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I hereby recommend that the thesis prepared under my supervision by Yasuo Sasaki

entitled THE EFFECT OF VITAMIN B_1 DEFICIENCY ON THE LIPIDES OF THE CENTRAL NERVOUS SYSTEM

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

Approved by:

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THE EFFECT OF VITAMIN B_1 DEFICIENCY ON THE LIPIDES OF THE CENTRAL NERVOUS SYSTEM

A dissertation submitted to the

Graduate School
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DOCTOR OF PHILOSOPHY

1936

by

Yasuo Sasaki

B.A. University of Utah, 1933.
M.Sc. University of Cincinnati, 1934.

TABLE OF CONTENTS

THE EFFECT OF VITAMIN B_1 DEFICIENCY ON THE LIPIDES OF THE CENTRAL NERVOUS SYSTEM

by

Yasuo Sasaki

Department of Biochemistry, University of Cincinnati.

INTRODUCTION

Studies by Mayer and Schaeffer (1914) and Terroine (1914) have been influential in promoting the widely accepted concept of the importance of phospholipids and cholesterol in the essential life processes of the cell; these substances are known to persist even in prolonged starvation. The central nervous system, which does not lose weight during starvation, is characteristically rich in these substances.

The physiological importance of these lipides is unquestionable despite the fact that the exact nature of their functions still remains in the dark. Thudichum's statement in 1884 -- "Phosphatides are the centre, life, and chemical soul of all bioplasm whatsoever, that of plants as well as animals" -- still carries weight. Bloor (1930) has advanced this theory: that the lecithin cholesterol ratio in a tissue may be taken as an index of its

metabolic activity. He has found (1934) that in increased muscular activity muscle phospholipides increase and the more active muscles are quantitatively richer in these substances. Schmidt (1934) likewise, has found an increase in muscle phosphatides in thyroxine-injected animals.

Neither the phospholipide nor cholesterol content of the brain and spinal cord, however, is altered by thyroxine injected intravenously in rabbits. This has been shown by the author in a previous study (1934).

These brain lipides while not changed by thyroxine are nevertheless not unaffected by other factors. Collazo and his coworkers (1934) obtained a nearly three-fold increase in cholesterol content of rat brain in hypervitaminosis A and 6% increase in vitamin A deficiency. Replacement of the normal anisotropic substance in the myelin by isotropic material has been shown by Setterfield and Sutton (1935), by use of polarized light, in nerve degeneration in the albino rat associated with vitamin A deficiency. Cholesterol and its esters are anisotropic when in the crystalline form. This result, therefore, is not in obvious agreement with that of Collazo. Halliburton (1916) and Weil (1933) present evidences that Wallerian degeneration results in phospholipide decrease.

May's studies (1931) on autopsy material have shown that degenerative changes in the chemical constitution of the brain consist largely in lowering the amount of phospholipides. The formation of water-soluble phosphorus compounds during decomposition processes in the brain have been demonstrated by Longo (1932), Stich (1933) and Epstein and Lorenz (1934).

Increase in the cholesterol content of polyneuritic pigeon brain has been demonstrated by Hotta (1923), Messerle (1925) and Verzar and von Beznak (1925). Westenbrink (1934), however, was not able to confirm these findings. Milbradt (1930), in connection with his lipemia studies, has presented data showing that a lowered phospholipide and an increased cholesterol content occur in the brain of pigeons fed on polished rice.

Since an exclusive diet of polished rice causes a deficiency in vitamin B_1 these results indicate changes in the lipides of the brain as a result of this defect in diet. This is a very important observation since taken in connection with the experiments of Peters and Kinnersley (1929) on the defective respiration of the brains of pigeons when ingesting too little vitamin B_1, they constitute almost the first evidence to be obtained that the chemical constitution of the brain and also its chemical powers can be altered detrimentally by diet. They thus

furnish a basis for the experimental study of the causes of mental disease. For there is abundant evidence that mental activity depends upon the chemical activity of the brain.

Object of the present investigation. The importance of these observations of Milbradt is so great, that it is necessary that they be repeated by others in other laboratories and with other animals in order to establish the general connection between vitamin B_1 deficient diets and the change in constitution of the brain.

I have attempted, therefore, to repeat Milbradt's observations on rats, rather than on pigeons; and to extend the examination to the protein nitrogen.

Recent histological studies have shown that in animals on a vitamin B_1 deficient diet, there are extensive degenerations in the central nervous system, as well as in the peripheral nerves. Indeed the changes in the latter probably follow the central nervous changes.

The outward symptoms manifested are clearly those of nervous origin. Although various investigators differ in their pathological findings, the recent study by Prickett (1934), in which he criticizes earlier work by others, provides evidence for lesions in the central nervous system responsible for the symptoms of rat beriberi,

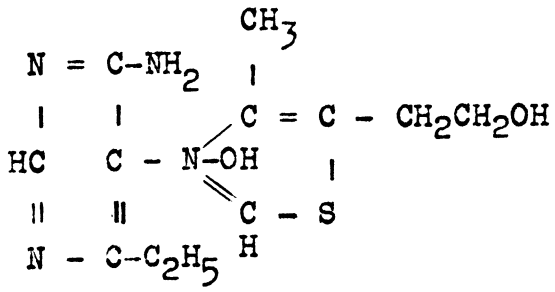
rather than in the peripheral system.

The result of my study has been at least a partial confirmation of that of Milbradt. The phospholipide content was reduced and the cholesterol increased in the rat brain and spinal cord during vitamin B_1 deficiency.

Historical: A widely prevalent disease, beriberi, has long been associated with an accessory food factor, found in the cortex of rice, which has now been isolated and identified as the antineuritic vitamin, B_1. A long list of investigators following Eijkman, who was able to reproduce this disease in fowls and called it polyneuritis gallinarum, have proved beyond doubt that the lack of this specific vitamin, now derived from various other sources -- yeast (torulin), legumes and grains -- is the etiological factor in beriberi. Most notable among the early investigators was Funk (1922), who correctly interpreted the findings of Eijkman and himself as due to a lack of some substance essential for normal nutrition, which he called

"vitamine".

The first crystalline substance, not entirely pure however, was obtained by Jansen and Donath in 1926. Later workers obtained a purer preparation; these were Chdake (1932), Van Veen (1931), Windaus and co-workers (1932), Kinnersley, Peters and O'Brien (1935) and Williams and co-workers (1935). Ohdake gave the formula $C_{12}H_{16}N_4SO_2$. Windaus and Tschesche have given it the empirical formula: $C_{12}H_{18}O_2N_4S$. Williams and his co-workers have assigned it the structural formula:



Vitamin B_1, $C_{12}H_{18}N_4SO_2$.

