARASA, 1975*a*). The antibody was partially purified from serum by ammonium sulfate precipitation and DEAE-Sephadex (BAUMSTARK *et al.*, 1964). Quantitative precipitin analyses (KABAT & MEYER, 1961; VOLPE *et al.*, 1973) and isotopic-immunochemical analyses (VOLPE *et al.*, 1973; VOLPE & MARASA, 1975*a*) were utilized to compare the amount of immunoprecipitable enzyme per unit of activity among various extracts and to measure synthesis of fatty acid synthetase, respectively. In the latter experiments, after cells were exposed to L-[4,5-^{3}H]leucine, 10 μCi/ml, for 1 h, 22–38% ammonium sulfate fractions of cell extracts were prepared and subjected to immunoprecipitation (VOLPE & MARASA, 1975*a*).

Sterol synthesis and digitonin precipitation. Synthesis of sterols was evaluated by measuring the incorporation of radioactivity into these products from [2-^{14}C]acetate (VOLPE & HENNESSY, 1977). The basic isolation procedure for digitonin-precipitable sterols (3-β-hydroxysterols) was modified from POPJAK (1969), as described (VOLPE & HENNESSY, 1977).

HMG-CoA reductase assay. Cellular extracts utilized for assay of this enzyme were prepared as described (VOLPE & HENNESSY, 1977). The enzyme was assayed by a modification of the method of BROWN *et al.* (1973) and the radioactive product, [^{14}C]mevalonate, isolated by ion-exchange chromatography essentially according to AVIGAN *et al.* (1975), as described (VOLPE & HENNESSY, 1977). One unit of enzyme activity is defined as the amount required to catalyse the formation of 1 pmol of mevalonate/min at 37°C.

Protein determination. Cellular protein was determined by the method of LOWRY *et al.* (1951) or by a micro-biuret procedure (MUNKRES & RICHARDS, 1965).

Statistical procedures. Statistical significance was determined by Student's *t* test (SNEDECOR, 1956). The *t* test was carried out for all the data for which means ± S.E.M. are presented, and all differences discussed in the paper are significant at the $P < 0.01$ level or better.

TABLE 1. EFFECT OF THIAMINE DEFICIENCY ON FATTY ACID SYNTHESIS IN C-6 GLIAL CELLS

Days in medium	Fatty acid synthesis (c.p.m./mg protein × 10^{-3}) Th+	Th−	Protein (mg/flask) Th+	Th−
5	6.5 ± 0.8	8.1 ± 1.0	0.48 ± 0.06	0.42 ± 0.05
7	91.1 ± 8.5	58.3 ± 6.1	1.01 ± 0.11	0.89 ± 0.08
9	137.2 ± 14.0	17.9 ± 1.4	2.40 ± 0.08	1.09 ± 0.11

Cells were transferred to 18 25-cm^2 flasks, 9 of which contained thiamine-deficient (Th−) medium, and 9, thiamine-supplemented (Th+) medium. At the times indicated in the table, rates of fatty acid synthesis from [2-^{14}C]acetate and protein concentration were determined in each of 3 flasks from the deficient and supplemented groups (see Methods). Values are means ± S.E.M. obtained from separate determinations on each of 3 flasks. Similar results were obtained in 3 separate experiments.

RESULTS

Effect of thiamine deficiency on fatty acid synthesis in C-6 *glial cells*

We evaluated first the effect of thiamine deficiency on fatty acid synthesis from [2-^{14}C]acetate in the glial cells (Table 1). After 7 days, cells in thiamine-deficient medium exhibited an approx 2-fold lower rate of fatty acid synthesis than did cells in thiamine-supplemented medium. No significant decrease in growth rate, as determined by the rate of increase in total protein in the culture flasks, was apparent at that time. Over the next 2 days both fatty acid synthesis and the growth rate decreased markedly. Thus, after 9 days, cells in thiamine-deficient medium exhibited an approx 7-fold lower rate of fatty acid synthesis than did cells in thiamine-supplemented medium.

