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I hereby recommend that the thesis prepared under my supervision by Nathaniel Brower.

entitled The influence of Vitamin B, on Pancreatic lipase and its relation to multiple sclerosis

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

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THE INFLUENCE OF VITAMIN B_1 ON PANCREATIC LIPASE AND ITS RELATION TO MULTIPLE SCLEROSIS

A dissertation submitted to the

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by

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I. INTRODUCTION

Multiple sclerosis is a degenerative disease of the central nervous system which has provoked a considerable amount of investigation during the last fifty years. The literature dates back to at least 1890 but the bulk of the more important published material is of the last twenty or twenty five years. Just as in the case of other diseases, a great advance can be made after scientific progress has supplied new techniques and new facts to provide better methods of study and throw more light on the subject, so will multiple sclerosis be understood more fully and perhaps a specific treatment will be found.

It is not within the scope of this paper to deal extensively with all the clinical and pathological aspects of multiple sclerosis. However, it is well to note the importance of multiple sclerosis as a disease and mention its chief features which will be pertinent to this thesis.

In England this disease is usually termed disseminted sclerosis. Its name is thus derived from the peculiar scattering of the lesions throughout the entire nervous system. Numerous areas of demyelination occur along the entire neuro-axis, and although parts of the lower brain are involved, the white and gray matter of the cerebrum are usually spared and the peripherial nerves are never involved (104).

Apparently this disease is not very common in the United States but Grinker (103) states that multiple sclerosis ranks third in frequency among neurological disorders. The same author states that it is seen with much more frequency in the British Isles and on the European Continent than in this country. Patients with this disease are seen between the ages of twenty and forty (103) although undoubted cases have been recognized as early as ten years and as late as fifty. Both sexes are affected with the same frequency according to most authors. In a statistical study of 689 patients, Gram (39) noted that 312 were males and 377 were females, while Grinker finds that more males than females were affected. The exact age at which the first symptoms appear is difficult to determine. The neurologist sees the patient when signs already denote an affection of a considerable length of time. The peculiar fleeting character of the earliest symptoms and the almost complete remissions of the disease are such that the patient the does not realize nor remember the actual onset. Grinker states that careful questioning of the patients brings out symptoms many years prior to the time when a physician was consulted.

Multiple sclerosis is a chronic progressive disease. There may appear very faint and fleeting symptoms which

completely disappear and appear again in a more pronounced form, indicating the progression. Later when enough of the nervous system is involved, the symptoms are continuous and the patient is bedridden. "The clinical picture as wellas the course of the disease are determined by two main pathological features: first, there are numerous and widespread patches or foci of sclerosis scattered throughout the central nervous system; second, there is a degeneration of the myelination sheaths and preservation of the axis-cylinder in the midst of the most extensive patches of sclerosis. The axis-cylinder is destroyed later so that the secondary degeneration occurs only after a long time". (104). Wechsler states that the destruction of the myelin sheaths explains the partial impairment of function while the preservation of the axis-cylinder accounts for the possibility of remissions. It is only when the myelin and the axis-cylinder are destroyed that the signs and symptoms become permanent. "These patches of degeneration are found everywhere in the nervous but with a special predilection for the white matter of the spinal cord, the pyramidal tract, structures of the medulla, pons, midbrain and the optic tract". (104). Siemerling and Raecke state that the olfactory nerve may also be affected (82). In regard to the nature lesions of

multiple sclerosis, Alexander (2) has made an interesting observation by an ingenious method. By a process of "micro-incineration" Alexander has found that the demyelinated areas are demineralized. A quotation from his publication reads as follows: "The plaques of multiple sclerosis are demineralized lesions.....It is of great interest to find that the plaques of multiple sclerosis in their mineral architecture most closely resemble foci of softening or of ischemic necrosis, all of these being essentially lesions of demyelination" (2).

After reviewing the literature on this subject it is sfae to state that no absolutely characteristic changes occur in the spinal fluid of multiple sclerosis. The cell count and the protein content may be normal; there is a negative Wassermann but there may be a luetic or paretic reaction to colloidal gold. (1). Even in the active and inactive stages of the disease there are no characteristic changes which are specific for multiple sclerosis.(31). However, Kerch (51) found in ten cases of multiple sclerosis seven positive globulin reactions in the spinal fluid and three instances of pleocytosis while the Wassermann reactions were negative. Luhan and Balser (60) claim to have demonstrated a myelolytic substance in the spinal fluid of patients suffering from

this disease. These authors also state that this myelolytic substance is present inthe urine. Sussner, on the other hand, could not find any lipase in the spinal fluid of either normal and abnormal subjects.(87). Swan and Myers (88) also state that no lipase could be demonstrated in the spinal fluid of multiple sclerosis.

It is often extremely difficult to make a diagnosis of this disease because of its similarity to other diseases of the central nervous system. In view of the negative findings of the spinal fluid and that no symptoms and laboratory findings are absolutely specific for this disease, the difficulty of a differantial diagnosis is obvious. However, there are certain symptoms which appear with constant frequency so that authorities such Grinker and Wechsler (103 and 104) diagnose the disease by the following group of symptoms:

1- Nystagmus (oscillation of the eyes).

2- Speech changes (slow or scanning speech).

3- Intention or action tremor.

4- Spasticity of gait.

Abscence of the abdominal reflexes.

6- Mental symptoms, not frequent, but there may be a spontaneous laughter and a feeling of well being.

7- Optic nerve is often the seat of affection.

8- Altered reflexes, especially of the extremities.

9- Cranial palsies.

10- Muscular weakness.

11- Bladder trouble (incontinence).

12- Laboratory findings are insignificant.

THEORIES OF THE ETIOLOGY

Research on multiple sclerosis has prompted a number of interesting but conflicting speculations. On investigation the literature one finds a number of theories as to the etiology of the disease and it is noteworthy that none of them have made much progress. However, it can be said that the theory of the infectious origin of the disease, i. e. , that the disease is caused by a spirochaete or a filterable virus, has reached its zenith and is now passing into the discard.

Weil (96, 97, and 98) has quite adequately summed up the theories on the etiology of multiple sclerosis by dividing them up into five groups as follows: (1) spirochaetal infection, (2) filterable virus infection, (3) myelolytic ferment as the etiologic agent, (4) endotoxins, and (5) a primary disease of the glia. Putnam (740 and Schukru (81Ø contend that perivascular disorders due to thromboses are improtant in aggravating the symptoms of the disease. In regard to the theory that toxins or noxious substances produce the demyelination, Dattner and

others have not been able to confirm the theory of Gerhartz (30) that tuberculo-toxin might be the demyelinating agent in multiple sclerosis. Dattner made a study of ninety patients suffering from multiple sclerosis for the purpose of studying the relation of the intestinal tract disorders. He found the interesting fact that 37% of the patients suffering from multiple sclerosis had a condition of anacidity of the gastric juice. He states: "This disturbance of the gastric juice is of great importance and may be compared with that of pernicious anemia and achylic chloranemia" (30). Pepper (67) also found the condition of anacidity of the gastric secretion in eleven cases of undoubted multiple sclerosis. This is extremely interesting in view of the similarity of pernicious anemia and multiple sclerosis, since in the former disease a demyelinating agent also occurs in the spinal fluid. Among other changes, according to Dattner, the clotting time of the blood is increased, but he states that this can be correlated with the metabolic disturbances depending on the liver.

Dattner also calls attention to another fact which has not been extensively investigated until recent years; namely, the similarity between multiple sclerosis and the diseases of avitaminosis such as beri-beri, pellagra, polyneuritis and scurvy. Lepine (56) obtained favorable

results in treating multiple sclerosis with a compound of ascorbic acid with iron and magnesium. Everyone is familiar with the importance of the vitamin B complex in in regard to the health of the nervous system; hence it is not at all unreasonable to venture the importance of this vitamin in connection with multiple sclerosis.

The possibility of a filterable virus being the etiologic agent is stressed mainly by the English workers Bullock (106), Purves-Stewart (70), Chevassut (26) and by Bernard (107). Most of the champions of this theory claim to have transmitted the disease experimentally to animals and some even state that they have cultured the virus. But Charmichael (25) and Weil (95, 96, 97 and 98) and others have quite conclusively disproved the theory of the infectious origin.

The theory that the causative agent is a"demyelinating ferment" is supported by Brickner (15, 16, 17 and 18), Crandall and Cherry (28 and 29), Camp (24) and by Weil who apparently would rather not commit himself to any pet theory, yet favors this trend. Camp even treats multiple sclerosis with small doses of potassium arsenite and the elixir of iron, quinine and strychnine. He states : "There is a possibility that arsenic and quinine are a benefit because of their action on the lipase of the blood". Brickner also employs the quinine therapy

and he states that the demyelinating ferment can be demonstrated in vitro with the plasma from multiple sclerosis patients. Brickner states that this lipase does not occur normally in the blood and that it differs in properties from the normal blood lipase.

