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The data obtained showed a statistically significant decrease in ^{35}S -sulfate uptake by the brain particulate sulfolipid in those animals fed a vitamin E-deficient diet. No significant difference was found in the radioactive sulfate uptake by liver particulate sulfolipid between vitamin E-sufficient and -deficient animals. Only traces of radioactivity were found in the soluble fraction of either liver or brain.

The data obtained were interpreted as an indication of a selective rather than a general effect of avitaminosis E. In sulfolipid metabolism it appears that vitamin E functions in the synthesis of new sulfolipid rather than in the sulfation of sulfolipid by exchange. Since those sulfolipids which are the most inert are the membrane sulfolipids, it was suggested that some of the symptoms of avitaminosis E may be a reflection of an alteration in the cerebroside-sulfolipid ratio in the myelin sheath.

Nutrit. Dept.

July 31, 1968

To the Graduate Council:

I am submitting herewith a thesis written by Brenda Pearl Todd entitled "The Effect of Avitaminosis E on the Incorporation of ^{35}S -Sulfate by Rat Tissue Sulfolipids." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

John T. Smith
Major Professor

We have read this thesis and
recommend its acceptance:

Mary Row Green

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Graduate Studies and Research

THE EFFECT OF AVITAMINOSIS E ON THE
INCORPORATION OF ^{35}S -SULFATE
BY RAT TISSUE SULFOLIPIDS

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Brenda Pearl Todd.

August 1968

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ABSTRACT

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CHAPTER I

INTRODUCTION

It is becoming increasingly evident that relationships exist between vitamin E and the metabolism of sulfur. Previous work in this laboratory has demonstrated decreased incorporation of radioactive ^{35}S -sulfate into the insoluble lipoprotein of the liver and the mucopolysaccharides isolated from cartilage and lung of rats fed diets deficient in vitamin E. It has also been shown that the sulfate incorporating components associated with the lipoprotein may be fractionated into a mucopolysaccharide, a sulfolipid, a nucleotide, a non carbohydrate precipitable (NCAP), and a residual fraction. Such fractionation of the lipoprotein prepared from rats fed a vitamin E-deficient diet has demonstrated a significant decrease in ^{35}S -sulfate incorporation into the associated sulfolipid and mucopolysaccharide. In contrast, the non carbohydrate precipitable fraction, which is the most active in incorporating sulfate, is unaffected by vitamin E deficiency.

If the effect of avitaminosis E were a general decrease in the rate of sulfur uptake, it would be logical to assume that this more active fraction would show the greatest change. Since no such change has been demonstrated, however, it appears that the action of vitamin E is involved in specific types of sulfur uptake rather than in a more generalized utilization of sulfur. This being the case, the effect of

avitaminosis E on the sulfation of such tissue fractions as the sulfolipids offers a tool for the further investigation of the role of vitamin E in sulfur metabolism.

The present investigation was undertaken, therefore, to compare the sulfation of soluble and particulate sulfolipid fractions isolated from brain and liver tissues of rats maintained on vitamin E-free diets with the sulfation of comparable sulfolipid fractions from rats fed a vitamin E-sufficient regimen.

CHAPTER II

REVIEW OF THE LITERATURE

The study of sulfur-containing lipids dates back to at least the 1870's, when Thudichum first reported the finding of such compounds in a preparation of cerebroside from brain (1). It has been only in recent years, however, with the development of new techniques of isolation and separation, that extensive work has been carried out on the biosynthesis and metabolism of these sulfolipids.

Early investigators of the chemistry and occurrence of the sulfolipids recognized these compounds as important constituents of brain tissue, and postulated that, because their increase in the brain was found to parallel the growth of the medullary sheath, they might be considered as essential constituents of that structure (2). At that time, the sulfolipids were thought to be combinations of phosphatides and cerebroside, linked by means of sulfuric acid groups in ester combination (Fig. 1).

Later work by Blix, whom Thannhauser et al. (3) cite as the first to isolate what was called cerebroside sulfate ester from beef brain, described the constituents of this compound as a cerebronic acid, sphingosine, and galactose (3). Since that time, work has been continuing to determine not only the exact configuration of the cerebroside sulfate molecule, but also its synthesis, its metabolism, and its role in the tissues in which it is found.

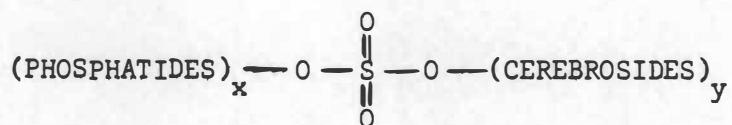


Figure 1. Early postulated structure of sulfolipid.

SOURCE: Koch, W., and M. L. Koch 1913 Contributions to the chemical differentiation of the central nervous system. III. The chemical differentiation of the brain of the albino rat during growth. J. Biol. Chem., 15: 423.

Sulfolipids, or sulfatides, are presently recognized as sulfur-containing glycolipids, a glycolipid being defined as a lipid containing one or more carbohydrate residues (4). As stated previously, Blix had described the constituents of the sulfolipid, but had not determined the position of the sulfate group (3). Thannhauser and co-workers (3) concluded from their studies of methylated brain cerebroside that the sulfate group was esterified to the primary hydroxyl of carbon atom 6 of the galactose moiety. They used as proof of this conclusion the finding of no 2,3,4,6-tetramethylgalactose after methylated cerebroside sulfate had been split and the methylgalactose compound identified. Later, however, Stoffyn and Stoffyn (5) found that when sulfatides are methylated with methyl iodide and silver oxide, a trimethyl compound, 2,4,6-trimethyl-D-galactose, is obtained. This, they state, demonstrates that the sulfate group is located not at the C-6 position as previously thought (3), but at the C-3 position of the galactopyranoside moiety of the sulfolipid.

It has also been shown (6) that isolated cerebroside sulfate contains no free amino group and no free reducing sugar group, thus substantiating Blix's assumption that the fatty acid, sphingosine, and galactose constituents are linked in the same way as in cerebroside (7). Moreover, these same workers have demonstrated by partitioning the sulfolipid between chloroform and water that the sulfur is actually part of the sulfolipid molecule and not just present as an inorganic salt which is carried along in the isolation process (6).