To determine whether these effects on the incorporation of radioactivity from [2-^{14}C]acetate into fatty acids reflected an alteration in *de novo* fatty acid synthesis and were not related to changes in uptake of acetate or conversion to acetyl-CoA, or in pool sizes of fatty acid precursors, we evaluated the effects of thiamine deficiency on the activities of acetyl-CoA carboxylase and fatty acid synthetase and on the synthesis of fatty acids from ^3H_2O (Table 2). Cells were grown in thiamine-deficient or supplemented medium for 7 days before enzymatic activities and fatty acid synthesis were determined. The decrease in fatty acid synthesis observed in the thiamine-deficient cells with ^3H_2O as precursor was similar to the decrease observed with [2-^{14}C]acetate. Moreover, a close correlation was apparent between the decreases in the activities of acetyl-CoA carboxylase and fatty acid synthetase and in the rate of fatty acid synthesis from ^3H_2O. Thus, the decrease in the fatty acid synthetic rate is apparently caused by the decrease in the activities of these two important lipogenic enzymes.

Effect of thiamine deficiency and replenishment on fatty acid and protein synthesis in C-6 *glial cells*

We next asked whether the effect on fatty acid synthesis might be a reflection of a nonspecific toxic effect of the deficient state, which might be reflected in a decrease in total protein synthesis. In addition we estimated the specificity of the effects by determining whether the cells could recover after marked effects on fatty acid synthesis were induced and by determining the quantity of thiamine needed to effect recovery (Table 3). No decrease in incorporation of [^{3}H]leucine into total protein was evident even after 10 days of growth in the thiamine-deficient medium. Despite this an approx 5-fold decrease in the rate of fatty acid synthesis occurred. Moreover, the exquisite sensitivity of this effect to thiamine was demonstrated by the observation that complete recovery of fatty acid synthesis resulted after growth for 3 days in just 0.01 μg/ml of thiamine.

TABLE 2. EFFECT OF THIAMINE DEFICIENCY ON FATTY ACID SYNTHETASE, ACETYL-CoA CARBOXYLASE AND FATTY ACID SYNTHESIS (FROM ^3H_2O) IN C-6 GLIAL CELLS

Thiamine in medium	Fatty acid synthetase activity (units/mg protein)	Acetyl-CoA carboxylase activity (units/mg protein)	Fatty acid synthesis (c.p.m./mg protein × 10^{-3})
+	6.56 ± 0.51	2.21 ± 0.11	2.00 ± 0.14
−	3.27 ± 0.22	1.21 ± 0.08	0.73 ± 0.06

Cells were transferred to 12 25-cm^2 flasks, 6 of which contained thiamine-deficient (−), and 6, thiamine-supplemented medium (+) for 7 days. At that time the activities of fatty acid synthetase and acetyl-CoA carboxylase and the rates of fatty acid synthesis from ^3H_2O were determined in each of 3 flasks from the deficient and supplemented group (see Methods). Values are means ± S.E.M. obtained from separate determinations on each of 3 flasks. Similar results were obtained in 2 separate experiments.

TABLE 3. EFFECT OF THIAMINE DEFICIENCY AND REPLENISHMENT ON FATTY ACID AND PROTEIN SYNTHESIS IN C-6 GLIAL CELLS

Days in medium	Thiamine in medium (μg/ml)	Fatty acid synthesis (c.p.m./mg protein $\times 10^{-3}$)	Protein synthesis (c.p.m./mg protein $\times 10^{-3}$)
7	0	34.0 ± 2.9	39.1 ± 4.4
7	400	65.7 ± 4.4	32.2 ± 4.8
10	400	84.1 ± 8.0	35.2 ± 3.9
10	0	16.2 ± 1.2	43.9 ± 5.2
3*	0.01	88.9 ± 7.7	35.6 ± 4.4
3*	400	86.0 ± 7.1	36.7 ± 4.7

Cells were transferred to 36 25-cm^2 flasks, 24 of which contained thiamine-deficient medium, and 12, similar medium supplemented with the standard quantity, i.e. 400 μg/ml, of thiamine. After 7 days the rates of fatty acid synthesis from [2-^{14}C]acetate and of protein synthesis from [^{3}H]leucine were determined in each of 3 flasks from the deficient and supplemented groups (see Methods). The remaining 6 flasks with the thiamine supplementation (400 μg/ml) were continued in similar medium for 3 additional days. The remaining 18 flasks with thiamine-deficient medium were divided into 3 groups: thiamine-deficient, thiamine-replenished, 0.01 μg/ml (see * in table), and thiamine-replenished, 400 μg/ml (see * in table), and grown for 3 additional days. At that time, 10 days after the start of the experiment, rates of fatty acid and protein synthesis were determined as described above. Values are means ± S.E.M. obtained from separate determinations on each of 3 flasks. Similar results were obtained in 2 separate experiments.