The daily vitamin B_1 requirements of mouse, rat, pigeon, and dog and of man have been accurately determined by Cowgill (1934). The formula applicable to man is: Gms. of vitamin B_1 required per day = 0.0000284 times total calories intake.

Beriberi is a disease characterized by degenerative changes in the nervous system, including a multiple peripheral neuritis, which may exist alone but is often, in

the "wet" form, combined with generalized edema and serous effusions, and by a tendency to the development of cardiac hypertrophy that frequently results in cardiac failure and sudden death. Complications that characterize the human "wet" form are usually not obtained in experimental animals, and the symptoms developed are mainly those of the "dry" type directly attributable to involvement of the nervous system.

These symptoms of nervous origin, including ataxia and paralysis, are apparent in both human and animal cases. Schaumann in 1910 was probably the first to describe beriberi or polyneuritis in rats. Among others were Langstein and Edelstein (1917), Funk and Macallum (1915), Drummond (1918) and Hofmeister (1922). The last-named described in detail the paralytic symptoms obtained in most of the rats placed on a vitamin B (B complex) diet. Sandels (1930) was probably the first to have definitely sought to supply the B_2 factor in the rat beriberi diet. Smith (1930) and Heyroth (1932), eliminating factor B_1 alone, have succeeded in reproducing the symptoms described by Hofmeister with remarkable regularity. In reporting their experiment with the curative rat test, Kinnersley, Peters and Reader (1930) state that "convulsions and paralysis of the hind legs appear to be the polyneuritic symptoms and are cured by

vitamin B_1 in its purest form.

Studies by Eijkman (1897), Voegtlin and Lake (1919), McCarrison (1921), Findlay (1921), Cully (1927), Vedder and Clark (1927), Woollard (1927), Stern and Findlay (1929), Zimmerman and Burack (1932), for the most part demonstrating myelin damage in the peripheral nerves of experimental animals, have been criticized by Prickett, who found lesions, as well as punctuate hemorrhages and intense congestion, especially in the vestibular nuclei and nucleus solitarius. Hofmeister (1922) likewise had noted previously that in the rat, degeneration of the peripheral nerves could but rarely be found and that multiple hemorrhages were present especially in the cerebellum and brain stem. More recently than either, Church (1935) confirmed the finding of perivascular hemorrhages chiefly in the region of the vestibular nuclei.

Of especial note is the fact generally observed, that regeneration of nerve fibers in the central nervous system and the restoration of their physiological function are not possible. Why this is so is one of the most perplexing questions in medical science. The proper attack in approaching this problem of degeneration and regeneration of nerve tissue must necessarily include an established knowledge of the biochemistry of nerve function and metabolism. How closely beriberi is allied to mental diseases

of organic nature, such as anemic softening, etc., remains still a matter of conjecture.

The exact roles of the vitamines in the biochemical processes of life and their physiological effects are not as yet well-defined, but it is most apparent, from abundant evidence, that they participate in the oxidation systems of the cell. At least, this has been shown most clearly for vitamin B_1 as well as the flavines (vitamin B_2) and ascorbic acid (vitamin C). Vitamin A is also readily oxidizable; carotene (a precursor) has been shown to act as a transporter of oxygen.

As early as 1918 Dutcher, and in 1921 Findlay, reported a fall in catalase and glyoxalase in polyneuritic pigeon tissues. Abderhalden (1921) and Hess (1921), the former manometrically and the latter colorimetrically, demonstrated a decrease in the intensity of tissue oxidation in vitamin B_1 deficiency, but this has been shown by them to occur to some extent in inanition. A critical review of experiments on tissue oxidation in relation to beriberi has been published by Westenbrink (1932).

That B_1 avitaminosis is independent of inanition has been discussed by Sinclair (1933) and by Rydin (1935). Sinclair showed that the respiratory quotient of brain tissue from polyneuritic pigeons is low and is raised nearly to the normal value by the addition in vitro of minute

amounts of crystalline vitamin B_1. The addition of the vitamin does not influence the R.Q. of tissue from normal pigeons fed on polished rice but not showing symptoms. In Rydin's experiments, although inanition produced a similar decrease in the oxidative capacity of pigeon muscles which paralleled results in the B_1 avitaminosis studies, specific decrease in oxidative capacity was found in the avitaminotic brain tissue.

It is hardly possible to produce B_1 avitaminosis in experimental animals without a complication of some degree of inanition, for loss of appetite is one of the acknowledged features of this disease. However, the role played by inanition seems overshadowed by the influences attributable to lack in vitamin B_1 alone.

Kinnersley and Peters (1929) have found in conjunction with lowered oxygen uptake of avitaminous pigeon brain an increased lactic acid content. Stare and Elvehjem (1933) confine this lowered oxygen uptake to the cerebellum in avitaminotic chicks and rats. Peters and Sinclair (1933) and Thompson (1934) have shown that the addition of vitamin B_1 in vitro to avitaminous brain tissue. This is the basis of the catatorulin test (Passmore, Peters and Sinclair, 1933), in which catatorulin is defined as the principle in vitamin B_1 crystals responsible for the

increase in oxygen uptake of avitaminotic brain tissue. The effect is at a maximum at pH 7.3, and is poisoned by cyanide and fluoride. Addition of pyrophosphate to the lactate and vitamin B_1 substrate further enhanced the rise in oxygen uptake. The authors concludes that vitamin B_1 takes part in some kind of a coupled oxidation system in the cell and influences more than one phase of energy metabolism of the cell.

Meiklejohn (1933) states that deficiency in vitamin B_1 affects the oxidative system concerned in lactate formation but not with the removal of lactate itself from the isolated brain tissue. Peters and Thompson (1935) in studying the metabolism of pyruvic acid in the brain, observed that the vitamin catalyzes the disappearance of pyruvate. They also found that pyruvic acid was produced by avitaminotic pigeon brain tissue respiring in a lactate solution. Thompson and Johnson (1934) moreover found an increase in bisulfite binding substances, suspected to be pyruvates, in the blood of polyneuritic pigeons. Vogt-Moller (1931) believes that polyneuritic symptoms are brought about by intoxication due to methyl glyoxal and that vitamin B_1 has to do with the conversion of methyl glyoxal to lactic acid.