Weil has an interesting publication (95) containing criticisms of the lipolytic theory of multiple sclerosis. His comments are based on a careful study of the phosphorus content of the serum as wellaas the lipase activity. He finds, as does Brickner and others, that the blood serum of multiple sclerosis patients exerts a lipolytic destruction of the myelin sheaths but emphasizes the fact that the same is true in a number of other diseases, for example, syphilis of the central nervous system and in liver diseases. Furthermore Weil states on the basis of his work that "it seems premature that we can assume a direct relationship between the increase in lipase in the sera of patients suffering from multiple sclerosis and the demyelinating action of such sera on rats' spinal cords". In regard to the inorganic phosphorus one would expect to find an increased amount in the serum of multiple sclerosis. Weil finds that the serum phosphorus is, on the average, 3.4 mg per 100 cc. as compared with 4.4 mg per 100 cc. in the normal subject. In 40% of the cases of multiple sclerosis the inorganic

phosphorus was below 3.3 mg per 100 cc. of the serum and in 65% of the cases it was 3.5 mg or below.

Other substances which are said to cause demyelination are hemolytic toxins and noxious substances such as sodium taurocholate, saponin and snake venom. In this connection it is interesting to note the work of Weil (98) in which he showed that lipoids may inhibit the action of hemolytic toxins on myelin; especially in the case of sodium taurocholate which is inhibited by lecithin.

This is a review of the most important literature as it is seen today. Other references which space does not permit to cite in detail are listed in the bibliography. The question of the myelolytic ferments is the main subject of this investigation. The questions for which answers are to be sought in this study are : (1) Is there any abnormal agent in the spinal fluid of multiple sclerosis which causes a myelolytic action or increases the activity of lipase and (2) what is the effect of vitamin B_1 ?

A vast amount of work is now being published in regard to the functions of vitamin B_1 in the central nervous system. The role of this vitamin in the carbohydrate metabolism of the brain is now confirmed (52, 63, 80, 92, 68 and many others). Vitamin B_1 must have an additional function in the nervous system for it seems

to be necessary for the preservation of normal myelin. Bertrand and Liber and Randoin (9) found in their experimental work on rats that a B_1 deficient diet produced changes in the myelinated motor neurones but not in the unmyelinated ones. They noted marked degenerative changes in the myelin sheath along with changes in the axis-cylinder. Lewy (57) confirms this view and adds that neuritis and pernicious anemia are also concerned with the vitamin. Thus many investigators are turning their attention to this vitamin in seeking the cause of numerous nervous disorders. Luhan and Balser (60) and Weil and Luhan (98) have shown that the spinal fluid and urine of patients suffering from multiple sclerosis contains a substance which dissolves myelin. These authors evaporated the spinal fluid in vacuo at 50 C and dissolved the residue in water. When the spinal cords of rats were incubated in the resulting solution the myelin of the cords was dissolved. Luhan showed the demyelination histologically but in preparing the spinal cords for histological study he embedded the specimens in paraffin. In this investigation it was found that the paraffin method of imbeddeing could not be used because the warm xylol and the paraffin dissolved the myelin out of the normal spinal cords as well as the experimental ones.

Many authors have shown the pathological changes which occur in vitamin B_1 deficiency but the purpose of this study is to showt that the vitamin is an important factor in inhibiting lipase activity, in this case the activity of pancreatic lipase, and thus may be a very important factor in the prevention of demyelination in the human subject, particularly in the case of multiple sclerosis.

II. PREPARATIONS AND MATERIALS.

PREPARATION OF PANCREATIC LIPASE. The method used in preparing the pancreatic lipase was essentially that of Willstatter and Waldschmidt-Leitz (108 and 109). Five pounds of hogs pancreas were freed as much as possible from the adhering fat then ground up in a meat grinder. The pulp was placed in a 2½ liter jar and extracted for thirty minutes with two liters of ether. This extraction with ether was repeated three times. The pulp was then extracted with a 1:1 mixture of acetone and ether; this extraction was also repeated three times. Finally the pulp was extracted three times with two liter quantities of acetone. This amount of extracting was enough to remove the fat from the pulp. After the final extraction the pulp was pressed into a Buchner funnel and the last traces of liquid filtered off by suction. The fat-free residue was then spread out in a vacuum desicator and allowed to dry for two days. At this time the pulp was throughly dried and powdery except for the coarse strings of connective tissue. The dry material was ground up as finely as possible in a motor and sifted through a very fine screen. This process gave a very

good product of a fine smooth powder having only a faint tinge of yellow. The greater part of this preparation was stored in a vacuum desicator in the refrigerator. A small amount , for immediate use, was kept in a tightly sealed, wide-mouthed bottle and removed from the refrigerator only when necessary. With these precautions and keeping the material in sterile containers the pancreatic powder can be kept indefinitly.

ENZYME SOLUTION. The pancreatic powder described above can be made up into a very active enzyme solution by the method described by Willstatter and Waldschmidt-Leitz (108) and by Weinstein and Wynne (99). These authors usually made up a mixture of 4 grams of powdered pancreas in 100 cc. of 50% glycerine. This was incubated for three hours at 37 C with frequent shakings and then centrifuged and filtered by suction. The 50% glycerine solution made a very viscous fluid which was difficult to filter and free from the fine powder. It was found in this work that it was more convenient to use a 25% solution of glycerine without altering the properties or activity of the enzyme preparation. If this enzyme preparation is prepared in sterile apparatus and kept in the refrigerator it will retain its activity for three or four days without the addition of any preservative.

The activity of this enzyme preparation was tested as follows: Clean Erlenmeyer flasks of 150 cc. capacity were stoppered with cotton plugs and sterilized by heating in the oven for three hours. After the flasks were cooled to room temperature the following materials were added: 2 cc. of olive oil, 1 cc. of bile salt solution containing 0.064 gms of bile salt per cc., 3 cc. of 4% sodium bicarbonate and 5 cc. of double distilled water. This made a total volume of 15 cc. when 2 cc. of the enzyme solution were added just before placing the flasks in the incubator. Controls were run on all of the constituents. Three samples were removed from the incubator at a time and titated at intervals of 2, 4, 8, 16, 24 and 36 hours. Before titrating 25 cc. of 95 % ethyl alcohol were added to each flask. The titrations were made with N/20 NaOH and phenolphthalein was used as the indicator. The activity curve showing the amount of oleic acid formed is shown in figure 1. The activity of this enzyme preparation is comparable to that of the authors cited above.

VITAMIN B_1. The vitamin B_1 used throughout this investigation was a pure, crystalline product from the Merk Chemical Company. The system of units employed in this paper is the international method of standardization; namely, one milligram of the pure, dry, crystalline

vitamin is equivalent to 300 international units. Since the vitamin is unstable in neurtal and alkaline solutions it was made up in 1% HCl so that 1 cc. of the solution contained 4 mgs of the vitamin. This was kept as the stock solution and stored in the refrigerator. The solution of vitamin B_1 used throughout this study was made up from the above stock solution by diluting 1 cc. to 200 cc. with double distilled water. Thus 1 cc of this solution contained six internaltion units of the vitamin or 20 gamma.

OLIVE OIL. The olive oil used was a product of the McKesson Robins Company. Unless otherwise indicated it was always used in 1 cc. quantities when used as a substrate in these lipase experiments.

LECITHIN. When lecithin was employed as a substrate a purified product from the Pfanstiehl Chemical Company was used. A one gram sample was dissolved in 125 cc. of a mixture of ethyl alcohol and ether (3:1). This solution was evaporated on the steam bath and shaken during the evaporation so that when all the liquid was evaporated the lecithin was deposisted on the bottom and the sides of the flask. In doing this the lecithin was easily emulsified when 100 cc. of distilled water were added. This gave a 1% emulsion of lecithin and was used as such in the subsequent experiments.

ETHYL BUTYRATE. This reagent was a C. P. product of the Eastman KodaK Company. When employed as a substrate it was used in quantities of 0.20 cc without dilution.

SPINAL FLUID. The spinal fluid for this investigation was obtained through the kind cooperation of Dr. H. McIntyre from patients at his office or from the wards at the General Hospital. It was collected under sterile procedures and with few exceptions was used immediately after the spinal puncture. When necessary the spinal fluid was stored in tightly sealed test tubes in the refrigerator over night. It is imperative to use the spinal fluid as soon as possible after it is collected because it increases in alkalinity upon standing.

III. EXPERIMENTAL

A. The Spinal Fluid of Multiple Sclerosis.

Altmann and Goldhammer (3), Brickner (16 and 18), Crandall and Cherry (28 and 29) and Sussner (87) and Weil and Cleveland (95) have demonstrated an abnormal lipase content of the blood serum of patients suffering from multiple sclerosis. In addition to this Luhan and Balser (60) and Weil and Luhan (98) have demonstrated that the spinal fluid of multiple sclerosis contains an agent which dissolves the myelin of normal rats' cords in vitro. Since this disease is one of the central nervous system it seemed only logical that the spinal fluid should be investigated for the presence of a lipase or any other abnormal changes. Hence the questions to be investigated in this phase of the work are three; namely, (a) is there an active lipase present in the spinal fluid of multiple sclerosis ? (b) If there is no lipase present does the spinal fluid of multiple sclerosis differ in any other respect from normal spinal fluid ? And (c) does the spinal fluid of multiple sclerosis differ from that of patients suffering from other diseases ?