Finally, Stoffyn and Stoffyn (7), by a mild process of desulfation of sulfolipids, with a resulting production of cerebrosides, have provided direct evidence for the heretofore unproved proposition that sulfolipids are sulfuric esters of cerebrosides.

From such findings as these, it becomes possible to formulate a structure for the sulfolipid molecule (Fig. 2) which is indeed different from that originally proposed by Koch and Koch (2).

The similarities between the structures of sulfolipids and cerebrosides have led investigators to seek a possible precursor-product relationship between the two compounds. Radin and co-workers (8), using ^{14}C -galactose and ^{35}S -sulfate, found that while cerebrosides undergo a slow but demonstrable turnover, sulfolipids show no breakdown in normal brain. This observation led to the suggestion by these workers that cerebrosides are direct precursors of sulfolipids. Likewise, Hauser (9) observed that the specific activity of labeled cerebrosides was so much greater than that of sulfolipids as to preclude the possibility that cerebrosides could be produced from sulfolipids. He was unable, on the other hand, to say whether the sulfate was attached to the galactose moiety at some earlier point in the synthetic process (9).

It is now recognized that the formation of cerebroside sulfate occurs by the transfer of sulfate to the sulfolipid by the compound 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as first observed by Goldberg and Delbrück (10); however, there remains some question as to whether it is actually the cerebrosides which are recipients of this

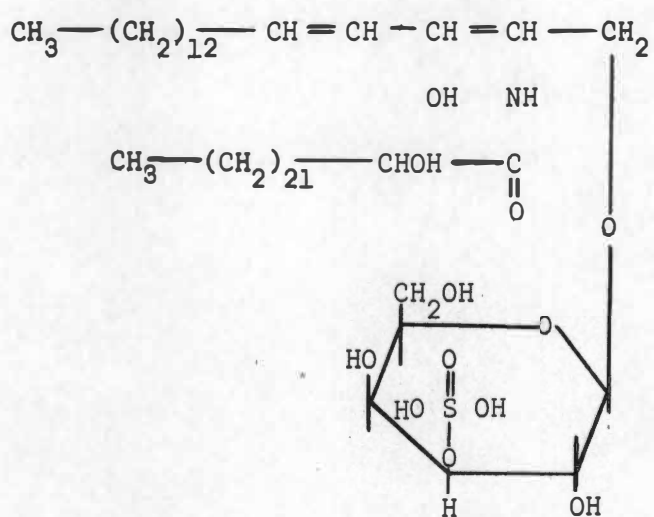


Figure 2. Presently accepted structure of sulfolipid.

sulfate, thus becoming sulfolipids. In vivo studies using both labeled sulfate and labeled acetate have provided evidence that brain tissue converts cerebroside to sulfolipids, presumably by direct sulfation of the former by PAPS (11,12,13). McKhann and co-workers (13) have even suggested that this sulfation of cerebroside by PAPS is controlled by a soluble enzyme, specific for galactocerebroside, which they call "galactocerebroside sulfokinase."

In contrast, there have also been recent reports of unsuccessful attempts to produce sulfolipids from labeled cerebroside when the latter were injected into rats either intracerebrally or intraperitoneally (14,15). This has led to the suggestion by these investigators that sulfolipids are not formed by the direct sulfation of cerebroside, but that the latter compounds are metabolized by an entirely different route (14). Similarly, no sulfate-accepting ability for either psychosine or natural cerebroside in vitro could be demonstrated by Goldberg (1), which led him to propose that some other synthetic mechanism is involved in sulfolipid formation.

More recently, it has been found by Balasubramanian and Bachhawat (16) that an enzyme prepared from the white matter of sheep brain could catalyze the formation of ^{35}S -cerebroside sulfate from ^{35}S -PAPS. Furthermore, they found that this sulfolipid formation seemed to involve only a transfer of sulfate from PAPS, since neither nucleotides such as ATP, UTP, UDPG, nor N-acylsphingosine had a stimulating effect on the reaction; that the reaction was a transfer of sulfate to a galactose-containing acceptor and not just a sulfate exchange reaction with any

sulfolipid present in the enzyme; and that this galactose-containing acceptor is present only in the bound form in the enzyme preparation, since attempts to isolate the enzyme activity from the acceptor were unsuccessful. From these results, they concluded that sulfolipids are formed by the direct sulfation of cerebroside by PAPS, but that this sulfation occurs only when the acceptor is bound to the protein part of the enzyme (16). Such observations account for previous failures (14, 15) to produce sulfolipids from injected cerebroside and suggest that the synthesis of sulfolipids proceeds as in Fig. 3.

In studying the synthetic mechanisms involved in the formation of sulfolipids, it has become increasingly apparent that these compounds are closely associated not only with the structure but also with the function of the tissues or organs in which they occur.

Early investigators found that the sulfolipids in the brain of the rat, dog, and man increased parallel to the development of the medullary sheath (2,17). Moreover, they also observed that once the period of myelination was over, the sulfolipid content of the brain did not decrease but tended to remain stationary throughout the remainder of the animal's life (2). This led to the suggestion that perhaps these compounds played a structural and/or operational role in the myelin network. Findings indicative of such a structural role for sulfolipids have been reported by a number of recent investigators. It has been found, for example, that the increase in sulfolipid content of the rapidly myelinating brain is accompanied by a more efficient incorporation

Galactocerebrosides + Enzyme* $\longrightarrow$ [Galactocerebroside-enzyme complex]

+PAPS $\longrightarrow$ sulfolipids + PAP

*Enzyme: galactocerebroside sulfokinase (13).

Figure 3. Synthetic route of sulfolipid.

of substances considered to be direct precursors of brain lipids--i.e., sulfate, glucose, galactose, and serine (18). At the same time, the fatty acids of cerebrosides and sulfolipids undergo a redistribution in the maturing animal suggestive of a rapid conversion of the former to the latter (11,19), and it is only after myelination is complete that a myelin lipid ratio characteristic of the adult animal is attained (20). Also paralleling the period of rapid myelination is an increase in the activity of the enzyme system responsible for the synthesis of active sulfate (21). It would seem, therefore, that since the formation of sulfolipids so closely corresponds to the maturation of brain tissue, these compounds could conceivably play a vital role in the structure of myelin.