The complexity of these varied findings, instead

of confusing investigators, prompts further interest in the search for a clearer knowledge not merely of the role of vitamins but of the systems through which oxidations take place in the cell. Vitamin B_1 probably participates somewhere and in some manner in the following chain of reactions, which occurs in the transformation of glucose to CO_2 and water:

Methyl glyoxal $\rightleftharpoons$ lactic acid $\rightleftharpoons$ pyruvic acid $\rightarrow$ acetaldehyde + CO_2

Boyland (1933) has attempted to identify vitamin B_1 with Banza and Szent-Gyorgi's co-enzyme for lactic acid dehydrogenase but without much success, although the co-enzyme itself is not definitely established in its purest state.

Of particular interest is the fact that fats have a sparing action on vitamin B_1, which is not dependent upon absorption factors (Evans and Lepkovsky, 1929, 1931, 1932). By this is meant that rats survive B_1 deficiency longer if given certain fatty acids in the food. Gregory and Drummond (1932) and Kemmerer and Steenbock (1933) have also made studies on this problem.

EXPERIMENTAL

Experimental animals. Female albino rats weighing between 80 and 100 grams were used in these experiments. The total number of animals was 48, in two sets marked series A and series B. Of these 34 were placed on the vitamin B_1 deficient diet, and 14 on the control diet. Eight in the former group were successfully brought back from a state of marked deficiency with the use of a vitamin B (complex) concentrate preparation. Several of the animals died of inanition or infection, or failed to respond to the treatment, and these were eliminated from the analyses.

Diet employed. The vitamin B_1-deficient diet chosen was the following:

Deactivated casein.....	18%
Argo corn starch.....	58%
Harris autoclaved brewer's yeast	10%
Olive oil.....	9%
Salt mixture (McCollum's 185)...	4%
Cod-liver oil (Patch's).....	1%

The casein was deactivated by washing for three days in distilled water and for seven days in 0.2% acetic acid and drying.

The Harris autoclaved yeast contained 13 Sherman units of vitamin B_2 per gram.

For the control diet, considered adequate in all respects, Fleischmann's pure dried brewer's yeast was used in place of the autoclaved yeast. Otherwise the constituents were the same.

All the animals had free access to water at all times.

Wire-mesh bottomed cages were used to prevent coprophagy, since this will prolong or prevent the onset of polyneuritis. Unfortunately the rats could eat an unknown amount of their feces. It is earnestly hoped that they ate very little.

For the purpose of curing a group of polyneuritic animals Parke-Davis vitamin B (complex) concentrate was administered by mouth. Two to three cc. of this preparation was fed with a small pipette to each rat for 3 to 5 days, the deficient diet being replaced simultaneously with the control diet. Many recovered from the paralytic symptoms within 24 hours, all others within 48 hours. These are regarded as the "cured" animals.

Criteria of polyneuritic manifestation. The signs and symptoms of rat beriberi obtained in the deficient groups agreed in the main part with those obtained by

Hofmeister (1922) and Heyroth (1932). Hofmeister has described four stages of the disease: (A) a prodromal phase of loss of appetite, diminishing weight, depression of spirit, and general inactivity; (B) a stage of ataxia, incoordination, and disturbance in gait; (C) a spastic phase of varied symptoms, marked usually by a tonic extension of the hind legs and often involving the fore legs; and finally (D) a phase of paralysis, weakness and coma, ending in death. In about half of the rats under my observation a moribund state directly followed stage (B); in these inanition was more marked than in those that reached the spastic stage. It may be well to remark here that animals in the weak, comatose state could not be brought back to normal with the vitamin B concentrate, whereas in stage (B) or (C) the vitamin preparation was invariably of avail.

The body weight response of the animals to the diets is presented in Graph I.

Insert Graph I.

The initial and final weights of individual animals are given in Table I.

Insert Table I.