(a). Lipase Activity of Multiple Sclerosis Spinal Fluid.

For this experiment the standard sized six inch test tubes were used. The test tubes were stoppered with cotton plugs and sterilized as described in the section on preparations and materials. Into each test tube was carefully measured 0.5 cc of olive oil, 0.5 cc. of 6% bile salt solution and 1 cc. of the spinal fluid to be investigated. The final volume was made up to 5 cc. by the addition of 3 cc. of distilled water. Duplicate samples were made up at the same time and titrated at once in order to get an initial reading (16, 18). Controls were run on each of the constituents used and titrated and incubated along with the samples. All tubes were shaken equally for mixing and placed in the incubator at the same time. The incubator was maintained at a temperature of 38 to 40 C. The samples were incubated for 24 hours with frequent shakings to keep the contents mixed. All titrations were made with N/100 NaOH after the addition of carefully neutralized ethyl alcohol (5 cc.). Phenolphthalein was used as the indicator and each sample was titrated to a faint pink and matched with a blank. This experiment was repeated using ethyl butyrate for the substrate; in this case no alcohol was added for the titrations.

The results of this experiment which was repeated twelve times, with different specimens of spinal fluid, are given in table 1. The experimental error in these titrations is plus or minus 0.20 cc of NaOH. In other words, at the end of the incubation, the titration value should show an increase of at least 0.2 cc NaOH in order to prove any lipasic hydrolysis. The results here indicate that there is no lipolytic hydrolysis caused by the presence of the spinal fluid of multiple sclerosis. This is in harmony with the results of Altmann and Goldhammer (3) and Sussner (87). The former authors studied sixteen different cases of multiple sclerosis without finding any evidence of a lipase in the spinal fluid.

Although no lipase could be demonstrated in the spinal fluid of multiple sclerosis there is a possibility that a lipase may be present in too small amounts to be demonstrated and there might also be present a substance which activates the lipase. On the other hand the spinal fluid of multiple sclerosis may not contain a substance which normally inhibits the lipase which might be present. In this respect it was decided to determine what effect, if any, the spinal fluid had on the hydrolysis of a substrate in the presence of pancreatic lipase. For this experiment 1 cc. of the spinal fluid of multiple sclerosis,

Table I.

The results of an experiment to show that there is no demonstrable amount of a lipase present in the spinal fluid of multiple sclerosis. An example of 12 trials.

Preparation	cc. N/100 NaOH Before	After
1 cc. sp. fld., 0.5 cc oil, 0.5 cc bile	0.05	0.06
Control on olive oil alone	0.04	0.06
" " bile salt alone	0.02	0.02
" " bile salt and oil.	0.04	0.07
" " spinal fluid alone	alkaline *	
1 cc sp. fld. & 0.20 cc. butyrate . . .	0.14	0.10
Control On ethyl butyrate	0.04	0.08
" " spinal fluid	0.02	alk. *

* The spinal fluid was sometimes just faintly alkaline to phenolphthalein at the beginning of the experiment and distinctly alkaline at the end of the experiment. However, it never took more than 0.04 cc. N/100 NaOH to neutralize the sample. This gain in the alkalinity of the spinal fluid was never enough to mask the increase in acidity that would result from the hydrolysis of the substrate; this can be determined by the controls.

0.20 cc. of ethyl butyrate, 0.20 cc. of the enzyme solution described in the section on preparation and materials, and 3.6 cc. of distilled water were carefully measured into each of six test tubes. This made a total volume of 5 cc. At the same time six tubes were prepared containing only the ethyl butyrate and the enzyme solution with enough water to make the total volume 5 cc. A control series was made up of each of the reagents; they were: a control of the enzyme alone made up to volume with water, ethyl butyrate alone made up to volume with water, spinal fluid alone with water and spinal fluid with ethyl butyrate and water as well as spinal fluid with enzyme solution and water. All the tubes were shaken equally. Three of the samples of each set were titrated immediately while the other three of each set were incubated for 24 hours before titrating with N/100 NaOH.

The above experiment is tabulated in table II. The results here show that the presence of the spinal fluid of multiple sclerosis enhanced the activity oft the pancreatic lipase or esterase. By calculating from the amount of NaOH used, the enzyme alone liberated 0.28 mgs. of butyric acid but in the presence of the spinal fluid 1.58 mgs of butyric acid were liberated by hydrolysis.

Table II.

The influence of multiple sclerosis spinal fluid on the esterase of the pancreatic enzyme preparation.

Each value listed is an average of six samples.

Preparation	cc. N/100 NaOH Before	After
1 cc. sp. fld., 0.2 cc enzy., 0.2cc buty.	0.47	2.01
0.2 cc. of enzyme soln. & 0.2 cc butyrate	0.36	0.65
Control on 0.2cc enzyme in water	0.11	0.13
" " 0.2cc butyrate and water. . . .	0.04	0.07
" " 1cc spinal fluid and water. . .	0.05	0.05
" " 0.2 cc enzyme & 1 cc sp. fld. .	0.12	0.14

Table III.

The influence of the spinal fluid of multiple sclerosis on the hydrolysis of olive oil by pancreatic lipase.

Each value listed is an average of three duplicate samples.

Preparation	cc. N/100 NaOH	Mgs acid formed	% Hydrol.
Lipase system without sp. fld. .	5.25	5.60	3.01
Control on enzyme solution . . .	0.45		
" " 1cc. of olive oil. .	0.00		
" " 1cc. spinal fluid. .	0.00		
" " spinal fluid & oil .	0.00		
Lipase system & 0.5 cc. sp. lfd.	5.90	7.42	4.07
" " 1.0 cc. " "	6.35	8.68	4.76
" " 4.0 cc. " " .	9.68	18.00	9.89
Spinal fluid specimen # 4			
Lipase system & 0.5 cc sp. fld. .	5.40	6.02	3.32
" " 1 cc. " " . .	7.00	10.50	5.76
" " 2 cc. " " . .	7.22	11.12	6.12
" " 4 cc. " " . .	7.48	11.84	6.50

The above experiment was repeated using olive oil as the substrate and it was performed on a larger scale in 150 cc Erlenmeyer flasks with the hope of making the results more distinct. Hereafter when the term "lipase system" is used it refers to the samples made up as follows: 2 cc. of enzyme solution, 1 cc. of olive oil, and water to make up to the volume indicated. Except where indicated, olive oil is the substrate used in this "lipase system". In the subsequent experiments the total volume of each flask was 15 cc. Table III gives the results obtained from two different specimens of spinal fluid of multiple sclerosis. Varying amounts of the spinal fluid were added to the lipase systems. Here it is again evident that the spinal fluid of multiple sclerosis activates the pancreatic lipase.

In all the above experiments no attempt was made to buffer the lipase systems to an optimum pH for the pancreatic lipase. Table IV shows the results obtained when using a phosphate sodium hydroxide buffer. Since the enzyme solution is acid in reaction and increases in acidity during the incubation it was decided to try a blank lipase system which contained boiled enzyme for a control. The initial pH of the samples was 8.35 as determined with the pH electrometer. After

incubating for 24 hours the samples were titrated back as nearly as possible to the original pH by matching themm with a blank which was tested on the pH lectrometer. By using these blanks for controls and titrating back to the initial pH all of the initial titrations were taken as zero.

Table IV.

The effect of the spinal fluid of multiple sclerosis on the hydrolysis of olive oil by pancreatic lipase.

The pH of each sample was 8.35 when made up.

Preparation	cc.N/20 NaOH	Mgs. acid formed	% Hydrol.
Lipase system alone	11.64	158.65	17.45
" " "	11.58	157.21	17.29
" " "	11.61	157.63	17.34
Blanks with boiled enzyme	0.35		
" " " "	0.31		
" " " "	0.32		
" " " "	0.32		
Lipase system & spinal fluid	11.90	161.88	17.80
" " " "	11.88	161.60	17.77
" " " "	12.00	163.08	17.94
Specimen # 7			
Lipase system alone	10.66	144.50	15.89
" " "	10.81	145.59	16.01
" " "	10.43	141.28	15.54
Blanks with boiled enzyme	0.32		
" " " "	0.33		
" " " "	0.33		
Lipase system & spinal fluid	11.21	152.18	16.74
" " " "	11.00	149.25	16.42
" " " "	10.96	148.55	16.34

Table IV again makes it obvious that the spinal fluid of multiple sclerosis exerts an activating influence on the pancreatic lipase. In the case of specimen number six of the above table the difference in the hydrolysis caused by the presence of the spinal fluid does not seem to be very great. Never-the-less the increased hydrolysis is significant when one considers the fact that 1 cc. of the N/20 NaOH is equivalent to 13.97 mgs. of the oleic acid and that the experimental error here does not exceed 0.20cc of the NaOH. It will be noticed in table IV that the amount of hydrolysis in the control lipase systems for the two different spinal fluid specimens, is quite different. This is due to the fact that, in the case of specimen number seven, the experiment was performed two days later and the enzyme solution had stood in the refrigerator for two days before using and had thus lost a little of its activity. This fact should be remembered in the subsequent experiments; since all of them could not be performed at the same time the amount of hydrolysis in the control lipase systems will vary. Whenever a specimen of spinal fluid was tested for its influence on the activity of the pancreatic lipase a control lipase system without the spinal fluid added was run simultaneously. Therefore the significant fact is to compare the amount.

of hydrolysis accomplished in the lipase systems alone with that of the lipase systems containing the spinal fluid. The amount of spinal fluid used to produce this increase was 1 cc. in table IV and in all the experiments which follow. In all cases the lipase system was made up of 2 cc. of the enzyme solution, 1 cc. of olive oil, and water to make the total volume up to 15 cc. When testing the spinal fluid 1 cc. of the spinal fluid replaced 1 cc. of the water, thus keeping the total volume the same in all samples.