Evidence for this structural role has also been accumulating from studies of the incorporation of radioactive sulfate into sulfolipid of various tissues. Green and Robinson (22) found that ^{35}S -sulfate injected into rats was very slowly incorporated into cerebroside sulfate, thus reflecting a slow turnover of the sulfolipids in brain tissue. Similar results were obtained by Heald and Robinson (23) who postulated that this slow incorporation of sulfate into brain actually represented new synthesis of sulfolipid and that this apparent metabolic stability would be required if the sulfolipids were actually a vital structural component of the myelin sheath. More recently, Davison and Gregson (24) have carried out a series of experiments on the metabolism of sulfolipids in each of the various subcellular fractions of the brain.

They, as other investigators, report that the incorporation of ^{35}S -sulfate into brain sulfolipid of rats is at a maximum in 20-day-old animals but also occurs in adult brain lipids. Moreover, this radioactivity was found to be more extensively incorporated into myelin of both young rats and adults when the animals were injected intracerebrally rather than intraperitoneally.

In the course of their experiments, these workers also found the existence of a small but rapidly exchanging pool of myelin sulfolipid, the presence of which seemed at variance with the idea of a metabolic stability in the myelin sheath. It was pointed out, however, that the size of this active pool is so small that, for all practical purposes, the myelin sulfolipids can be considered inert (24).

Additional evidence for the structural role of sulfolipids comes from recent investigations into the biosynthesis of total lipids in both brain and liver of rats. Hajra and Radin (25) postulate from their findings that the brain contains two groups of lipids having separate roles: a slowly metabolizing fraction which may be "structural" and which is used in myelin formation; and a rapidly metabolizing fraction, "functional" in that it serves a role in brain metabolism. This latter "functional" lipid perhaps corresponds to the rapidly metabolizing sulfolipid which Davison and Gregson (24) reported in both microsomal and supernatant fractions of brain, and which they suggested was an active biosynthetic mechanism for non-membrane sulfolipid.

Similarly, the finding by Vyas and Tanksdale (26) that lipid formation in brain decreased after the first 10 days of life has led

these investigators to the conclusion that, since the structural framework of the brain is laid during the first 10 days of life and prior to myelination, a decrease in the rate of lipid formation parallel to a decrease in the growth of brain tissue after 10 days tends to support the idea that lipids are mainly for structural framework with limited metabolic participation. It should be pointed out, however, that these investigators are speaking of total lipids and, because they do not differentiate among the various types of lipids, their results at first appear contradictory to the findings for increased sulfolipid values with increased growth reported by the workers previously mentioned.

From the above statements it can be seen that the sulfolipids are important constituents of brain tissue. Further evidence of their importance is indicated by reports of their occurrence in such widely differing tissues as horse kidney, liver, testes, submaxillary glands, and muscle, as well as nervous tissue (1,22). Yet only recently has there been any indication of a possible connection between the metabolism of the sulfolipids and that of the tocopherols.

Vitamin E, a fat-soluble vitamin, is stored in the liver and fatty tissues of the body (27), and recent studies with labeled α -tocopherol have shown that when this substance is injected, the radioactivity is associated mainly with the structural components of liver and spleen cells (28). Much of the present research into the role of this vitamin in the body has centered around its function in lipid metabolism (29-34), or its effect in degenerative diseases of the

nervous system as previously reviewed (30,34). Recently, however, Green and co-workers have challenged the "lipid peroxidation" prevention theory and have suggested that this antioxidant activity is not the sole, or even the primary, function of vitamin E in the tissues (35). It is thought, therefore, that perhaps this fat-soluble vitamin plays an important role in sulfur metabolism, as indicated by studies of its effect on the sulfation of mucopolysaccharides and cellular lipoproteins (36,37).

Fractionation of the cellular lipoprotein into a mucopolysaccharide, a sulfolipid, a nucleotide, a non carbohydrate alcohol precipitable fraction, and a residual fraction enabled Fulton (37) to determine the effect of avitaminosis E on the fixation of ^{35}S -sulfate by the mucopolysaccharides and sulfolipids of the cellular lipoprotein but not by the other fractions. One of the other fractions, the non carbohydrate alcohol precipitable fraction, was more active with respect to sulfate fixation than either the mucopolysaccharide or the sulfolipid fractions; however, it was unaffected by the absence of vitamin E from the diet. If a generalized reduction in ^{35}S -sulfate fixation were the effect of avitaminosis E, this most active fraction should have been affected. These data indicate, then, that the effect of avitaminosis E on sulfate fixation may be selective rather than general.

It has been shown that sulfate incorporation into brain sulfolipid occurs slowly, indicating new synthesis (22), while the more rapid turnover of sulfate in the liver indicates exchange (23). Therefore, a

comparison of the effect of avitaminosis E on the fixation of a test dose of $^{35}\text{SO}_4^{=}$ into sulfolipids should show the relative effects of a lack of vitamin E on both synthetic and exchange reactions in brain and liver sulfolipids.

CHAPTER III

METHODS

I. GENERAL PLAN

Because recent work in this laboratory had indicated a vitamin E-related difference in the incorporation of ^{35}S -sulfate into certain lipoprotein fractions of rat tissues (37), the effect of both vitamin E-sufficient and -deficient diets on the sulfation of sulfolipid isolated from the liver and brain tissues of experimental animals was investigated.

Forty male, albino rats of the Sprague-Dawley strain were used as tissue donors. The animals were divided randomly into two groups, the first of which was fed a low inorganic sulfate (0.002 per cent), vitamin E-free diet similar to that used by Fulton (37) and outlined in Tables 1 and 2. The other 20 rats were fed the same diet which had been supplemented with 28 mg. DL- α -tocopherol acetate per 100 g. of diet. The animals were housed in wire cages, 5 animals per cage, and given food and distilled water ad libitum. Care was taken at all times to see that the vitamin E-free diet was in no way contaminated with the supplemented diet. Fourteen of the animals died during the course of the experiment, apparently from exposure suffered during shipment; the remaining rats, however, appeared healthy, and no difference in growth rate between the animals on the two diets was detected.