To examine the rapidity of recovery of fatty acid synthesis in thiamine-deficient cells after thiamine replenishment and to evaluate further the relation between the effects on fatty acid synthesis and those on cell growth, we determined the time course of recovery after 8 days of thiamine deficiency (Fig. 1). Two major points are apparent from the data. First, a sharp increase in the rate of fatty acid synthesis occurred in the thiamine-replenished cells between 6 and 12 h after addition of the vitamin. The rapid recovery continued over the next 36 h, and after 48 h, the rate of fatty acid synthesis in the replenished cells was identical to the rate in cells that were not thiamine-deficient during the 10 days of the experiment (data not shown). Second, in contrast to the response of fatty acid synthesis, the rate of cell growth, based upon the amount of protein in the culture flasks, did not increase sharply until 24–48 h of replenishment.

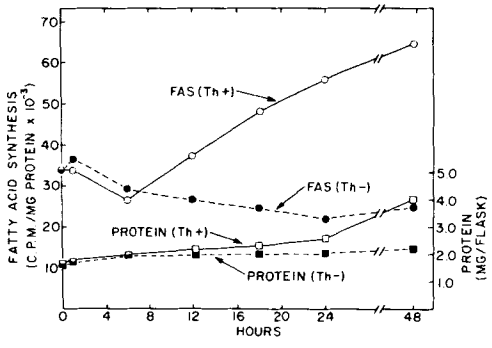

FIG. 1. Recovery of fatty acid synthesis after replenishment of thiamine-deficient C-6 glia with the vitamin. C-6 glial cells were transferred to 39 25-cm^2 flasks and grown for 8 days in thiamine-deficient medium. At that time (zero h) the rate of fatty acid synthesis (FAS) and the protein concentration were determined in each of 3 flasks. The medium was changed in the remaining 36 flasks so that 18 contained 400 μg/ml of thiamine (Th+), and 18, fresh thiamine-deficient medium (Th−). At the indicated times the rate of fatty acid synthesis and the protein concentration were determined in each of 3 flasks from the deficient and the replenished cells. Values are means obtained from separate determinations on each of 3 flasks. Similar results were obtained in a separate experiment.

Effect of thiamine deficiency on synthesis of fatty acid synthetase in C-6 glial cells

To determine the mechanism whereby thiamine deficiency caused the decrease in the activity of fatty acid synthetase, we utilized immunochemical tech-

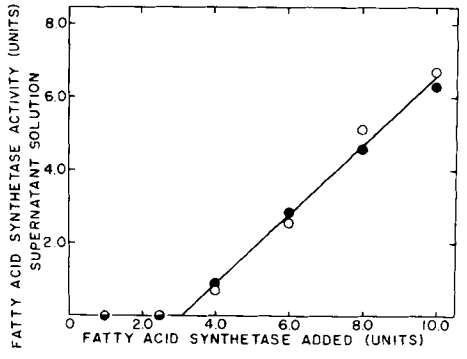

FIG. 2. Quantitative precipitin reactions of fatty acid synthetase of C-6 glial cells grown in thiamine-deficient (○) or thiamine-supplemented (●) medium. Cells were grown in the respective media for 7 days before assay of fatty acid synthetase and performance of the quantitative precipitin reactions (see Methods). Values are means obtained from separate determinations on each of 3 flasks.

TABLE 4. EFFECT OF THIAMINE DEFICIENCY ON SYNTHESIS OF FATTY ACID SYNTHETASE IN C-6 GLIAL CELLS

Thiamine in medium	Fatty acid synthetase activity (units/mg protein)	Specific radioactivity Fatty acid synthetase (A) (c.p.m./mg protein × 10^{-3})	Specific radioactivity Total protein (B) (c.p.m./mg protein × 10^{-5})	Relative rate of synthesis (A/B × 100)
+	6.18	7.23	2.73	2.65
−	2.37	4.03	3.20	1.26

Cells were transferred to 8 75-cm^2 flasks, 4 of which contained thiamine-deficient (−) medium, and 4, thiamine-supplemented (+) medium. After 8 days an isotopic-immunochemical analysis was carried out (see Methods). Values for fatty acid synthetase activity are means obtained from separate determinations of the 4 flasks from each group. Values for specific radioactivity of synthetase and protein are means obtained from separate determinations of 2 pooled pairs of flasks from each group. Values did not vary more than 5–10%. A similar result was obtained in a separate experiment.

niques. Quantitative precipitin analyses, performed with a constant amount of antibody and increasing amounts of enzyme extract, revealed identical equivalence points for extracts from cells grown either in thiamine-deficient or thiamine-supplemented medium for 7 days, although specific enzymatic activities of the extracts differed by 2.4-fold. This finding indicates that the difference in specific activities reflect a difference in *content* rather than in catalytic efficiency of the enzyme.