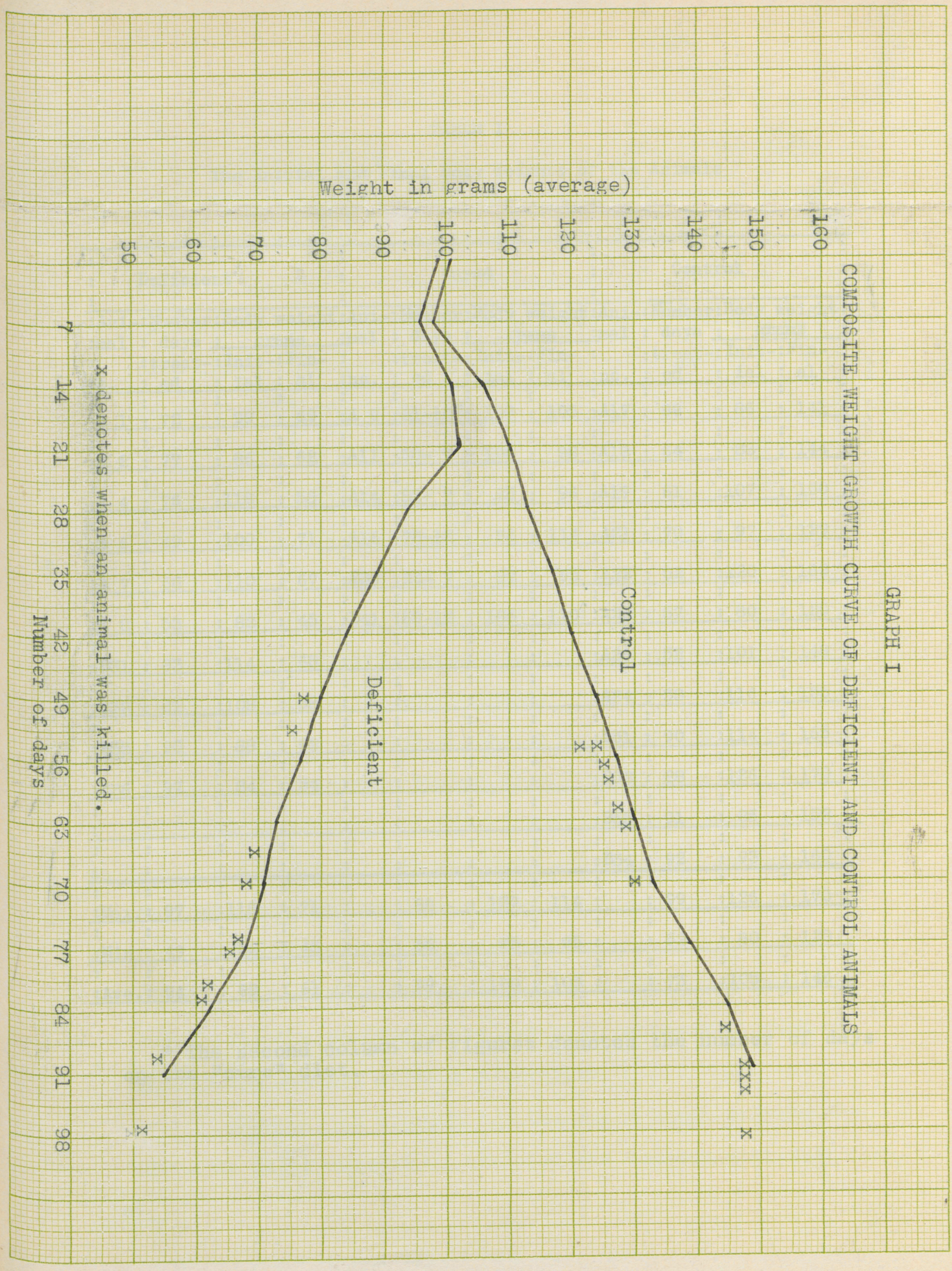

GRAPH I
COMPOSITE WEIGHT GROWTH CURVE OF DEFICIENT AND CONTROL ANIMALS
Weight in grams (average)
160
150
140
130
120
110
100
90
80
70
60
50
Control
Deficient
x denotes when an animal was killed.
7 14 21 28 35 42 49 56 63 70 77 84 91 98
Number of days

TABLE I

The Initial and Final Body weights of the Animals

Deficient				Cured				Control			
Animal no.	Number days treated	Body weight grams Initial	Final	Animal no.	Number days treated	Body weight grams Initial	Final	Animal no.	Number days treated	Body weight grams Initial	Final
A 1	72	99	69	A 9	81-10*	107	105	A17	99	86	141
A 2	77	86	62	A11	50- 7	106	98	A18	64	88	144
A 4	76	118	58	A14	82- 7	85	96	A19	61	107	130
A 6	49	100	71	A16	78-12	96	114	A21	70	97	129
A 7	66	110	67	B39	84- 7	99	95	A22	52	120	162
A 8	53	82	45	B41	88-12	105	110	A23	57	80	120
B31	83	114	65					A26	52	101	148
B32	81	108	58					A24	58	116	135
B33	90	88	62					B51	90	114	165
B36	97	84	54					B52	88	126	170
								B53	91	85	152
								B54	92	93	160
Max.	97	118	71		88	107	114		99	126	170
Min	49	82	45		50	85	95		52	80	120
Ave.	74	99	61		77	100	103		73	105	146

* The second column of figures denotes the number of days on the "cure" diet.

Killing of animals. The animals on the deficient diet were closely followed as to weight decrease and onset of nervous symptoms characteristic of rat beriberi. The majority responded with definite signs of nervous affection, manifested primarily by incoordination and flaccidity of the hind legs. In about half of the animals, as stated above, in which complications of possible inanition seemed least marked, spastic paralysis was developed. In nearly all cases a weak, moribund stage was soon reached. Wherever possible the animal was killed at this stage (about 60 to 90 days).

The control rats were killed at such times when the duration of the diet treatment corresponded approximately to that of the deficient animals.

Eight rats were successfully cured out of 12 attempted. These were killed from 7 to 12 days following the first administration of the vitamin B concentrate.

A sharp blow on the head and bleeding by cutting the vena cava proved an efficient method of killing. In order to avoid possible brain hemorrhages in obtaining tissue, the animals for histological examination were anesthetized with chloroform and bled to death. Each animal was weighed at time of killing.

Removal of tissues. The whole brain and the whole or major portion of the cord was removed with the

aid of necessary instruments. The whole brain was cut off from the cord at the level of the foramen magnum and was immediately weighed to the nearest milligram.

Determination of solids. Immediately following the removal of the brain and cord and the weighing of the whole brain, a portion (approximately a half; the other half to be used for extraction) of the cerebrum was accurately weighed in a 30 cc. beaker. In alternate animals (as designated in Table III) the whole of the cerebellum and the whole or major part of the spinal cord were, besides the portion of cerebrum, likewise used for solid content determination. To the weighed samples 10 cc. (enough to cover tissue) of absolute alcohol-ether mixture (3:1) was added as dehydrating agent to facilitate drying. After most of the liquid had evaporated off on a steam bath the beakers were placed in a drying oven and dried in air at 106° C., from 24 to 36 hours -- i.e., until consecutive weighings were constant within 0.5 mg. The water loss was thus determined and the percentage of solids.

Extraction of tissues. The remainder of the cerebrum, and the cerebellum and spinal cord not used for solid content determination were separately weighed to within 0.5 mg. The cerebrum samples (1/2 of each cerebrum) weighed in the neighborhood of .5 g.; while cerebellum, .2 g.; cord, .3 g. The samples of fresh tissue

from animals in series A were ground in fine, fat-free sand and extracted with five successive 20 cc. portions of boiling 3:1 alcohol-ether mixture (as recommended by Bloor and used by the author in previous studies). C.P. absolute alcohol and anhydrous ether were used. The filtered (six cm. Whatman extracted filter paper was used) extractions of each sample were united and made up to mark in a 100 cc. volumetric flask. This was then set aside in a dark, cool place for subsequent analyses.

Each sample of fresh tissue from series B, cut with small, sharp scissors to allow better penetration of solvents, was placed in an Erlenmeyer flask and covered with the 3:1 alcohol-ether mixture and allowed to stand for 24 - 48 hours. The solvent was then filtered into a 100 cc. volumetric flask and made up to volume with the addition of four successive boiling extractions of the same sample.

(In series A, subsequent extractions on 2 samples, making up a total of 50 cc., on analysis gave very faint indication of a lipide phosphorus, too small in amount to be measured in the colorimeter with the regular standard chosen. In a previous work extraction by this method left a residual amount in subsequent two extractions, of .04 - .05% of the lipide phosphorus value in the original

extract. In series B the extraction was also practically complete. In two combined extractions of 3 samples (cerebrum, cerebellum and cord), each making up 100 cc. only a faint trace of phosphorus was found. The Liebermann-Burchard color for cholesterol was not obtained using 10 cc. portions of these extracts.)

The tissues in series B, exhaustively treated with the alcohol-ether mixture, were subsequently used for total protein nitrogen determination.