TABLE 1
COMPOSITION OF EXPERIMENTAL DIETS

	g./100 g.
Vitamin free casein	15.00
Methionine	0.35
Sucrose	31.00
Cornstarch	32.00
Non-nutritive bulk ^a	10.00
Stripped lard ^b	6.00
Cod-liver oil	2.00
Vitamin mixture ^c	1.00
Salt mixture ^d	3.00

^aAlphacel, Nutritional Biochemicals Corporation, Cleveland.

^bDistillation Products Industries, Inc., Rochester, New York.

^cSynthetic vitamins added per 100 g. diet were: (in mg.) nicotinic acid, 20.0; pyridoxine·HCl, 0.5; thiamine·HCl, 0.5; riboflavin, 0.5; Ca pantothenate, 1.0; folic acid, 0.5; biotin, 0.005; 2-methylnaphthoquinone, 0.025; vitamin B₁₂, 0.0045; choline chloride, 100.0; i-inositol, 100.0; p-aminobenzoic acid, 7.5. The vitamins were triturated with sufficient sucrose to make 1.0 g. DL- α -tocopheryl acetate was added to the E-sufficient diet at the level of 28 mg./100 g. diet.

^dSee Table 2.

TABLE 2
COMPOSITION OF SALT MIXTURE^a

	g.
CaCO ₃	44.750
MgCO ₃	3.060
NaCl	6.900
KCl	11.200
KH ₂ PO ₄	21.200
FePO ₄ ·2H ₂ O	2.050
KI	0.008
NaF	0.010
AlK(SO ₄) ₂	0.017
Cu(C ₂ H ₃ O ₂) ₂ ·H ₂ O	0.072
MnCl ₂ ·4H ₂ O	0.040
Cornstarch	10.693

^aModification of Hubbell, et al. (38).

After the animals had been fed the diet for a minimum of five weeks, the erythrocyte hemolysis test was used to determine whether the rats on the vitamin E-free diet were vitamin E-deficient. Hemolysis by dialuric acid of at least 85 per cent in erythrocytes from vitamin E-free animals, as compared to 0 per cent hemolysis in vitamin E-sufficient animals, was used for judging a deficiency state. As soon as a vitamin E-deficiency had been established, the rats were injected subcutaneously with 10 to 25 μ c. of $\text{Na}_2^{35}\text{SO}_4$ in 0.5 ml. isotonic saline and killed 48 hours later. At the same time an equal number of vitamin E-sufficient animals, similar in weight to the deficient rats, were injected and killed 48 hours later. Following decapitation, the livers, brains, and ribs were removed from the animals, placed in individual labeled jars, and stored at -20°C . until analyses could be run.

Animals on the vitamin E-free diet were paired for analysis with those vitamin E-sufficient rats of equal or nearly equal weight which had been killed on the same day. Isolation of both particulate and soluble sulfolipid was carried out on partially thawed livers and brains from each pair of animals. The soluble and particulate sulfolipid samples from 20 of the animals (10 pairs) were counted by liquid scintillation to determine the $^{35}\text{SO}_4 =$ distribution; the remaining samples (2 pairs of animals) were used to determine the degree of radioactive purity of the isolated material by thin layer chromatography.

II. ERYTHROCYTE HEMOLYSIS

The susceptibility of the erythrocytes of the vitamin E-deficient rats to hemolysis by dialuric acid was determined by the method of Friedman et al. (39). About 40 λ blood collected from an incision in the rat's tail were put into a 15 ml. centrifuge tube containing 5 ml. saline phosphate buffer made by combining equal quantities of 0.9 per cent saline solution and 0.1 M Na_2HPO_4 solution adjusted to pH 7.4 by the addition of 20 ml. 1.0 N HCl per liter. The tubes were swirled gently to prevent clotting and to insure thorough mixing of the blood and buffer solution. This suspension was then centrifuged at 1000 x g. for 10 minutes, after which the supernatant fluid was removed by suction and the cells resuspended in 3.5 ml. of the buffer.

A 1 ml. aliquot of the erythrocyte suspension was placed in each of 3 test tubes. To tubes 1 and 2 was added 1 ml. dialuric acid solution which was prepared by adding 10 mg. dialuric acid to 50 ml. saline-phosphate buffer; to tube 3 was added 1 ml. of the saline-phosphate buffer. All tubes were incubated in a 37°C. water bath for 1 hour after which they were allowed to cool at room temperature for another hour. Following this cooling period, 5 ml. of distilled water were added to tube 2 for complete hemolysis of the erythrocytes; at the same time, an addition of 5 ml. saline-phosphate buffer was made to tubes 1 and 3. The tubes were then centrifuged at 1000 x g. for 5 minutes and the supernatant fluid poured into cuvettes. A Beckman Model B Spectrophotometer which had been set at zero optical density with a distilled

water blank was used for absorptivity readings at a wavelength of 420 mμ. Per cent hemolysis was calculated by the formula

$$\text{Per cent hemolysis} = \frac{\text{Optical density tube 1} - \text{Optical density tube 3}}{\text{Optical density tube 2} - \text{Optical density tube 3}} \times 100$$

III. ISOLATION OF SULFOLIPID

Rats of similar weights were paired for the isolation of soluble and particulate sulfolipid fractions from both brain and liver tissue. The methods employed for this isolation were somewhat different for the two tissues; therefore, both procedures are outlined below. Following this initial analysis, all samples were treated similarly in regards to purification and preparation for counting.

Liver Sulfolipid

Partially thawed livers from each pair of rats were weighed and a 10 per cent homogenate of the individual livers was prepared using 0.067 M phosphate buffer, pH 7.4. Homogenization was carried out using a motor-driven Potter-Elvehjem glass homogenizer with a Teflon pestle. An ice bath and cold buffer solution were used during the homogenization process to keep the tissue chilled.

The homogenate was centrifuged at 1200-1500 x g. for 10 minutes in a refrigerated centrifuge with the Lourdes 9RA head. The supernatant fluid was decanted, with care taken to remove any white fatty layer with cotton swabs which were extracted with chloroform-methanol and added to the supernatant fluid. The centrifuge cake was also removed and saved.