To determine whether the decrease in synthetase content was related to a change in enzyme *synthesis*, we performed an isotopic-immunochemical experiment (Table 4). Cells were grown in thiamine-deficient or supplemented medium for 8 days before exposure to [^{3}H]leucine and subsequent isolation of fatty acid synthetase by immunoprecipitation and total protein by trichloroacetic acid precipitation. A close correlation was observed between the decreases in the relative rate of synthesis and the specific activity of the enzyme in the deficient cells. Thus, the decrease in synthetase content in the deficient cells is caused by a decrease in rate of synthesis of the enzyme.

Effect of thiamine deficiency on faty acid synthesis in neuroblastoma cells

In view of neuropathological observations of the lesions observed in states of thiamine deficiency *in vivo* (see Discussion) that indicate earlier involvement of the glial than the neuronal cell, we next asked whether thiamine deficiency affected lipid synthesis in neuroblastoma cells in a manner different from the glial cells (Table 5). Indeed, there was no difference in the rate of fatty acid synthesis observed in the neuronal cells after 7 days in the deficient or supplemented medium and only a 24% lower rate in the deficient cells after 10 days. These observations should be contrasted with the 2-fold and 7–8-fold lower rates observed in the glial cells after similar periods of deficiency (Table 1). In contrast to the lesser effect on fatty acid synthesis in the neuronal vs the glial cells, cell growth was affected more in the former than in the latter cell type. This lends additional support to the data indicating that the changes in fatty acid synthesis in the glial cells are not secondary to changes in cell growth.

Effect of thiamine deficiency on sterol synthesis and HMG-CoA reductase in C-6 glial and neuroblastoma cells

To determine whether sterol synthesis and the rate-limiting enzyme thereof, HMG-CoA reductase, are affected by thiamine deficiency, the glial and neuronal cells were studied after 7 days in the thiamine-deficient medium (Table 6). In the thiamine-deficient glial cells the rate of sterol synthesis was reduced to approx 60% of that in the supplemented cells. As observed for fatty acid synthesis, the neuronal cells did not exhibit the responsiveness of the glial cells, i.e. no significant reduction in sterol synthesis occurred in the deficient neuroblastoma. That the reduction in sterol synthesis in the glial cells is mediated at the level of HMG-CoA reductase is indicated by the concomitant decrease in activity of this enzyme, the rate-limiting step in cholesterol biosynthesis in these cells (VOLPE & HENNESSY, 1977).

DISCUSSION

This investigation has dealt with the role of thiamine in the regulation of fatty acid and cholesterol biosynthesis in cultured cells of neural origin. The

TABLE 5. EFFECT OF THIAMINE DEFICIENCY ON FATTY ACID SYNTHESIS IN NEUROBLASTOMA CELLS

Days in medium	Fatty acid synthesis (c.p.m./mg protein × 10^{-3}) Th+	Fatty acid synthesis (c.p.m./mg protein × 10^{-3}) Th−	Protein (mg/flask) Th+	Protein (mg/flask) Th−
5	10.5 ± 1.2	10.8 ± 1.1	0.21 ± 0.02	0.21 ± 0.02
7	12.0 ± 1.0	10.9 ± 1.0	0.30 ± 0.04	0.23 ± 0.01
10	12.5 ± 0.9	9.5 ± 0.7	0.48 ± 0.03	0.28 ± 0.02

See legend to Table 1.