(b) The Influence of Normal Spinal Fluid on Pnacreatic Lipase.

The spinal fluid for these experiment was also obtained from patients at Dr. McIntyre's office and from the wards of the General Hospital. The specimens used were those that were found to be normal by all the laboratory examinations. Occasionally a specimen was found to be contaminated because the technicians did not use sterile pipettes or the sample was exposed to the air of the room for too long a time. These samples were not included in the results.

These experiments were performed as usual in sterle Erlenmeyer flasks of 150 cc. capacity. The lipase systems were composed of 2 cc. of enzyme solution, 1 cc. of olive oil and water to make the volume up to 15 cc.

Controls were run on simultaneously on each of the constituents and all the flasks were incubated for 24 hours. Before titrating 25 cc of 95% ethyl alcohol was added to each flask. The titration value of the alcohol was determined beforehand and all the values listed in the following tables are the corredted values.

An expample of the results obtained when useing normal spinal fluid is shown in table V. It will be noticed that the increased hydrolysis due to the presence of the normal spinal fluid amounts to an addition of only 3.57 mgs of oleic acid. This is only slightly more than enough to make a significant increase in the hydrolysis. In terms of oleic acid the experimental error is plus or minus 2.82 mgs of oleic acid; this experiment then shows a significant increase of only 0.75 mgs of oleic acid. In terms of percent hydrolysis this value means an increase of only 0.38%.

The slight increase in the hydrolysis when adding normal spinal fluid to the lipase system may be due to the presence of the small amounts of protein in the spinal fluid and the other normal constituents of the specimen. The presence of the protein would influence the hydrolysis by causing a more complete emulsification of the substrate. If the amount of hydrolysis is increased by the presence of some activater in the spinal

fluid, one would expect a greater increase than is shown in the results of table V. For a comparison of the results obtained when useng the spinal fluid of multiple sclerosis in the lipase system, the results of table six are given. This experiment was performed with a specimen of spinal fluid taken from an active case of multiple sclerosis. Here the increased hydrolysis due to the presence of the spinal fluid of multiple sclerosis amounts to 30.34 mgs. of oleic acid. The hydrolysis was accelerated by 17.35% in this case.

Table VII is a summary table showing the effect of normal spinal fluid on the activity of pancreatic lipase, compared with the influence of the spinal fluid of multiple sclerosis. Each figure given is a value obtained from a different specimen of spinal fluid and represents an average of three duplicate samples. For the sake of convenience and brevity the results are listed only in terms of the milligrams of oleic acid formed and the percent hydrolysis. The average values of all the experiments are listed at the bottom of the table.

Table V.

The effect of normal spinal fluid on the activity of pancreatic lipase. The values for the control samples are averages of triplicate samples.

T Preparation	cc.N/20 NaOH Before	After	Mgs acid formed	% Hydrol.
Lipase system alone.	2:50	10:52	111:05	12:21
" " "	2:52	10:50	111:47	12:26
" " "	2.49	10.47	111.05	12.21
Lipase system & lcc sp. fld.	2:48	10:70	113:87	12:52
" " " " "	2:49	10:73	115:12	12:66
" " " " "	2.49	10.75	115.29	12.67
Control on enzyme.	2:39	2:52		
" " olive oil. . .	0:26	0:28		
" " spinal fluid .	0:20	0:25 *		
lcc. sp. fld. & lcc. oil .	0.26	0.28		

* These values are in terms of N/20 HCl because the spinal fluid was alkaline and had to be titrated back.

Table VI.

The effect of multiple sclerosis spinal fluid on the activity of pancreatic lipase.

Preparation	cc.N/20 NaOH Before	After	Mgs acid formed	% Hydrol.
Lipase system alone.	3:00	14:45	144:91	15:91
" " "	2:98	14:44	145:05	15:95
" " "	2.98	14.45	145.19	15.97
Lipase system & lcc sp. fld	2:97	16:55	175:11	19:26
" " " " "	2:98	16:61	175:67	19:32
" " " " "	2.98	16.59	175.39	19.29
Control on enzyme.	2.50	3.64		
" " Olive oil . . .	0:35	0:39		
" " spinal fluid . .	0:03	0:04 *		
Spinal & olive oil. . . .	0.33	0.28		

* N/20 HCl.

A comparative study on the effect of normal and multiple sclerosis spinal fluid on the activity of pancreatic lopase.

Sample	Mgs Oleic acid formed		Percent hydrolysis		Diff. in	% increase
	Controls	With sp. fld.	Controls	With sp. fld.	Mgs.	in hydrol.
Normal.	111.19	114.76	12.23	12.62	3.57	0.38
"	110.29	114.60	12.13	12.61	4.31	0.48
"	132.02	149.75	14.51	16.52	17.73	2.01
"	145.27	131.22	15.97	14.43	14.05	154
"	144.91	127.88	15.94	14.06	27.03	1.88
Average	128.74	127.45	14.16	15.05	17.34	-0.75
Mult. sclerosis	89.68	111.15	9.89	12.22	21.47	2.33
" "	145.09	164.00	15.96	18.04	18.91	2.08
" "	145.05	175.39	15.94	19.27	30.34	3.35
" "	145.14	183.19	15.97	20.14	38.05	4.17
" "	187.90	222.46	20.66	24.50	34.56	3.84
" "	131.27	191.28	14.44	21.01	60.01	6.60
Average.	140.69	174.58	15.46	19.20	33.89	3.73

(c) Influence of Spinal Fluid of Other Diseases.

The spinal fluids from patients suffering from diseases other than multiple sclerosis were obtained from the same sources as those used in the above experiments. The specimens were studied in the same manner as those described in the foregoing section. The results are compared in table VIII with the results obtained by using the spinal fluid taken from patients suffering from multiple sclerosis. In these results it seems as though the spinal fluids taken from patients afflicted with hysteria, cerebral arterial sclerosis, brain tumor or attacks of coma increased the activity of the pancreatic lipase. But this may be due to the high protein content of the spinal fluid as shown by the laboratory examination. This is not true of the spinal fluids of multiple sclerosis.

Table VIII

The effect of the spinal fluid of various diseases on the activity of pancreatic lipase as compared to the effect of the spinal fluid of multiple sclerosis.

Sample used	Mgs oleic acid formed		Percent hydrolysis		Diff. in Mgs.	% increase in hydrol.
	Controls	With sp. fld.	Controls	With sp. fld.		
Luteic meningitis	119.19	114.76	12.23	12.62	3.57	0.38
Multiple neuritis	145.00	159.72	15.94	17.57	14.72	1.63
Hysteria . . .	148.44	176.98	16.32	19.47	28.54	3.15
Cerebral arterial sclerosis . .	148.44	173.83	16.32	19.17	25.39	2.85
Epilepsy. . . .	110.29	114.60	12.13	12.61	4.31	0.48
Brain tumor	132.02	149.75	14.51	16.52	17.73	2.01
Attacks of coma	187.90	213.25	20.66	23.45	25.35	2.79
Epilepsy. . . .	142.92	149.55	15.72	16.45	6.63	0.73
Diabetes. . . .	141.56	147.92	15.57	16.27	6.36	0.70
Average.	140.86	155.59	15.69	17.12	14.73	1.64
Average for mult. scleroaia	140.69	174.58	15.46	19.20	33.89	3.73

B. The Effect of Vitamin B_1 on Pancreatic Lipase.

It was demonstrated in the experiments shown on the preceeding pages that the spinal fluid of multiple sclerosis had no lipolytic activity but that it did accelerate the activity of pancreatic lipase. Bertrand and Liber and Randoin (9) and Lewy have shown the necessity of vitamin B_1 in maintaining the normal health of the central nervous system. The former authors demonstrated degenerative changes in myelin during a vitamin B_1 deficiency. In as much as demyelination is a lipolytic process it was decided to investigate the following question: What effect does vitamin B_1 have on the activity of lipolytic hydrolysis ? In this, as in the previous experiments pancreatic lipase was used.