The supernatant fluid was extracted with about 50 ml. chloroform-methanol (2:1 v/v), followed by centrifugation at 1200 x g. for 10 minutes. This centrifugation resulted in three layers, the first two of which were decanted and discarded. The remaining chloroform layer was then washed in a separatory funnel with an additional 20 ml. chloroform-methanol (2:1 v/v), and the bottom layer containing the soluble sulfolipid fraction removed and saved.

To obtain the particulate sulfolipid fraction, the centrifuge cake which had been saved from the initial centrifugation above was treated with 20 ml. chloroform-methanol (1:1 v/v) and heated in a 60°C. water bath for 1 hour. Following this heating period the treated centrifuge cake suspension was centrifuged at 1200 x g. for 10 minutes, and the supernatant fluid decanted and saved. The centrifuge cake was again treated with chloroform-methanol (2:1 v/v) and heated in a 60°C. water bath for 1 hour, followed by centrifugation at 1200 x g. for 10 minutes. The supernatant fluid was decanted and combined with that above. This combined fraction was then washed in a separatory funnel with 20 ml. chloroform-methanol (2:1 v/v) and the bottom layer containing the particulate sulfolipid fraction drawn off and saved.

Brain Sulfolipid

Sulfolipid was extracted from brains of paired animals in a manner similar to that used for liver sulfolipid extraction.

The brains of each rat in the pair were partially thawed and homogenized for approximately 30 seconds (low speed) with 25 ml.

phosphate buffer. A motor-driven Eberbach Monel metal Semi-Micro blender cup with Teflon coating was used for this operation. The blender cup was rinsed with 10-20 ml. of the buffer, and care was taken to remove all of the homogenate. The homogeneous suspension was then centrifuged for 10 minutes at 1200 x g., after which the supernatant fluid was decanted and the centrifuge cake removed and saved. Any fat clinging to the sides of the centrifuge tubes was removed with cotton swabs which were extracted with chloroform-methanol and added to the supernatant fluid. Ten milliliters of chloroform-methanol (2:1 v/v) were shaken with the supernatant fluid, followed by centrifugation for 10 minutes at 1200 x g. Of the three resulting layers, the top two were decanted and discarded, and the remaining chloroform layer washed in a separatory funnel with 10 ml. chloroform-methanol (2:1 v/v). This washing resulted in two layers; only the bottom layer containing the soluble portion of the brain sulfolipid was drawn off and saved.

The brain particulate sulfolipid fraction was isolated from the centrifuge cake which had been saved from the initial centrifugation. This cake was treated with 10 ml. chloroform-methanol (1:1 v/v) and heated in a 60°C. water bath for 1 hour. This was followed by centrifugation for 10 minutes at 1200 x g. after which the supernatant was decanted and saved, and the centrifuge cake was treated with 10 ml. chloroform-methanol (2:1 v/v). A second heating period of 1 hour in a 60°C. water bath was followed by centrifugation as above. The supernatant fluid obtained was combined with that from the first treatment,

and the combined fractions washed in a separatory funnel with 20 ml. chloroform-methanol (2:1 v/v). Again two layers resulted, and only the bottom layer containing the particulate sulfolipid fraction was removed and saved.

IV. PURIFICATION OF SULFOLIPID FRACTIONS

The particulate and soluble sulfolipid fractions from both the brains and livers of each animal obtained as outlined above were further purified by dialysis against running tap water for approximately 24 hours. The contents of each dialysis tube were centrifuged at 1200 x g. for 10 minutes; the bottom layer was then removed by suction and prepared for counting.

V. PREPARATION FOR COUNTING

Each dialyzed sample was quantitated to 10 ml. by evaporation or addition of chloroform-methanol (2:1 v/v). Two 5 ml. aliquots were put into small glass counting vials and evaporated to dryness. Twenty milliliters of scintillation solution¹ were added to each vial, after which the vials were sealed and shaken thoroughly to dissolve the sample. Radioactivity was determined by a Picker Nuclear Liquimat 220 liquid scintillation detector set for a confidence level of 3 per cent.

¹Picker Nuclear packaged scintillator [98 per cent 2,5-diphenyl-oxazole and 2 per cent p-bis (o-methylstyryl benzene)] mixed in 1 liter toluene.

VI. RADIOACTIVE PURITY CHECK

Radioactive purity of the isolated sulfolipid was determined by thin layer chromatography on Silica Gel G. A sample of brain particulate sulfolipid which had been isolated as described previously but not prepared for counting was used for the determination. The sample to be chromatographed was quantitated to 5 ml. by the addition of chloroform-methanol. A 2 ml. portion was removed to a glass counting vial, evaporated to dryness and prepared for counting. A second 2 ml. portion was removed and evaporated to dryness, after which 500 λ of chloroform-methanol were added to dissolve the sample. About 300 λ of this sulfolipid sample were stripped on a Silica Gel G plate along with a spot of brain sulfolipid standard. The plate was developed with a petroleum ether-diethyl ether-glacial acetic acid (90:10:1 v/v/v) solvent, and the bands visualized by charring in a 100°C. oven after the plate had been sprayed with chromic sulfuric acid reagent. Each band was removed from the plate and placed in a glass counting vial. The radiation was determined as previously described.

VII. STATISTICS

The method of paired comparisons as described by Steel and Torrie (40) was used to determine statistical significance of the data.

CHAPTER IV

RESULTS AND DISCUSSION

The relationship between vitamin E and the sulfation of brain and liver sulfolipid has been investigated by comparing the fixation of a test dose of ^{35}S -sulfate by the sulfolipids of brain and liver in rats fed vitamin E-sufficient and -deficient diets. Both diets were low in inorganic sulfur since previous investigations have shown that feeding diets low in inorganic sulfur enhances the effect of avitaminosis E on sulfate utilization (36,37).

The differences in the incorporation of sulfate into sulfolipid obtained in this investigation probably would have been greater if the animals had been fed one of the more drastic vitamin E-deficient diets used by other investigators (33,34,41,42). However, because these diets often result in inanition, it appears that some difficulty in distinguishing between the effects of inanition and those of avitaminosis E would arise if such regimens are used. As can be seen from Table 3, the vitamin E-deficient animals used in this investigation attained final weights almost identical to those attained by the vitamin E-sufficient animals, with no signs of inanition.