TABLE 6. EFFECT OF THIAMINE DEFICIENCY ON STEROL SYNTHESIS AND HMG-CoA REDUCTASE IN C-6 GLIAL AND NEUROBLASTOMA CELLS

Cell type	Sterol synthesis (c.p.m./mg protein × 10^{-3})		HMG-CoA reductase activity (units/mg protein)	
Glia	6.05 ± 0.51	3.61 ± 0.29	6.28 ± 0.41	3.70 ± 0.29
Neuroblastoma	4.91 ± 0.48	4.69 ± 0.54	10.7 ± 1.1	10.1 ± 0.9

C-6 glial and neuroblastoma cells each were transferred to 6 25-cm^2 flasks and 6 75-cm^2 flasks, 3 of which contained thiamine-deficient medium (Th−), and 3, thiamine-supplemented (Th+) medium. After 7 days the rate of sterol synthesis from [2-^{14}C]acetate (25-cm^2 flasks) and the activity of HMG-CoA reductase (75-cm^2 flasks) were determined in each of 3 flasks from the deficient and supplemented groups (see Methods). Values are means ± S.E.M. obtained from separate determinations on each of 3 flasks. Similar results were obtained in 2 separate experiments.

data indicate that thiamine deficiency has a major effect on these biosyntheses in the glial cells. Thus, the rate of fatty acid synthesis in thiamine-deficient glia was reduced to as low as approx 15% of the rate in thiamine-supplemented cells. The decrease in fatty acid synthetic rate was accompanied by a comparable alteration in the activity of acetyl-CoA carboxylase and fatty acid synthetase, the two enzymes that function in sequence in the synthesis of fatty acids. The mechanism of the effect on fatty acid synthetase activity was shown to involve a decrease in enzyme synthesis. The rate of cholesterol biosynthesis was also reduced in the thiamine-deficient glial cells, i.e. to approx 60% of the rate in thiamine-supplemented cells. The enzymatic locus of this effect was shown to be HMG-CoA reductase. In contrast to the effects in the glial cells, under similar conditions of thiamine deficiency neuronal cells exhibited either no or only a slight reduction in fatty acid and cholesterol synthesis.

These data may have major implications for the genesis of the neuropathological lesions in conditions of thiamine deficiency (e.g. Wernicke's encephalopathy) or altered thiamine metabolism (e.g. Leigh's disease). Fatty acids and cholesterol are major constituents of cellular membranes, and alterations in these critical membrane constituents can result in marked effects on a variety of cellular functions (FARIAS *et al.*, 1975). Indeed, it is quite apparent in the present studies that the disturbances of fatty acid and cholesterol biosyntheses are followed closely by a disturbance of cell growth. The relation of lipid synthesis and cell growth is a highly important one, and it is clear that the study of thiamine-deficient and replenished glial cells provides an excellent model to define this relationship further. At any rate, the fact that the syntheses of fatty acids and cholesterol are severely disturbed in the glial cells is compatible with data derived from experimental studies (COLLINS, 1967), which indicate that the glial cell is the site of the early lesion in thiamine deficiency. Thus, the data provide at least one rational hypothesis for the previously undefined biochemical basis for the disturbance of cellular integrity in disorders of thiamine homeostasis.

A role for thiamine in the regulation of the biosyntheses of fatty acids and cholesterol is established clearly for the first time. The precise basis for this regulatory role is not definitely known, although the ultimate effect is exerted at the level of the critical enzymes in the two biosynthetic pathways. The effect on fatty acid synthetase clearly involves *synthesis* of this enzyme. Our previous observations of the regulation of fatty acid synthesis in extraneural tissues *in vivo* (VOLPE & VAGELOS, 1974; VOLPE & MARASA, 1975*b*, *c*) and glial cells in culture (VOLPE & MARASA, 1976*a*, *b*) suggest that the synthesis of fatty acid synthetase is regulated by intermediates of carbohydrate metabolism at the triose phosphate step or beyond, including perhaps acetyl-CoA. The present data support that hypothesis and perhaps specifically implicate levels of acetyl-CoA, since pyruvate decarboxylase activity has been shown to be severely depressed in glial cells subjected to a degree of thiamine deprivation similar to that produced in the present work (SCHWARTZ & MCCANDLESS, 1976). However, pyruvate decarboxylase activity is also depressed in thiamine-deficient neuroblastoma cells (SCHWARTZ & MCCANDLESS, 1976), which did not exhibit the marked effects on fatty acid synthetic rate. Determinations of intracellular acetyl-CoA levels, and particularly the specific pool that might regulate the synthesis of fatty acid synthetase, are needed to resolve this issue decisively. Whether similar or alternative mechanisms account for the effect of thiamine deficiency on HMG-CoA reductase is a provocative topic for future research.

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