To study the effect of vitamin B_1 on the activity of pancreatic lipase the experiments were performed in the usual manner in 150 cc Erlenmeyer flasks. The lipase system was comprised of 2 cc of the enzyme solution, 1 cc of olive oil and water to make up to volume. At the same time additional samples were made up containing varying amounts of the vitamin. The vitamin solution contained six international units of vitamin per cubic centimeter. This amounts to 20 gamma

of the vitamin per cubic centimeter. All flasks were treated in the same manner and incubated for 24 hours. Enough samples were made up so that titrations could be made both before and after the icubation. The titrations were made with N/20 NaOH and phenolphthalein was used as the indicator. At this stage of the study it was not deemed necessary to titrate the samples in alcohol, consequently the amount of hydrolysis shown in tables IX, X, and XI is perhaps unduely low. The results shown in table IX are plotted in figure 2 and the results of tables X and XI are plotted in figure 3. The amount of hydrolysis shown in tables X and XI are much lower than the amount shown in table IX. This is due to the fact that in the former cases the enzyme solution was not freshly prepared as was the case in the latter. It was therefore decided that it was necessary to prepare a fresh enzyme solution for each experiment. All the experiments that follow those listed in tables IX, X, and XI were titrated in alcohol and a correction made for the amount of NaOH necessary to neutralize the alcohol used.

The improved results obtained by using a freshly prepared enzyme solution and titrating all the samples in alcohol are shown in tables XII, XIII and XIV. The results shown in table XII are plotted in figure 4.

Table IX.

The effect of vitamin B_1 on the activity of pancreatic lipase.

preparation	cc. N/20 NaOH Before	cc. N/20 NaOH After	Mgs. acid formed	% Hydrol.
Lipase system alone. . .	0.85	6.14	69.15	7.60
" " " . . .	0.85	6.30	71.39	7.85
" " " . . .	0.83	6.28	71.39	7.85
Lipase & 3 units of B_1 .	0.84	5.61	61.89	6.80
" " " " " .	0.84	5.62	62.03	6.82
" " " " " .	0.85	5.64	62.17	6.95
" " 6 units of B_1 .	0.84	5.56	61.19	6.73
" " " " " .	0.85	5.56	61.05	6.71
" " " " " .	0.85	5.56	61.05	6.71
" " 12 units B_1 .	0.85	4.63	48.06	5.28
" " " " " .	0.85	4.57	47.22	5.19
" " " " " .	0.85	4.38	45.96	5.05
" " 18 units B_1 .	0.85	4.07	40.23	4.42
" " " " " .	0.85	4.17	41.63	4.57
" " " " " .	0.86	4.13	40.93	4.50
" " 24 units B_1 .	0.86	4.92	51.97	5.71
" " " " " .	0.87	5.03	53.37	5.87
" " " " " .	0.86	4.98	52.81	5.80
" " 30 units " .	0.87	3.35	29.90	3.28
" " " " " .	0.87	3.40	30.59	3.36
" " " " " .	0.87	3.36	30.04	3.30
" " 36 units B_1 .	0.87	3.00	25.01	2.75
" " " " " .	0.86	3.00	25.15	2.76
" " " " " .	0.87	3.00	25.01	2.75
" " 48 units B_1 .	0.88	1.74	7.26	0.79
" " " " " .	0.87	1.74	7.40	0.81
" " " " " .	0.87	1.69	7.71	0.73
Control on enzyme. . .	6.57	0.77		
" " olive oil . .	0.02	0.04		
" " 6 units B_1 . .	0.01	0.02		
" " 18 units B_1 .	0.02	0.02		
" " 48 " " .	0.02	0.02		
" oil & 24 " " .	0.03	0.04		
" enzyme & 24 units B_1	0.58	0.80		

Table X.

The effect of vitamin B_1 on the activity of pancreatic lipase.

Preparation	cc. N/20 NaOH Before	cc. N/20 NaOH After	Mgs. acid foremed	% Hydrol.
Lipase system alone. . .	0.65	2.75	32.27	3.55
" " " . . .	0.65	2.70	31.57	3.47
" " " . . .	0.65	2.73	31.99	3.51
Lipase & 3 units B_1. . .	0.65	1.76	18.44	2.02
" " " " " . . .	0.65	1.77	18.58	2.04
" " " " " . . .	0.65	1.55	15.61	1.71
" " 6 " " . . .	0.64	1.62	16.62	1.82
" " " " " . . .	0.65	1.62	16.48	1.81
" " " " " . . .	0.66	1.64	16.62	1.82
" 12 " " . . .	0.65	1.21	10.76	1.18
" " " " " . . .	0.65	1.23	11.04	1.21
" " " " " . . .	0.64	1.22	10.90	1.19
" 18 " " . . .	0.64	1.15	10.06	1.10
" " " " " . . .	0.64	1.16	10.20	1.12
" " " " " . . .	0.64	1.16	10.20	1.12
" " 24 units B_1 . .	0.65	0.76	4.47	0.49
" " " " " . .	0.64	0.75	4.33	0.47
" " " " " . .	0.64	0.78	4.89	0.53
" " 30 " " . .	0.65	0.59	2.10	0.23
" " " " " . .	0.65	0.59	2.10	0.23
" " " " " . .	0.64	0.56	1.82	0.20
" " 36 " " . .	0.65	0.54	1.40	0.15
" " " " " . .	0.64	0.49	0.84	0.09
" " " " " . .	0.66	0.50	0.70	0.07
" " 48 " " . .	0.66	0.81	5.03	0.55
" " " " " . .	0.66	0.81	5.03	0.55
" " " " " . .	0.64	0.80	5.17	0.56
" " 60 " " . .	0.65	0.78	4.75	0.52
" " " " " . .	0.65	0.80	5.03	0.55
" " " " " . .	0.65	0.80	5.03	0.55
Control on enzyme	0.57	0.04	(average) of three)	
" " olive oil . .	0.02	0.02	" " "	
" " 6 units of B_1	0.02	0.02	" " "	
" " 30 " " "	0.02	0.02	" " "	
" " 60 " " "	0.04	0.04	" " "	
Enzyme & 6 units B_1 . . .	0.57	0.88		
" " 30 " " . . .	0.57	0.88		
" 60 " " . . .	0.57	0.87		

Table XI

The effect of vitamin B_1 on the activity of pancreatic lipase.

Preparation	cc. N/20 NaOH Before	cc. N/20 NaOH After	Mgs. acid formed	% Hydrol.
Lipase system alone. . .	0.67	2.53	30.73	3.38
" " " . . .	0.65	2.50	30.59	3.36
" " " . . .	0.64	2.51	30.87	3.39
Lipase & 3 units B_1 . . .	0.65	1.61	18.16	1.99
" " " " " . . .	6565	1.63	18.44	2.02
" " " " " . .	0.65	1.68	19.14	2.10
" " 6 " " . .	0.65	1.64	18.58	2.04
" " " " " . .	0.65	1.80	20.82	2.29
" " " " " . . .	0.65	1.60	18.02	1.98
" " 12 " " . . .	0.65	1.20	12.42	1.36
" " " " " . . .	0.65	1.22	12.71	1.39
" " " " " . . .	0.65	1.20	12.43	1.36
" " 18 " " . . .	0.65	1.15	11.73	1.29
" " " " " . .	0.65	1.15	11.73	1.29
" " " " " . . .	0.65	1.14	11.60	1.27
" " 24 units B_1 . .	0.65	0.76	6.29	0.69
" " " " " . .	0.66	0.72	5.73	0.63
" " " " " . .	0.66	0.74	5.87	0.64
" " 30 " " . . .	0.66	0.59	3.77	0.41
" " " " " . .	0.66	0.53	2.93	0.31
" " " " " . .	0.66	0.56	3.35	0.36
" " 48 " " . .	0.66	0.86	7.54	0.82
" " " " " . .	0.66	0.87	7.68	0.84
" " " " " . .	0.66	0.85	7.40	0.81
" " 60 " " . .	0.66	0.85	7.40	0.81
" " " " " . .	0.66	0.84	7.26	0.79
" " " " " . .	0.66	0.85	7.40	0.81
Control on enzyme. . . .	0.65	0.00	(average of three)	
" " olive oil . .	0.02	0.02	" " "	
" " 30 units B_1 .	0.02	0.02	" " "	
Enzyme & 6 units B_1 . . .	0.64	0.93	" " "	
" 30 " " . . .	0.64	0.95	" " "	
" 60 " " . . .	0.65	0.95	" " "	

It was noticed when plotting the results that although the vitamin inhibits the action of pancreatic lipase, the amount of inhibition is not directly proportional to the amount of vitamin B_1 used. There is a low point in the curve where 18 units of the vitamin are used and a rise to the point where 24 units are used. This is followed by an increasingly less amount of hydrolysis with an increased amount of the vitamin present in the lipase system. In order to find the lowest and the highest points of the curve the experiment shown table XIV. was performed. The results are plotted in figure 5. This experiment shows low points at 17, 23, 30 and on to 100 units and peaks at 22 and 25. These were consistent throughout the entire study and remain to be explained. However, the inhibitive effect of the vitamin is very significant; in some cases there is over fifty percent inhibition. All of the experiments which were performed to show the inhibitory effect of vitamin B_1 on the activity of pancreatic lipase when olive oil was used as the substrate, are shown in a summary form in table XV. Each figure given in this table is an average value computed from at least 180 trials with the exception of those listed when using 90, 100, and 150 units of the vitamin. The average values of all the experiments are plotted in figure 6.

Table XII.

The effect of vitamin B_1 on the activity of pancreatic lipase. All titrations made in alcohol. The values listed are the corrected values after subtracting the titration value of the alcohol.