Although these weight gains appear normal for 5 to 6 week old rats, it has been suggested that the removal of the natural antioxidant (vitamin E) from the deficient diets produces rancidity and thus

TABLE 3
WEIGHT OF RATS WHEN PLACED ON DIET AND WHEN KILLED

Rat Pair Number	Sufficient E		Deficient E	
	Weanling g.	Killed g.	Weanling g.	Killed g.
1	38	330	45	330
2	40	270	38	270
3	36	320	36	320
4	38	304	36	310
5	34	280	36	280
6	40	280	34	280
7	36	260	36	250
8	36	280	40	270
9	41	307	40	309
10	37	295	42	300
	38±1 ^a	293±7	38±1	292±8

^aMean ± standard error of mean.

rejection of the diet by the animals.² While feed consumption records were not kept for each rat, total food consumption per cage of animals was recorded and used in conjunction with total weight gains to calculate average feed efficiency. These data, presented in Table 4, show no significant differences in the feed efficiency between those rats fed the vitamin E-sufficient and -deficient diets. Some rather large differences in weight gain among certain cages of animals are reported in Table 4, particularly in the deficient group. It may be recalled, however, that several of the animals died during the early part of the experiment from exposure during shipment; therefore each cage did not always contain the same number of animals. These data, then, are a reflection only of the size and number of animals per cage and not of a variability in growth possibly caused by a rejection of the diet.

Since the rats were not suffering from inanition and showed no visible symptoms of avitaminosis E, hemolysis by dialuric acid was used as the criterion for a deficiency state. Each time animals from the vitamin E-deficient group were tested, one of the vitamin E-sufficient rats was also tested to ascertain the susceptibility of normal erythrocytes to dialuric acid hemolysis. In every case the deficient animals demonstrated a hemolysis of 90 per cent or greater while the erythrocytes from the vitamin E-sufficient animals consistently showed no hemolysis; therefore, a vitamin E-deficiency state was established.

²Personal communication, J. T. Smith.

TABLE 4

TOTAL WEIGHT GAIN, FEED CONSUMPTION AND FEED EFFICIENCY FOR
VITAMIN E SUFFICIENT AND DEFICIENT RATS

Sufficient E			Deficient E		
Total Weight Gain/ Cage g.	Total Feed Consumption/ Cage g.	Feed Efficiency/ Cage	Total Weight Gain/ Cage g.	Total Feed Consumption/ Cage g.	Feed Efficiency/ Cage
Cage 1 901	2812	0.29	Cage 5 817	2799	0.32
2 757	2686	0.32	6 1174	3714	0.28
3 944	2755	0.33	7 916	2757	0.34
4 791	2271	0.40	8 598	1506	0.35
		0.32±0.02 ^a			0.33±0.02

^aMean ± standard error of the mean.

The brain is a very stable organ and changes little with dietary manipulation. In contrast, the liver is very susceptible to changes in diet and may undergo rapid fluctuations in fat, glycogen, and cytoplasmic protein content (4). Such changes are often used as indices of a deficiency or a diseased state, as in the liver necrosis reported by some workers as a classic symptom of avitaminosis E (42,43). In the present investigation, livers from the vitamin E-deficient rats showed no signs of necrosis. As can be seen in Table 5, the liver weights from the vitamin E-deficient rats tended to be higher than those of the vitamin E-sufficient animals. The difference, however, was not statistically significant ($P < 0.1 > 0.05$).

Several investigators have suggested that the sulfolipids are membrane lipids (22,23,24,43), while others have also reported small amounts of rapidly metabolizing cytoplasmic sulfolipids (10,24,25,28). Therefore, both particulate and cytoplasmic preparations were made and evaluated in this investigation. With the level of radioactivity used (10 - 25 μ c.), however, no ^{35}S -radioactivity could be detected in the lipid preparations from the cytoplasm of either the liver or the brain. Therefore, the data which are shown in Table 6 were obtained exclusively from the particulate fraction of the liver. Since total ^{35}S in the sulfolipid fraction of the particulate preparation did not differ significantly ($P < 0.4 > 0.3$) between vitamin E-deficient and -sufficient animals, the data were recalculated on the basis of liver weight. It appeared that the differences in liver weight might decrease the high standard

TABLE 5
WEIGHT OF LIVERS FROM VITAMIN E-SUFFICIENT AND -DEFICIENT RATS

Rat Pair Number	Liver Weight	
	Sufficient E g.	Deficient E g.
1	12.0	12.1
2	12.2	12.5
3	13.4	18.6
4	12.0	13.4
5	10.0	11.2
6	11.3	13.9
7	10.2	9.7
8	11.5	11.4
9	14.0	14.3
10	12.2	13.3
	11.9±0.4 ^{a,b}	13.0±0.8

^aMean ± standard error of the mean.

^bP<0.1>0.05.

TABLE 6

TOTAL ³⁵S-RADIOACTIVITY IN SULFOLIPID FROM LIVER PARTICULATE FRACTION

Rat Pair Number	Total Counts/Min. $\times 10^{-3}$	
	Sufficient E	Deficient E
1	1.0	0.4
2	0.5	1.0
3	2.0	1.2
4	1.5	1.2
5	0.8	0.9
6	1.0	0.9
7	2.7	2.0
8	1.1	0.9
9	0.7	0.8
10	0.1	1.2
	1.2 $\pm$ 0.2 ^{a,b}	1.1 $\pm$ 0.1

^aMean $\pm$ standard error of the mean.

^bP<0.4>0.3.

error which was obtained. As can be seen from Table 7 the differences in the data remained statistically insignificant ($P < 0.2 > 0.1$).

The lack of a statistically significant difference in the fixation of a test dose of ^{35}S by the sulfolipid fraction from the liver particulate preparation was unexpected. Fulton (37) obtained a significant decrease in the ^{35}S fixation by the sulfolipid fraction from cellular lipoprotein prepared from the livers of rats fed vitamin E-deficient diets when the animals were killed 8 hours after injection of the radioactivity; however, she found no significant difference in the ^{35}S uptake between the deficient and sufficient animals when they were killed 24 hours after injection. Similarly, Green and Robinson (22) reported that maximal incorporation of radioactivity into liver sulfolipids occurred 12 hours after injection, with only a small fraction of the radioactivity remaining after 48 hours. In the present investigation, therefore, the fact that the animals were killed 48 hours after injection could account for the lack of significant differences in liver sulfolipid.