Phospholipide determination. The phospholipides were determined oxidatively by the method devised by Bloor (1929), the principle of which is the oxidation of the phospholipids, separated from fats and sterols by actone precipitation, by potassium dichromate in the presence of sulfuric acid and a catalyst, silver dichromate (Nicloux's reagent). Ten cc. portions, in duplicates, of the lipide extract were evaporated to dryness, taken up in petroleum ether, precipitated with acetone, redissolved in ether and again evaporated to dryness. An excess (exactly 3 cc. of one normal) of potassium dichromate solution plus 5 cc. Nicloux's reagent was added to samples contained in glass-stoppered flasks, and after heating to 106° C. from 30 to 45 minutes in the oven the excess of dichromate was titrated with 0.1 N. sodium thiosulfate, using starch-iodine as indicator. Boyd (1931) represents the reaction

by the following equation:

$2C_{42}H_{84}O_9NP + 118O_2 + H_2SO_4 = 84CO_2 + 78H_2O + 2H_3PO_4 + (NH_4)_2SO_4$,

from which it may be calculated that 1 mg. oleo-palmityl lecithin would require 3.03 cc. of 0.1 N. dichromate solution for oxidation.

Phospholipide phosphorus determination. For the phosphorus determination the ether extract of phospholipides from the 10 cc. aliquots of each lipide extract, also in duplicates, was evaporated to dryness in a Pyrex test tube, and the residue ashed in sulfuric and nitric acids. Fiske-Subbarow's (1925) method was used in determining the phosphorus. Amino-naphthol sulfonic acid was the reducing agent in the color formation.

Free and combined cholesterol determination. The free cholesterol determinations were made by the method of Schoenheimer and Sperry (1934) as adapted for the colorimeter by Fritz (1935). In the cerebrum samples of series A the total cholesterol content was also determined. The difference of the total cholesterol value and the free value gave the combined cholesterol content for the sample. Ten cc. aliquot portions of the extracts were used in duplicates.

Protein nitrogen determination. The direct Nesslerization method was adopted for the determination of protein nitrogen. The tissue after the extraction of the lipids was utilized. Where the tissue was used for solid

content determination it was subsequently extracted with the alcohol and ether. The fat-free sample was then digested with 10 cc. of a mixture of 1 part concentrated solution of K_2SO_4: 1 part concentrated sulfuric acid plus the catalyst, selenium oxychloride. 30% H_2O_2 was also used to hasten complete digestion. This was then made up to exactly 100 cc. and 10 cc. portions thereof were used for Nesslerization and the nitrogen determined colorimetrically.

The tissues from the animals in series B were used for the protein nitrogen determination.

The method of determination adopted, however, gives only a relatively rough measure of the protein nitrogen content. The nitrogen of organic extractives not removed by the alcohol and ether is included in the determinations and provides a source of error.

Histological. The slide preparations were made using Herzheimer's Sharlach R, fat staining, technique. The tissue was fixed in formalin, sectioned with a freezing microtome and stained with Scharlach R. Tissues from 3 deficient, 1 "cured" and 3 control animals were thus prepared and examined for possible lipoid degeneration.

The Scharlach R stain is a fat stain and is not suitable for demonstrating possible cellular damage. In the preparations studied no accumulation of fat globules was found.

RESULTS

At the time of killing all the animals in the deficient group were in a generally weakened condition and exhibited signs of dizziness and paralysis. The paralysis was apparent in hind-legs and in most cases was of a flaccid type. In animals A2, A8, B32 and B36 the spastic type was observed, and when these animals were placed on their backs they would roll convulsively in an attempt to right themselves.

The effect of vitamin B_1 deficiency on the brain weight. Table II compares the weights of the brain of the deficient, "cured" and control animals, weighed immediately after removal from the body.

Insert Table II.

There is an apparently significant difference between the deficient and control animals. The percentage difference of the two averages is 8.2%. The average value of the "cured" animals is found to be intermediate but much nearer that of the deficient animals.

The solid content of cerebrum, cerebellum cord. Table III gives the solid content values of the samples

Insert Table III.

analyzed. The small magnitude of the individual

TABLE II

The Brain Weights of the Animals

Deficient			Cured			Control		
Animal no.	Number days treated	Weight of brain grams	Animal no.	Number days treated	Weight of brain grams	Animal no.	Number days treated	Weight of brain grams
A 1	72	1.270	A 9	81-10*	1.289	A17	99	1.260
A 2	77	1.135	A11	50- 7	1.174	A18	64	1.270
A 4	76	1.212	A14	82- 7	1.153	A19	61	1.302
A 6	49	1.190	A16	78-12	1.298	A21	70	1.227
A 7	66	1.318	B39	84- 7	1.279	A22	52	1.286
A 8	53	1.099	B41	88-12	1.315	A23	57	1.325
B31	83	1.242				A24	58	1.406
B32	81	1.220				A26	52	1.270
B33	90	1.285				B51	90	1.414
B36	97	1.226				B52	88	1.423
						B53	91	1.365
						B54	92	1.327
Max.	97	1.318		88	1.315		99	1.423
Min.	49	1.099		50	1.133		52	1.227
Ave.	74	1.220		77	1.248		73	1.318

* The second column of figures denote the number of days on the "cure" diet.

TABLE III

Per cent. of Solids of Cerebrum, Cerebellum and Cord

Deficient				Cured				Control			
Animal no.	Cerebrum Per cent.	Cerebellum Per cent.	Cord Per cent.	Animal no.	Cerebrum Per cent.	Cerebellum Per cent.	Cord Per cent.	Animal no.	Cerebrum Per cent.	Cerebellum Per cent.	Cord Per cent.
A 1	23.20	--	--	A 9	22.82	23.42	32.33	A17	22.71	22.78	29.40
A 2	22.72	--	--	A11	22.05	22.12	28.29	A18	21.93	--	--
A 4	24.02	22.40	31.21	A14	23.40	--	--	A19	22.97	23.13	32.10
A 6	23.60	22.69	30.52	A16	22.89	23.11	29.11	A21	22.76	--	--
? A 7	22.70	--	--	B39	22.48	--	--	A22	21.92	--	--
A 8	22.61	22.68	30.56	B41	22.15	--	--	A23	23.01	22.87	29.34
B31	22.53	--	--					A24	22.40	--	--
B32	22.78	22.85	30.47					A26	22.10	23.22	30/03
B33	22.40	22.59	30.19					B51	21.89	22.64	30.10
B36	23.14	--	--					B52	22.95	--	--
								B53	23.00	--	--
								B54	22.24	22.80	30.35
Max.	24.02	22.85	31.21		23.40	23.42	32.33		23.01	23.13	32.10
Min.	22.40	22.93	30.19		22.05	22.12	28.29		21.92	22.64	29.34
Ave.	22.97	22.64	30.59		22.63	22.88	29.91		22.49	22.95	30.39

Where blanks occur in the cerebellum and cord columns, the tissue sample was used for extraction.

variations and the closeness of the average values indicate little if any change.