Preparation	cc. N/20 NaOH Before	cc. N/20 NaOH After	Mgs acid formed	% Hydrol.
Lipase system alone	4.20	16.85	164.78	18.11
" " "	4.19	17.10	168.90	18.58
" " "	4.19	16.90	152.11	16.73
Lipase & 6 units B_1	4.22	14.06	124.73	13.72
" " " " "	4.21	14.10	125.44	13.79
" " " " "	4.19	16.90	152.11	13.72
" " 12 " "	4.21	13.30	113.59	12.49
" " " " "	4.20	13.24	112.88	12.41
" " " " "	4.21	13.24	112.74	12.40
" " 24 " "	4.21	13.50	116.69	12.83
" " " " "	4.22	13.50	115.55	12.71
" " " " "	4.22	13.50	115.55	12.71
" " 48 " "	4.22	12.25	998.91	10.11
" " " " "	4.21	12.90	108.22	11.99
" " " " "	4.22	11.60	90.30	9.93
" " 100 " "	4.22	10.70	77.04	8.47
" " " " "	4.22	10.72	77.32	8.50
" " " " "	4.22	10.05	67.87	7.46
Control on enzyme	3.70	4.62	(average of three)	
" " olive oil	0.50	0.53	" " "	
" " 12 units B_1	0.00	0.04	" " "	
" " 24 " "	0.00	0.04	" " "	
" " 48 " "	0.00	0.05	" " "	
" " 60 " "	0.00	0.05	" " "	
Enzyme & 6 units B_1	3.70	4.60	" " "	
" 24 " "	3.68	4.62	" " "	
" 60 " "	3.70	4.65	" " "	

Table XIII.

The effect of vitamin B_1 on the activity of pancreatic lipase. All titrations made in alcohol.

Preparation	cc. N/20 NaOH Before	cc. N/20 NaOH After	Mgs acid formed	% Hydrol.
Lipase system alone. . . .	2.43	11.65	125.30	13.78
" " "	2.24	11.70	125.44	13.79
" " "	2.42	11.72	127.13	13.98
Lipase & 6 units B_1 . . .	2.43	8.90	87.48	9.62
" " " " " . . .	2.43	8.90	87.48	9.62
" " " " " . . .	2.42	8.90	87.62	9.63
" " 12 " " . . .	2.42	9.50	96.09	10.57
" " " " " . . .	2.41	9.58	97.36	10.71
" " " " " . . .	2.42	9.60	97.50	10.72
" " 17 " " . . .	2.42	10.25	106.67	11.73
" " " " " . . .	2.42	10.25	106.67	11.73
" " " " " . . .	2.42	11.00	117.25	12.89
" " 18 " " . . .	2.42	11.43	123.32	13.56
" " " " " . . .	2.44	11.43	123.04	13.53
" " " " " . . .	2.43	11.40	122.76	13.50
" " 90 " " . . .	2.43	9.20	91.15	10.02
" " " " " . . .	2.43	9.20	91.15	10.02
" " " " " . . .	2.42	9.20	91.15	10.02
" "150 " " . . .	2.43	8.80	85.93	9.45
" " " " . . .	2.44	8.80	84.66	9.30
" " " " . . .	2.42	8.82	86.49	9.62
* Control on enzyme . . .	2.15	2.27		
" " olive oil . .	0.25	0.28		
" " 90 units B_1 .	0.02	0.02		
" "150 " " .	0.03	0.04		
Enzyme & 6 units B_1 . . .	2.15	2.28		
" " 90 " " . .	2.15	2.30		
" " 150 " " . .	2.15	2.29		

* All the values listed for the controls are averages of three samples.

Table XIV.

The effect of varying amounts of vitamin B_1 on the activity of pancreatic lipase.

Preparation	cc N/20 NaOH Before	cc N/20 NaOH After	Mgs acid formed	% Hydrol.
Lipase system alone. . . .	3.84	17.24	185.36	20.39
Lipase & 3 units B_1. . . .	3.85	14.55	146.74	16.14
Control on enzyme.	3.79	4.00		
" " olive oil . . .	0.15	0.21		
" " 3 units B_1. . .	0.02	0.02		
" enzyme & B_1. . . .	3.84	4.02		
" oil & 3 units B_1	0.14	0.21		
Lipase system alone. . . .	3.83	17.24	185.36	20.39
Lipase & 6 units B_1. . . .	3.84	14.16	140.52	15.49
Control on enzyme.	3.78	4.00		
" " olive oil . . .	0.14	0.20		
" " 6 units B_1 . . .	0.02	0.03		
" enzyme & B_1. . . .	3.78	3.86		
" oil & 6 units B_1. .	0.14	0.21		
"				
Lipase system alone. . . .	3.83	17.24	185.36	20.39
Lipase & 12 units B_1 . . .	3.83	13.72	134.33	14.77
Control on enzyme.	3.79	4.00		
" " Olive oil . . .	0.14	0.20		
" enzyme & B_1.	3.79	4.10		
" oil & 12 units B_1. .	0.15	0.22		
Lipase system alone. . . .	3.85	17.22	183.99	20.24.
Lipase & 16.8 units B_1 . .	3.86	12.88	121.63	13.38
Control on enzyme.	3.79	4.06		
" " olive oil . . .	0.14	0.21		
" " 16.8 units B_1	0.02	0.03		
" enzyme & B_1. . . .	3.78	4.12		
" oil & B_1.	0.12	0.15		
Lipase system alone. . . .	3.86	17.25	184.98	20.35
Lipase & 18 units B_1 . . .	3.89	13.45	130.24	14.32
Control on enzyme.	3.78	4.02		
" " olive oil . . .	0.11	0.15		
" " 18 units B_1 . .	0.02	0.02		
" enzyme & B_1	3.78	4.12		
" oil & 18 units B_1 .	0.12	0.16		

Table XIV (continued)

Preparation	cc. N/20 NaOH Before	After	Mgs acid formed	% Hydrol.
Lipase system alone. . . .	3.88	17.26	184.56	20.30
Lipase & 19.2 units B_1 . .	3.90	13.40	128.54	14.14
Control on enzyme.	3.78	4.02		
" " olive oil . . .	0.11	0.16		
" " 19.2 units B_1 .	0.02	0.03		
" enzyme & B_1	3.78	4.12		
" oil & B_1.	0.12	0.16		
Lipase system alone. . . .	3.88	17.27	184.56	20.30
Lipase & 22.8 units B_1 . .	3.90	14.76	147.45	16.21
Control on enzyme.	3.76	4.00		
" " olive oil . . .	0.11	0.16		
" " 22.8 units B_1 .	0.02	0.04		
" enzyme & B_1. . . .	3.79	4.16		
" oil & B_1.	0.12	0.16		
Lipase system alone. . . .	3.86	17.25	185.12	20.36
Lipase & 24 units B_1 . . .	3.89	14.31	141.38	15.55
Control on enzyme.	3.78	4.02		
" " oil	0.11	0.14		
" enzyme & B_1	3.81	4.18		
" oil and B_1.	0.12	0.16		
Lipase system alone. . . .	3.83	17.25	185.69	20.42
Lipase & 25.2 units B_1 . .	3.88	14.16	152.95	16.82
Control on enzyme.	3.78	4.02		
" " olive oil . . .	0.11	0.15		
" " 25.2 units B_1 .	0.03	0.03		
" enzyme & B_1.	3.79	4.19		
" oil and B_1.	0.12	0.16		
Lipase system alone. . . .	3.84	17.25	185.36	20.39
Lipase & 30 units B_1 . . .	3.87	13.00	122.19	13.44
Control on enzyme.	3.78	4.02		
" " olive oil . . .	0.11	0.14		
" " 30 units B_1 . .	0.03	0.04		
" enzyme & B_1. . . .	3.80	4.21		
" oil and B_1.	0.12	0.16		

Table XIV (continued)

Preparation	cc N/20 NaOH Before	cc N/20 NaOH After	Mgs acid formed	% Hydrol.
Lipase system alone. . . .	3.85	17.23	185.36	20.39
Lipase & 36 units B_1 . . .	3.87	12.79	120.78	13.28
Control on enzyme.	3.79	4.00		
" " olive oil . . .	0.11	0.15		
" " 36 units B_1 . .	0.02	0.02		
" enzyme & B_1	3.90	4.22		
" oil and B_1	0.12	0.15		
Lipase system alone. . . .	3.85	17.23	184.98	20.35
Lipase & 48 units B_1 . . .	3.87	12.86	121.49	13.41
Control on enzyme.	3.79	4.02		
" " olive oil . . .	0.11	0.15		
" " 48 units B_1 . .	0.02	0.03		
" enzyme & B_1	3.91	4.22		
" oil and B_1	0.11	0.16		
Lipase system alone. . . .	3.88	17.24	184.62	20.31
Lipase & 60 units B_1 . . .	3.91	12.81	118.81	13.07
Control on enzyme.	3.79	4.02		
" " olive oil. . .	0.11	0.15		
" " 60 units B_1 . .	0.03	0.03		
" enzyme & B_1	3.80	4.24		
" oil and B_1	0.12	0.17		
Lipase system alone. . . .	3.86	17.25	185.36	20.39
Lipase & 100 units B_1. . .	3.90	12.22	110.90	12.20
Control on enzyme.	3.78	4.01		
" " olive oil. . .	0.11	0.14		
" " 100 units B_1. .	0.02	0.03		
" enzyme & B_1.	3.82	4.25		
" olive oil and B_1 . .	0.12	0.16		

6 units B_1 equals 20 gamma of the vitamin
12 " " " 40 " " " "
48 " " " 160 " " " " etc.