The total ^{35}S -radioactivity isolated from the particulate fraction of brains is shown in Table 8. In contrast to the data for the liver sulfolipid fraction (Tables 6 and 7), there is a highly significant difference ($P < 0.01 > 0.001$) in the fixation of ^{35}S by this fraction. Other investigators (22,24) have also reported the ready incorporation of labeled sulfate into brain tissue; however, these workers do not report any relationship between this incorporation and the vitamin E status of the animal.

TABLE 7

³⁵S-RADIOACTIVITY IN THE PARTICULATE SULFOLIPID FRACTION
FROM RAT LIVER PER GRAM OF LIVER

Rat Pair Number	Counts/Min./g. Liver	
	Sufficient E	Deficient E
1	86	29
2	22	92
3	149	67
4	125	92
5	83	83
6	89	64
7	264	201
8	99	83
9	53	57
10	113	90
	108±20 ^{a,b}	87±14

^aMean ± standard error of the mean.

^bP<0.2>0.1.

TABLE 8
TOTAL ^{35}S -RADIOACTIVITY IN THE PARTICULATE SULFOLIPID
FRACTION FROM RAT BRAIN

Rat Pair Number	Total Counts/Min. $\times 10^{-3}$	
	Sufficient E	Deficient E
1	1.0	1.0
2	1.1	0.8
3	1.2	1.0
4	1.3	1.2
5	0.9	0.8
6	0.9	1.0
7	0.7	0.1
8	1.4	0.9
9	1.1	0.7
10	1.4	1.1
	$1.1 \pm 0.1^{a,b}$	0.9 ± 0.1

^aMean $\pm$ standard error of the mean.

^b $P < 0.01 > 0.001$.

The data obtained in this investigation indicate that the effect of avitaminosis E on the sulfation of sulfolipid is on de novo synthesis rather than exchange since it has been suggested that the slow uptake by the sulfolipids of the brain represents new synthesis while the comparatively rapid uptake by the liver represents exchange (22,23). The decreased uptake by the sulfolipid of the cellular lipoprotein prepared from the livers of vitamin E-deficient rats observed by Fulton (37) would seem to contradict the data obtained in this investigation since an avitaminosis E effect was obtained with sulfolipid from brain but not from liver. A review of the method used by Fulton to prepare cellular lipoprotein, however, shows extensive extraction by salt solutions; therefore, only the most tightly bound sulfolipid would be expected to remain with the "membrane" preparations. This tightly bound sulfolipid may be sulfated by synthesis rather than exchange.

One of the symptoms of severe avitaminosis E is a paralysis which in chicks is related to the sulfur nutrition of the animal (44). Since it has become generally accepted that sulfolipids have an important role in the integrity of the myelin sheath (23,24,41), these data suggest an important role for vitamin E in the sulfation of sulfolipids. If the presence of the charged sulfate group of sulfolipids is necessary for proper functioning of the myelin tissue, it is conceivable that the paralysis and muscular dystrophy of avitaminosis E is a reflection of an alteration in the cerebroside-sulfolipid ratio in nervous tissue, with an associated change in electrical potential and a disruption of function.

CHAPTER V

SUMMARY

The relationship between the sulfation of brain and liver sulfolipids was investigated by comparing the fixation of a test dose of ^{35}S -sulfate by the sulfolipid fractions of brain and liver from rats fed vitamin E-deficient and -sufficient diets. Brain and liver tissue was divided into particulate and soluble fractions and the sulfolipid was extracted from each with chloroform-methanol. Purification of the sulfolipid fractions by dialysis against running tap water was followed by determination of radioactivity by liquid scintillation.

The data obtained showed a statistically significant decrease in ^{35}S -sulfate uptake by the brain particulate sulfolipid in those animals fed a vitamin E-deficient diet. No significant difference was found in the uptake of radioactive sulfate by liver particulate sulfolipid between vitamin E-sufficient and -deficient animals. Only traces of radioactivity were found in the soluble fraction of either liver or brain.

Based on previous data obtained in this laboratory and conclusions in the literature, these data were interpreted as an indication of a selective rather than a general effect of avitaminosis E. In sulfolipid metabolism vitamin E appears to function in the synthesis of new sulfolipid rather than in the sulfation of sulfolipid by exchange.

Since those sulfolipids which are the most inert are the membrane sulfolipids, it was suggested that some of the symptoms of avitaminosis E may be a reflection of an alteration in the cerebroside-sulfolipid ratio in the myelin sheath.



LITERATURE CITED

LITERATURE CITED

1. Goldberg, I. H. 1961 The sulfolipids. J. Lipid Res., 2: 103.
2. Koch, W., and M. L. Koch 1913 Contributions to the chemical differentiation of the central nervous system. III. The chemical differentiation of the brain of the albino rat during growth. J. Biol. Chem., 15: 423.
3. Thannhauser, S. J., J. Fellig and G. Schmidt 1955 The structure of cerebroside sulfuric ester of beef brain. J. Biol. Chem., 215: 211.
4. White, A., P. Handler and E. L. Smith 1964 Principles of Biochemistry. McGraw-Hill Book Company, New York.
5. Stoffyn, P., and A. Stoffyn 1963 Structure of sulfatides. Biochim. Biophys. Acta, 70: 218.
6. Lees, M., J. Folch, G. H. Sloane-Stanley and S. Carr 1959 A simple procedure for the preparation of brain sulphatides. J. Neurochemistry, 4: 9.
7. Stoffyn, P., and A. Stoffyn 1963 Direct conversion of sulfatides into cerebroside. Biochim. Biophys. Acta, 70: 107.
8. Radin, N. S., F. B. Martin and J. R. Brown 1957 Galactolipide metabolism. J. Biol. Chem., 224: 499.
9. Hauser, G. 1964 Labeling of cerebroside and sulfatides in rat brain. Biochim. Biophys. Acta, 84: 212.
10. Goldberg, I. H., and A. Delbrück 1959 Transfer of sulfate from 3'-phosphoadenosine-5'-phosphosulfate to lipids, mucopolysaccharides, and aminoalkyl phenols. Federation Proc., 18: 235 (abstract).
11. Kishimoto, Y., and N. S. Radin 1966 Metabolism of brain glycolipid fatty acids. Lipids, 1: 47.
12. McKhann, G. M., R. Levy and W. Ho 1965 Biosynthesis of sulfatides in rat brain. Federation Proc., 24: 361 (abstract).
13. McKhann, G. M., R. Levy and W. Ho 1965 Metabolism of sulfatides. I. The effect of galactocerebroside on the synthesis of sulfatides. Biochem. Biophys. Res. Commun., 20: 109.