Phospholipide content of cerebrum, cerebellum and cord. Tables IV, V and VI show the phospholipide values as found in the cerebrum, cerebellum and cord respectively.

Insert Tables IV, V and VI.

The decrease in phospholipide content (dry tissue) of the deficient group is 22.4% in the cerebrum, 18.1% in the cerebellum and 15.1% in the cord. The "cured" group values (dry tissue) are 9.7%, 9.8% and 6.1% less than those of the control group.

The dry weight phospholipide content values for the cerebrum samples were computed from their individual solid content values. However, in the case of the cerebellum and the cord, as the solid content value was not obtained for every sample, the average solid content value for each group was used in computing the values in terms of dry tissue. This applied to all subsequent tables.

Phospholipide phosphorus content of cerebrum, cerebellum and cord. Tables VII, VIII and IX present the phospholipide phosphorus contents in the three parts of the nervous system.

Insert Tables VII, VIII and IX.

TABLE IV

Phospholipide Content of Cerebrum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	4.53	19.51	A 9	4.95	21.70	A17	5.60	25.06
A 2	3.94	17.33	A11	5.01	22.72	A18	5.45	24.85
A 4	4.45	18.60	A14	5.28	22.56	A19	5.93	25.82
A 6	4.66	19.75	A16	4.54	19.84	A21	5.75	25.27
A 7	3.98	17.26	B39	4.68	20.80	A22	4.74	21.60
A 8	4.89	21.60	B41	4.82	21.76	A23	5.51	23.98
B31	4.20	18.66				A24	5.56	24.80
B32	3.91	17.15				A26	5.43	24.57
B33	4.14	18.04				B51	4.97	22.70
B36	3.99	17.27				B52	4.88	21.26
						B53	5.36	23.30
						B54	5.20	23.38
Max.	4.89	21.60		5.28	22.72		5.93	25.82
Min.	3.91	17.15		4.54	19.84		4.74	21.26
Ave.	4.27	18.52		4.88	21.56		5.37	23.88

TABLE V

Phospholipide Content of Cerebellum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	5.10	22.54	A14	5.39	23.54	A18	5.41	23.57
A 2	4.10	18.11	B39	4.74	20.70	A21	6.12	26.67
A 7	4.58	20.23	B41	4.86	21.22	A22	5.63	24.09
B31	4.47	19.74				A24	5.72	24.49
B36	4.17	18.42				B52	5.13	22.35
						B53	5.50	23.96
Max.	5.10	22.54		5.39	23.54		6.12	26.67
Min.	4.10	18.11		4.74	20.70		5.13	22.35
Ave.	4.48	19.81		5.00	21.82		5.59	24.19

TABLE VI

Phospholipide Content of Cord

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	13.10	42.81	A14	11.90	39.80	A18	14.30	47.04
A 2	9.65	31.54	B39	12.02	40.20	A21	13.26	43.62
A 7	10.93	35.52	B41	10.90	36.45	A22	13.36	43.95
B31	10.40	33.99				A24	11.40	37.50
B36	9.66	31.57				B52	11.48	37.76
						B53	12.57	38.06
Max.	13.10	42.81		12.02	40.20		14.30	47.04
Min.	9.65	31.54		10.90	36.45		11.40	37.50
Ave.	10.79	35.09		11.94	38.82		12.73	41.32

TABLE VII

Phospholipide Phosphorus Content of Cerebrum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	.213	0.92	A 9	.224	0.98	A17	.247	1.09
A 2	.180	0.72	A11	.231	1.05	A18	.238	1.09
A 4	.206	0.86	A14	.248	1.06	A19	.260	1.13
A 6	.218	0.92	A16	.223	0.97	A21	.258	1.13
A 7	.193	0.85	B39	.214	0.95	A22	.232	1.06
A 8	.241	1.07	B41	.212	0.96	A23	.260	1.13
B31	.190	0.84				A24	.255	1.14
B32	.184	0.81				A26	.250	1.13
B33	.195	0.87				B51	.221	1.02
B36	.183	0.79				B52	.230	1.00
						B53	.258	1.12
						B54	.254	1.14
Max.	.241	1.07		.212	1.06		.260	1.14
Min.	.180	0.72		.248	0.95		.221	1.00
Ave.	.200	0.87		.229	1.00		.247	1.10

TABLE VIII

Phospholipide Phosphorus Content of Cerebellum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	.230	1.01	A14	.254	1.11	A18	.245	1.07
A 2	.188	0.83	B39	.217	0.95	A21	.260	1.13
A 7	.209	0.92	B41	.219	0.96	A22	.246	1.07
B31	.201	0.89				A24	.245	1.07
B36	.186	0.82				B52	.240	1.05
						B53	.256	1.11
Max.	.230	1.01		.254	1.11		.260	1.13
Min.	.186	0.82		.217	0.95		.240	1.05
Ave.	.203	0.89		.230	1.01		.247	1.08

TABLE IX

Phospholipide Phosphorus Content of Cord

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	.435	1.42	A14	.438	1.43	A18	.490	1.61
A 2	.392	1.28	B39	.395	1.32	A21	.456	1.50
A 7	.396	1.29	B41	.404	1.35	A22	.483	1.59
B31	.400	1.31				A24	.432	1.42
B36	.386	1.26				B52	.427	1.40
						B53	.430	1.41
Max.	.435	1.42		.438	1.43		.490	1.61
Min.	.386	1.26		.395	1.32		.427	1.40
Ave.	.402	1.31		.412	1.40		.453	1.49

The amounts of decrease correspond favorably with those obtained by determining the phospholipide oxidatively, being 20.9%, 17.6% and 12.1%, respectively for the deficient cerebrum, cerebellum and cord, expressed in terms of the dry weight of the tissue. The "cured" animals were less 9.1, 8.3 and 6.0%.

In Table X are submitted the values obtained for the ratio of phospholipides/phospholipide phosphorus.

Insert Table X.

On one hand it affords a comparison between the two methods of analysis, while on the other, granting that the errors in either method are not sufficiently great, it affords a rough index as to the nature of the phospholipides -- more specifically, the molecular weights of the fatty acids. We see that the fatty acids are lightest in the cerebrum, next in the cerebellum and heaviest in the cord. There is a greater degree of unsaturation of the fatty acids of the cerebrum than of the cord, as determined by the iodine number obtained by myself on the phospholipides of the rabbit brain (1934).