Table XV.

A summary table of 180 trials to demonstrate the inhibitory effect of varying amounts of vitamin B_1 on the activity of pancreatic lipase. Olive oil was used as the substrate.

Unist B_1	Mgs acid formed Lipase	Control	Diff. in Mgs.	% hydrolysis with B_1	Contr.	Diff. % hydrol.
3	146.74	185.66	38.92	16.14	20.42	4.28
6	111.14	164.75	53.61	12.22	18.12	5.90
12	109.21	164.76	55.55	12.01	18.12	6.11
17	117.17	185.30	68.13	12.89	20.38	7.49
18	127.19	165.56	38.37	13.98	18.21	4.23
19	127.61	185.26	57.65	14.03	20.38	6.35
23	148.99	185.47	36.48	16.39	20.40	3.91
24	133.11	177.67	44.56	14.64	19.54	4.90
25	145.10	185.82	40.12	15.96	20.44	4.48
30	122.19	185.36	63.17	13.44	20.39	6.95
36	120.78	185.36	64.58	13.28	20.39	7.11
48	104.86	173.45	68.59	11.53	19.08	7.55
60	85.26	173.27	88.01	9.47	19.06	9.59
90	91.15	125.56	34.61	10.02	13.81	3.79
100	111.90	185.36	73.46	12.31	20.39	8.08
150	85.69	125.76	40.07	9.42	13.83	4.41

A question arose concerning the effect of the vitamin in the presence of some activator such as bile salts. To show the effectiviness of the vitamin under this condition an experiment was performed in which bile salts were added to the samples as well as vitamin B_1. Additional samples were prepared without the addition of the bile salts to show the usual effect of the vitamin. The results of this experiment are shown in table XVI. Here 60 international units, or 200 gamma, of the vitamin were employed in the presence of bile salts and the inhibitory effect of the vitamin was not apperciably affected. A 6.4% bile salt solution was used ; one cubic centimeter of this solution was added to each sample.

In this experiment the decrease in hydrolysis caused by the presence of the vitamin without the addition of bile salt amounts to 34.09 mgs. of oleic acid or 11.31 % hydrolysis in contrast to 8.34 % when vitamin B_1 is present. This is equivalent to an inhibition of 26.25 %. When the bile salts are present there is a decrease in hydrolysis of 52.06 mgs oleic acid or a difference in percent hydrolysis of 5.36%. This is an inhibition of. 20.26%.

Table XVI

The effect of vitamin B_1 on the activity of pancreatic lipase in the presence of bile salts.

Preparation	cc. N/20 NaOH	Mgs acid formed	% Hydrol.
Lipase sysetm alone	8.00	96.70	10.64
" " "	8.80	108.08	11.86
" " "	8.50	103.85	11.42
Lipase system with bile salt	18.50	243.96	26.83
" " " " "	18.50	243.96	26.83
" " " " "	17.70	232.67	25.59
Lipase system with 30 i.u. B_1	5.50	61.24	6.74
" " " " "	7.00	82.42	9.06
" " " " "	7.10	83.81	9.22
Lipase, 30 i.u. B_1 & bile salt	15.30	198.53	21.81
" " " " "	14.62	188.93	20.78
" " " " "	14.50	187.24	20.59
Controls			
Enzyme in water.	1.10		
Olive oil control.	0.04		
Control on 30 units B_1	0.02		
Bile salt control	0.02		
Enzyme and bile salt	1.32		
Olive oil and bile salt.	0.17		
Bile salt and 30 units B_1	0.03		
Enzyme and 30 units B_1	1.11		
olive and 30 units B_1	0.18		
Enzyme, 30 units B_1 and bile salt	1.11		
Olive oil, B_1 and bile salt.	0.19		

30 units of vitamin equals 100 gamma.

It is clearly evident from the foregoing experiments that vitamin B_1 has a decided inhibitory effect on the action of pancreatic lipase. The inhibition amounting to as high as 26% depending upon the amount of the vitamin used. Is this inhibitory effect also manifested in the case of esterases? This question is answered in the experiments which follow.

To study the effect of the vitamin on the activity of esterases pancreatic esterase was used; this is contained in the same preparation as described in the section on materials and perparations. The experiments were performed in exactly the same manner as those described above except that 0.20 cc. of ethyl butyrate was substituted for the olive oil substrate. The control esterase flasks contained 2cc. of enzyme, 0.2 cc of ethyl butyrate, and water to make up to volume. To show the effect of the vitamin additional flasks were prepared in which varying amounts of vitamin B_1 were substituted for the correct amount of water to keep the total volume 15 cc. Controls were run on each of the ingredients of the samples. All flasks were shaken equally and incubated for 24 hours. The results are shown in tables XVII, XVIII and XIX.

The effect of vitamin B_1 on the hdyrolysis of ethyl butyrate by pancreatic esterase.

preparation	cc N/20 NaOH Before	After	Mgs acid formed	% Hydrol.
Enzyme and butyrate. . .	1.05	10.00	38.72	29.10
" " " . . .	1.05	10.05	38.94	29.21
" " " . . .	1.05	10.15	39.38	29.54
" " " . . .	105	10.11	39.20	29.41
" " " . . .	1.05	10.15	39.38	29.54
" " " . . .	1.05	10.20	39.60	29.74
Averages" . . "	1.05	10.11	39.21	29.46
Enzyme, butyrate & 18 B_1	1.05	9.53	36.65	27.56
" " " "	1.05	9.53	36.65	27.56
" " " "	1.05	9.80	37.84	28.42
" " " "	1.05	9.55	36.74	27.58
" " " "	1.05	9.75	37.62	28.25
" " " "	1.05	9.94	38.46	28.93
Averages.	1.05	9.68	37.33	28.05
Control on enzyme . . .	1.00	1.14		
Control on butyrate . .	0.01	0.02		
Control on 18 i.u. B_1 .	0.06	0.06		
Control: enzyme & B_1. .	1.09	1.22		
Butyrate and B_1.	0.07	0.07		

Table XVIII

The effect of varying amounts of vitamin B_1 on the activity of pancreatic esterase.

Preparation	cc N/20 NaOH Before	cc N/20 NaOH After	Mgs. acid formed	% Hydrol.
Enzyme & butyrate	0.94	10.00	43.21	32.41
" "	0.94	9.88	42.68	32.10
" "	0.94	10.10	43.65	32.79
Enzyme, butyrate, 6 i.u.B_1	.94	9.80	42.33	31.75
" " "	0.94	9.65	41.67	31.38
" " "	0.95	9.90	41.23	31.93
Enzyme & butyrate	0.97	9.94	42.94	32.21
" "	0.96	9.97	43.12	32.40
" "	0.97	10.04	43.38	32.54
Emzy. & 12 i.u. B_1 & buty.	0.97	9.83	42.46	31.81
" " " "	0.97	9.87	42.64	32.15
" " " "	0.97	9.91	42.81	32.20
Enzyme & butyrate	0.95	10.04	43.38	32.54
" "	0.95	10.14	43.82	32.87
" "	0.95	10.89	47.12	35.34
With 118 units B_1	0.95	10.00	43.21	32.41
" " " "	0.95	10.01	43.25	32.44
" " " "	0.95	9.90	41.23	30.93
Enzyme & butyrate	0.99	10.12	39.16	29.39
" "	0.98	10.06	38.96	29.18
" "	0.99	10.15	39.29	29.47
With 24 units B_1	0.98	10.00	38.68	29.08
" " " "	0.98	9.98	37.49	28.84
" " " "	0.98	10.02	38.76	29.11

Table XVIII (continued)

Preparation	cc N/20 NaOH Before	cc N/20 NaOH After	Mgs acid formed	% Hydrol.
Enzyme & butyrate	0.98	10.20	39.56	29.63
" "	0.98	10.25	39.78	29.82
" "	0.98	10.10	38.91	29.20
With 30 units B_1	0.98	9.89	38.19	28.62
" " " "	0.98	9.85	38.02	29.10
" " " "	0.98	10.01	38.72	29.10
Enzyme & butyrate	0.99	10.15	39.29	29.47
" "	0.97	10.20	39.36	29.52
" "	0.[illegible]	10.42	40.56	30.43
" with 60 i.u. B_1	0.98	9.45	36.26	27.20
" " " "	0.97	9.[illegible]	35.38	26.54
" " " "	0.98	8.99	34.23	27.03
Enzyme & butyrate	0.97	10.16	39.42	29.57
" "	0.99	10.25	39.74	29.81
" "	0.98	10.25	39.78	2982
With 100 units B_1	0.99	9.10	34.67	26.00
" " " "	0.97	9.00	34.32	25.74
" " " "	0.98	8.99	34.23	25.68
Controls				
Controlon enzyme	0.90	1.13	(average of three)	
" on ethyl butyrate	0.02	0.02	" " "	
" 6 units B_1	0.02	0.03	" " "	
" 24 " "	0.03	0.03	" " "	
" 100 " "	0.04	0.04	" " "	
Enzyme & 100 " "	0.93	1.16	" " "	
Butyrate & 100 units B_1	0.06	0.06		

Table XIX.