14. Kopaczky, K. C., and N. S. Radin 1965 In vivo conversions of cerebroside and ceramide in rat brain. J. Lipid Res., 6: 140.
15. Kanfer, J. 1965 Observations on cerebroside metabolism in vivo. J. Biol. Chem., 240: 609.
16. Balasubramanian, A. S., and B. K. Bachhawat 1965 Formation of cerebroside sulfate from 3'-phosphoadenosine-5'phosphosulfate in sheep brain. Biochim. Biophys. Acta, 106: 218.
17. Koch, W., and M. L. Koch 1917 Contributions to the chemical differentiation of the central nervous system. IV. The relative amount of sheathing substance in the corpus callosum and intradural nerve roots (man and dog). J. Biol. Chem., 31: 395.
18. Kishimoto, Y., W. E. Davies and N. S. Radin 1965 Turnover of the fatty acids of rat brain gangliosides, glycerophosphatides, cerebroside, and sulfatides as a function of age. J. Lipid Res., 6: 525.
19. Menkes, J. H., M. Philippart and M. C. Concone 1966 Concentration and fatty acid composition of cerebroside and sulfatides in mature and immature human brain. J. Lipid Res., 7: 479.
20. Davison, A. N., M. L. Cuzner, N. L. Banik and J. Oxberry 1966 Myelinogenesis in the rat brain. Nature, 212: 1373.
21. Balasubramanian, A. S., and B. K. Bachhawat 1961 Formation of active sulfate in the rat brain. J. Sci. Industr. Res., 20c: 202.
22. Green, J. P., and J. D. Robinson, Jr. 1960 Cerebroside sulfate (sulfatide A) in some organs of the rat and in mast cell tumor. J. Biol. Chem., 235: 1621.
23. Heald, P. J., and M. A. Robinson 1961 The metabolism of sulphatides in cerebral tissues. Biochem. J., 81: 157.
24. Davison, A. N., and N. A. Gregson 1966 Metabolism of cellular membrane sulfolipids in the rat brain. Biochem. J., 98: 915.
25. Hajra, A. K., and N. S. Radin 1963 Isotopic studies of the biosynthesis of the cerebroside fatty acids in rats. J. Lipid Res., 4: 270.
26. Vyas, M. K., and K. G. Tanksdale 1967 Post-natal changes in liver and brain lipids of rats. Current Sci., 36: 73.
27. Herting, D. C. 1966 Perspective on vitamin E. Amer. J. Clin. Nutr., 19: 210.

28. Mellors, A., and M. McC. Barnes 1966 The distribution and metabolism of α -tocopherol in the rat. Brit. J. Nutr., 20: 69.
29. Witting, L. A. 1965 Biological availability of tocopherol and other antioxidants at the cellular level. Federation Proc., 24: 912.
30. Alfin-Slater, R. B., and R. S. Morris 1963 Vitamin E and lipid metabolism. Adv. Lipid Res., 1: 183.
31. Kummerow, F. A. 1964 Possible role of vitamin E in unsaturated fatty acid metabolism. Federation Proc., 23: 1053.
32. Bieri, J. G., and E. L. Prival 1966 Effect of deficiencies of α -tocopherol, retinol and zinc on the lipid composition of rat testes. J. Nutr., 89: 55.
33. Harrill, I., and E. D. Gifford 1966 Effect of vitamin E, arginine and methionine on free amino acids and lipids in selected rat tissues. J. Nutr., 89: 247.
34. Morris, M. D., C. F. Fitch and E. Cross 1966 Abnormalities of lipid metabolism in the vitamin E-deficient monkey. J. Lipid Res., 7: 210.
35. Green, J., A. T. Diplock, J. Bunyan, D. McHale and I. R. Muthy 1967 Vitamin E and stress. I. Dietary unsaturated fatty acid stress and the metabolism of α -tocopherol in the rat. Brit. J. Nutr., 21: 69.
36. Smith, J. T., and B. J. Pendergrass 1962 A relationship between vitamin E and the sulfation of mucopolysaccharides and cellular lipoproteins. Federation Proc., 21: 170 (abstract).
37. Fulton, S. F. 1966 The effect of avitaminosis E on the incorporation of ^{35}S -sulfate by rat tissue components. Unpublished Master's Thesis, The University of Tennessee.
38. Hubbell, R. G., L. B. Mendel and A. J. Wakeman 1937 A new salt mixture for use in experimental diets. J. Nutr., 14: 273.
39. Friedman, L., W. Weiss, R. Wherry and O. K. Kline 1958 Bioassay of vitamin E by the dialuric acid hemolysis method. J. Nutr., 65: 143.
40. Steel, R. G. D., and J. H. Torrie 1960 Principles and Procedures of Statistics. McGraw-Hill Book Company, New York.

41. Century, B., and M. K. Horwitt 1965 Biological availability of various forms of vitamin E with respect to different indices of deficiency. Federation Proc., 24: 906.
42. Forbes, M., and P. György 1957 Effect of yeast and of yeast extracts on liver necrosis and hemolysis by dialuric acid of red blood cells of rats on a necrogenic diet. J. Nutr., 63: 461.
43. Davison, A. N. 1961 The role of cerebroside sulphate (sulphatide) in the brain. Biochem. J., 78: 28P.
44. Scott, M. L., and C. C. Calvert 1962 Evidence of a specific effect of nutritional muscular dystrophy in vitamin E-deficient chicks. J. Nutr., 77: 105.

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