Free cholesterol content. The free cholesterol data are presented in Tables XI, XII and XIII.

Insert Tables XI, XII and XIII.

TABLE X

Phospholipides/Phospholipide Phosphorus Ratio in Cerebrum, Cerebellum and Cord

Deficient				Cured				Control			
Animal no.	Cerebrum	Cerebellum	Cord	Animal no.	Cerebrum	Cerebellum	Cord	Animal no.	Cerebrum	Cerebellum	Cord
A 1	21.4	22.2	30.1	A 9	22.1	--	--	A17	22.6	--	--
A 2	21.8	21.8	24.6	A11	21.7	--	--	A18	22.9	22.0	29.4
A 4	21.8	--	--	A14	21.3	21.2	27.8	A19	22.8	--	--
A 6	21.4	--	--	A16	20.4	--	--	A21	21.6	23.6	29.8
A 7	21.0	21.9	27.5	B39	21.9	21.8	30.4	A22	20.4	22.5	27.6
A 8	20.4	--	--	B41	22.7	22.2	27.0	A23	21.2	--	--
B31	22.4	22.2	26.0					A24	21.4	22.9	27.5
B32	21.2	--	--					A26	21.8	--	--
B33	21.1	--	--					B51	21.3	--	--
B36	21.8	22.5	25.1					B52	20.8	21.6	28.6
								B53	20.8	21.6	28.6
								B54	20.3	--	--
Max.	22.4	22.2	30.1		22.7	22.2	30.4		22.9	23.6	29.8
Min.	20.4	21.8	25.1		20.4	21.2	27.0		20.3	21.5	26.8
Ave.	21.4	22.1	26.5		21.7	21.7	28.4		21.5	22.4	28.3

TABLE XI

Free Cholesterol Content of Cerebrum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent of Wet tissue	Per cent of Dry tissue
A 1	2.34	10.50	A 9	2.02	8.86	A17	1.85	8.15
A 2	2.50	10.92	A11	2.05	9.30	A18	1.90	8.68
A 4	2.47	10.23	A14	1.81	7.74	A19	1.83	7.98
A 6	1.92	8.14	A16	2.13	8.85	A21	1.86	8.17
A 7	2.30	9.75	B39	1.92	8.54	A22	2.07	9.45
A 8	2.06	9.12	B41	2.01	9.08	A23	1.96	8.52
B31	1.98	8.80				A24	2.05	9.15
B32	2.36	10.35				A26	2.00	9.05
B33	1.99	8.89				B51	1.90	8.63
B36	2.29	9.90				B52	1.87	8.15
						B53	1.83	7.95
						B54	1.82	8.18
Max.	2.50	10.92		2.13	9.30		2.07	9.45
Min.	1.92	8.14		1.81	7.74		1.82	7.95
Ave.	2.22	9.66		1.99	8.75		1.91	8.57

TABLE XII

Free Cholesterol Content of Cerebellum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	2.19	9.67	A14	2.04	8.91	A18	2.07	9.02
A 2	2.42	10.70	B39	1.93	8.43	A 21	1.85	8.06
A 7	2.37	10.47	B41	2.18	9.52	A22	1.98	8.67
B31	2.29	10.16				A24	1.92	8.37
B36	2.39	10.56				B52	2.09	9.11
						B53	1.97	8.59
Max.	2.42	10.70		2.18	9.52		2.09	9.11
Min.	2.19	9.67		1.93	8.43		1.85	8.06
Ave.	2.33	10.31		2.05	8.95		1.98	8.64

TABLE XIII

Free Cholesterol Content of Cord

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
A 1	5.11	16.70	A14	4.63	15.15	A18	4.57	15.03
A 2	5.58	18.24	B39	4.77	15.95	A21	4.59	15.10
A 7	5.28	17.29	B41	5.02	16.79	A22	4.80	15.79
B31	4.69	15.33				A24	4.91	16.15
B36	4.91	16.05				B52	4.63	15.23
						B53	4.38	14.41
Max.	5.58	18.24		5.12	16.79		4.91	16.15
Min.	4.69	15.33		4.63	15.15		4.38	14.41
Ave.	5.11	16.70		4.77	15.96		4.65	15.30

In contrast to the phospholipide content the cholesterol is increased in the deficient organs. The increase is, for the cerebrum 13.5%, cerebellum 20.0%, cord 9.35%. The values for the "cured" animals were but slightly higher than those of the controls: 2.8, 3.6 and 4.3%, all near the margin of experimental error.

Combined cholesterol content. Determinations were made on the cerebrum samples. The values computed from the difference of the total and free cholesterol data (see Table XIV) were so small as to make them unreliable for proving any significance.

Insert Table XIV.

In three cases negative values were obtained. It is clearly evident that the cholesterol in the nervous system occurs preponderantly in the free, unesterified state. This is also the observation of other investigators, such as Chanutin and Ludewig (1933) and Onizawa (1929). Because these values are so nearly within the margin of experimental error, determinations upon the other samples were not made. The amount of combined cholesterol is therefore considered negligible, and the free cholesterol values can be used to show the influence of B_1 deficiency on the total cholesterol content.

TABLE XIV

Combined Cholesterol Content of Cerebrum

Deficient		Cured		Control	
Animal no.	Per cent. of Wet tissue / Dry tissue	Animal no.	Per cent. of Wet tissue / Dry tissue	Animal no.	Per cent. of Wet tissue / Dry tissue
A 1	-0.16	A 9	0.08	A19	0.17
A 2	0.02	A11	-0.02	A21	0.13
A 4	-0.05	A14	0.19	A22	0.07
A 6	0.10	A16	0.06	A23	0.03
A 7	0.19			A24	0.03
A 8	0.23			A26	0.12
Max.	0.23		0.19		0.17
Min.	-0.16		-0.02		0.03
Ave.	0.05		0.08		0.09

Phospholipide/cholesterol ratio. The following table gives the phospholipide/cholesterol ratios for the three groups of animals computed from the averages (dry tissue).

TABLE XV.

Phospholipide/Cholesterol Ratio

	Deficient	Cured	Control
Cerebrum	1.92	2.46	2.78
Cerebellum	1.92	2.44	2.80
Cord	2.10	2.43	2.70

A remarkable uniformity in the ratios for each group with a significant decrease in the deficient animals is here observed.

Protein nitrogen content. Tables XVI, XVII and XVIII present the data on the protein nitrogen content.

Insert Tables XVI, XVII and XVIII.

There is indicated an influence of some significance upon the percentage compositions. The averages of the cerebrum, cerebellum and cord of the deficient rats were respectively 7.6, 5.6 and 8.4% higher than those of the control. The averages of the "cured" animals were intermediate between the corresponding averages of the other

TABLE XVI

Protein Nitrogen Content of Cerebrum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
B31	1.71	7.60	B39	1.72	7.76	B51	1.52	6.94
B32	1.66	7.28	B41	1.50	6.67	B52	1.41	6.15
B33	1.68	7.50				B53	1.64	7.13
B36	1.53	6.62						
Max.	1.71	7.60		1.72	7.76		1.64	7.22
Min.	1.53	6.62		1.50	6.67		1.41	6.15
Ave.	1.65	7.25		1.66	7.22		1.52	6.74

TABLE XVII

Protein Nitrogen Content of Cerebellum

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue	Animal no.	Per cent. of Wet tissue	Per cent. of Dry tissue
B31	1.65	7.29	B39	1.54	6.73	B51	1.46	6.38
B32	1.69	7.47	B41	1.49	6.51	B52	1.44	6.27
B33	1.38	6.19				B53	1.55	6.76
B36	1.44	6.36						
Max.	1.69	7.47		1.54	6.73		1.55	6.76
Min.	1.38	6.19		1.49	6.51		1.41	6.16
Ave.	1.54	6.83		1.52	6.63		1.49	6.47

TABLE XVIII

Protein Nitrogen Content of Cord

Deficient			Cured			Control		
Animal no.	Per cent. of Wet tissue	Dry tissue	Animal no.	Per cent. of Wet tissue	Dry tissue	Animal no.	Per cent. of Wet tissue	Dry tissue
B31	1.34	4.22	B39	1.38	4.63	B51	1.28	4.21
B32	1.50	4.90	B41	1.23	4.13	B52	1.47	4.83
B33	1.43	4.67				B53	1.29	4.24
B36	1.47	4.80						
Max.	1.50	4.90		1.38	4.63		1.47	4.83
Min.	1.34	4.22		1.23	4.13		1.28	4.21
Ave.	1.44	4.65		1.36	4.38		1.35	4.33

two groups.

Amounts of phospholipides, cholesterol and protein nitrogen in whole brain. To show whether there is any actual change in the total weight of phospholipides, cholesterol or protein nitrogen per whole brain, Table XVIII is submitted. These values were derived from the averages (dry tissue) of the groups. The percentage contents of the constituents used in computing these figures were those of the cerebrum, from which the percentage content values in the much smaller cerebellum differ but little.

TABLE XIX

	Deficient	Control	Difference
Average dry weight of brain	.2802g.	.2964g.	-.0162g., 8.25%
Phospholipides in whole brain	.0519g.	.0718g.	-.0199g.,27.7 %
Cholesterol	.0271g.	.0254g.	.0017g., 6.7 %
Protein nitrogen	.0203g.	.0200g.	.0003g., 1.5 %

Here it is seen that the decrease in the brain weight of the deficient animal can be almost wholly accounted for by the depletion in phospholipides and increase in cholesterol.

DISCUSSION

The alteration in the percentage composition of the two main lipide constituents, phospholipides and cholesterol, noted in the brain and spinal cord of polyneuritic albino rats indicates a manifestation of faulty metabolism resulting from the deprivation of vitamin B_1. This alteration, represented by decrease in phospholipide content and increase in cholesterol, confirms the results obtained by Milbradt for polyneuritic pigeon brains. That this is not due to mere inanition may be adduced from the fact that depletion was found in the phospholipide content and not in the cholesterol -- i.e., if we are to take into account a recent study by Gillum and Okey (1936) which show a depletion in both cholesterol and lecithin contents in the brain tissue of fasting rats, amounting to 9.4% in the former and 15.7% in the latter. A litter of eight rats was used by them for each group, undernourished and normal. However, the studies of Mayer and Schaeffer and Terroine demonstrate that these constituents are maintained at a constant proportion even in prolonged starvation.

That these chemical findings do not afford a measure of possible myelin damage, such as is typical in Wallerian degeneration and in multiple sclerosis, is

supported by the work of W. Koch and M. Koch (1917) on rat brain and of Backlin (1933) on rabbit brain. Both, studying the changes in the lipides during development, have found that at birth the cerebrosides are lacking in the brain and that their appearance and increase parallel the growth of the myelin sheath. The Kochs have found that the same is true of the sulfatides. These two lipides are probably more indicative of the myelin sheath. The phospholipides and cholesterol are probably important constituents of the nerve cell and axis cylinder as well as of the myelin.

The partial, incomplete restoration of phospholipides in the "cured" animals may mean that the normal store is considerably greater than is adequate for normal physiological function or that despite the irretrievable loss of that portion of phospholipides contained in the impaired nervous structure, if this does occur, the animals can still become apparently normal. One must remember, however, that the period for "cure" may not have been of sufficient length and that possibly in time complete restoration of phospholipides to that proportion found in control animals does result.

The low respiratory quotient of avitaminous brain tissue has led some to suspect that the fats are the fuel used. It may be that as the result of difficulties in

the carbohydrate metabolism arising from the lack of this essential vitamin undue stress is placed upon the fat metabolism mechanism. This is a mere conjecture for which a more factual basis is required.

In conclusion, it may be said from the numerous aspects of the problem of vitamin deficiency, such as its effect on growth, the occurrence of intermediary metabolic substances, the probable catalytic role of vitamins, etc., that the neuropathology of B_1 avitaminosis is probably an expression of a defect in a general metabolic process rather than a specific damaging effect on the neurons, that because of the peculiarities of the nervous system the effects are manifested to a greater degree there than in other tissues. The probable role of vitamin B_1 in the oxidation-reduction mechanism has been reviewed, and this and other considerations, stated above, lead one to this view-point.

SUMMARY

1. A decrease in the percentage content of phospholipides is noted in the cerebrum, cerebellum and spinal cord of the vitamin B_1 deficient albino rats, 22.4%, 18.1%, 15.1%, respectively.

2. An increase in the percentage content of free cholesterol is noted in the above tissues, 13.5%, 20.0%, 9.35%.

3. An increase in the percentage content of protein nitrogen was found for a smaller group of animals, 7.6%, 5.6%, 8.4%.

4. The amounts of the above constituents expressed in terms of their total weight per brain show that only the phospholipide and cholesterol contents have been influenced.

5. The percentage content values of the above constituents in "cured" animals are presented; these show a range intermediate between the deficient and control animals.

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