A summary table showing the effect of vitamin B_1 on the activity of pancreatic esterase. Each figure listed represents an average of 21 trials.

Units B_1	Mgs acid formed With B_1	Control*	Diff. in Mgs.	% Hydrolysis With B_1	Contr.	Diff. in % hydrol.
3	36.43	40.24	3.81	27.32	30.19	2.87
6	41.74	43.18	1.44	31.02	32.43	1.41
12	42.78	43.14	0.36	32.05	32.38	0.33
18	42.56	44.77	2.21	32.00	33.59	1.59
24	38.31	39.12	0.81	28.73	29.35	0.62
30	38.31	39.42	1.11	28.73	29.57	0.84
36	38.94	39.22	0.28	28.46	29.42	0.96
48	39.18	39.22	0.04	29.39	29.42	0.04
60	36.62	39.61	2.99	27.49	29.72	2.23
100	34.41	39.55	5.15	25.81	29.73	3.93

* The systems or samples made with enzyme and ethyl butyrate only are here designated as the controls.

Lecithin is an important constituent of the nervous tissue and particularly of the myelin sheaths. In multiple sclerosis the primary lesion consists of a degeneration of the myelin, and in the advanced stages of the disease the myelin is completely destroyed in numerous patches throughout the spinal cord. It was only natural, therefore that lecithin was used as substrate in this investigation.

A one percent solution of lecithin, described in the section on materials and preparationss, was employed as a substrate in these experiments. The experiments were performed in the same manner as those in which olive oil or ethyl butyrate were used as the substrate. However, the amount of the lecithin present was so small that N/100 NaOH was used in these titrations. The control lipase systems were made up as follows: 2 cc of enzyme solution, 2 cc. of lecithin solutin and water to make the total volume 15 cc. To show the effect of the vitamin the desired amount of vitamin solution was sub- for the correct amount of water. Controls were run on each of the constituents as shown in tables XX and XXI where the results are listed. Here it is again evident that the vitamin inhibits the action of the enzyme on the lecithin substrate.

Table XX

The effect of vitamin B_1 on the activity of pancreatic lipase when useing lecithin as a substrate.

Preparation	cc N/100 NaOH Before	cc N/100 NaOH After	Mgs acid formed	% Hydrol.
Lipase system alone. . . .	0.96	7.18	9.38	46.90
Lipase system, 18 i.u. B_1.	90	6.25	7.89	39.45
Control on enzyme.	0.50	0.85		
" " lecithin . . .	0.10	0.10		
" " 18 units B_1. .	0.04	0.04		
" enzyme & B_1.	0.55	0.91		
" lecithin & B_1	0.15	0.16		
Lipase system alone . . .	0.95	7.20	9.31	46.50
Lipase system, 18 i.u. B_1	0.92	6.24	7.78	38.90
Control on enzyme.	0.50	0.87		
" " lecithin. . . .	0.10	0.16		
" " 18 i. u. B_1. .	0.05	0.05		
" enzyme & 18 i. u. B_1	0.56	0.93		
" lecithin & B_1	0.15	0.20		
Lipase system alone. . . .	0.94	6.95	9.04	45.20
Lipase system, 18 i. u. B_1	0.92	6.25	7.90	39.50
Control on enzyme.	0.51	0.85		
" " lecithin . . .	0.10	0.12		
" " 18 i.u. B_1 . .	0.05	0.05		
" enzyme & B_1.	0.55	0.90		
" lecithin "	0.15	0.17		
Lipase system alone. . . .	0.95	7.19	9.39	46.95
Lipase system, 18 i.u. B_1.	0.92	6.26	7.89	35.45
Control on enzyme.	0.51	0.87		
" " lecithin . . .	0.11	0.11		
" " 18 i. u. B_1 . .	0.05	0.05		
" enzyme & B_1	0.55	0.90		
" lecithin & B_1	0.15	0.17		
Lipase system alone. . . .	0.94	7.28	9.57	47.85
Lipase system, 18 i.u. B_1.	0.92	6.80	8.70	43.95
Control on enzyme.	0.50	0.85		
" " lecithin . . .	0.10	0.11		
" " 18 i.u. B_1. . .	0.05	0.05		
" enzyme & B_1.	0.55	0.90		
" lecithin & B_1	0.15	0.16		

Table XXI.

The effect of varying amounts of vitamin B_1 on the activity of pancreatic lipase when lecithin is used as the substrate.

Preparation	cc N/100 NaOH Before	cc N/100 NaOH After	Mgs acid formed	% Hydrol.
Lipase system alone. . . .	0.83	5.62	6.88	34.00
" " "	0.83	5.90	7.33	36.65
" " "	0.85	5.56	6.98	33.60
Average.	0.85	5.69	6.98	34.42
Lipase system & 3 units B_1	0.84	5.50	6.64	33.40*
" " 6 " "	0.82	5.30	6.35	31.75
" " 12 " "	0.83	4.80	5.54	27.70
" " 18 " "	0.85	4.70	5.50	27.50
" " 24 " "	0.0.85	5.35	6.38	31.90
" " 30 " "	0.83	4.90	5.73	28.65
" " 36 " "	0.86	5.00	5.81	29.05
" " 48 " "	0.84	4.65	5.28	26.40
" " 60 " "	0.85	4.45	4.94	24.70
" " 100 " "	0.86	3.55	3.49	17.45
Control on enzyme.	0.61	1.20		
" lecithin	0.10	0.10		
" 6 units B_1. . . .	0.04	0.04		
" 18 " "	0.04	0.04		
" 48 " "	0.06	0.07		
" 100 " " . . .	0.06	0.06		

* All values listed below the star are averages of three triplicate samples.

C. Spinal Cord Studies.

From the results in the preceeding pages it is obvious that the vitamin has a decided inhibitive effect on the activity of pancreatic lipase and esterase. In order to correlate this work with multiple sclerosis and show a similar relation between the vitamin and myelin, spinal cords were used as substrates. Supposing then, that the demyelination which occurs in multiple sclerosis is an enzymatic hydrolysis, does the vitamin inhibit the action of the enzyme as it does in the preceeding experiments and thereby prevent demyelination ?

The spinal cords of rats were used first in these experiments. Three, adult normal rats were chosen for each experiment. After the rats were killed suddenly by a blow on the head their spinal cords were removed by sterile technique. Each cord was placed in a separate 150 cc. Erlenmeyer flask which contained 30 cc of an 0.85% saline solution. The three flasks were then labelled "A", "B", and "C" respectively. To flask "B" was added 30 units (100 gamma) of vitamin B_1. After the flasks stood at room temperature for three hours 4cc of the enzyme solution was added to flasks "A" and "B" and the flasks were shaken gently for the purpose of mixing the contents. Nothing was added to flask "C" which

was kept for a control. Now flask "A" contained the saline solution and 4 cc. of the enzyme solution in addition to the spinal cord; flask "B" contained the spinal cord in physiological saline with 4 cc of the enzyme solution but 100 gamma of the vitamin also, while flask "C" contained only the spinal cord in the 0.85% saline solution. All flasks were placed in the incubator at the same time and incubated for 24 hours at 38 to 40 C. After the incutbation of 24 hours the flasks were removed for observation. The very cloudy and turbid appearence of the solution in flask "A" indicated considerable digestion; in fact the spinal cord in this flask was so macerated that bits of the white matter were floating around in the solution. Flask "B" which was prepared in the same manner as "A" except for the addition of the vitamin, showed only traces of cloudiness or turbidity indicating less digestion than in flask "A". The control flask "C" had remained clear. No putrification could be detected in any of the flasks.

The spinal cords were removed from their respective flasks and fixed in 10% formalin. The solution left in each of the three flasks was titrated with N/20 NaOH, phenolphthalein being used for the indicator. The solution of flask "A" required 2.38 cc. of the NaOH to neutralize it. In contrast to this the solution of

flask "B" required only 1.85 cc of the alkali and the control flask required only 0.05 cc. This experiment was repeated nine time with the spinal cords of rats and similar results were obtained each time. The results of the titrations are shown in table XXII. Plate 1 is an illustration to show the appearence of the contents of the three flasks at the end of the incubatin period. The contents of each flask were transferred to large test tubes so that this photograph could be taken.

The spinal cords were prepared for histological study and stained to demonstrate myelinatinn by the method of Weigert, modified by Pal (110). The samples of the spinal cords were sectioned lengthwise in this case. With this technique the myelin shows up as darkly stained areas and the demyelinated areas as gray or white areas depending upon the extent of the demyelination. This is illustrated in plate 2. This illustration shows that considerable demyelinatin also occurred in cord from flask "B", but the greater amount of demyelination in cord "A" is very striking.

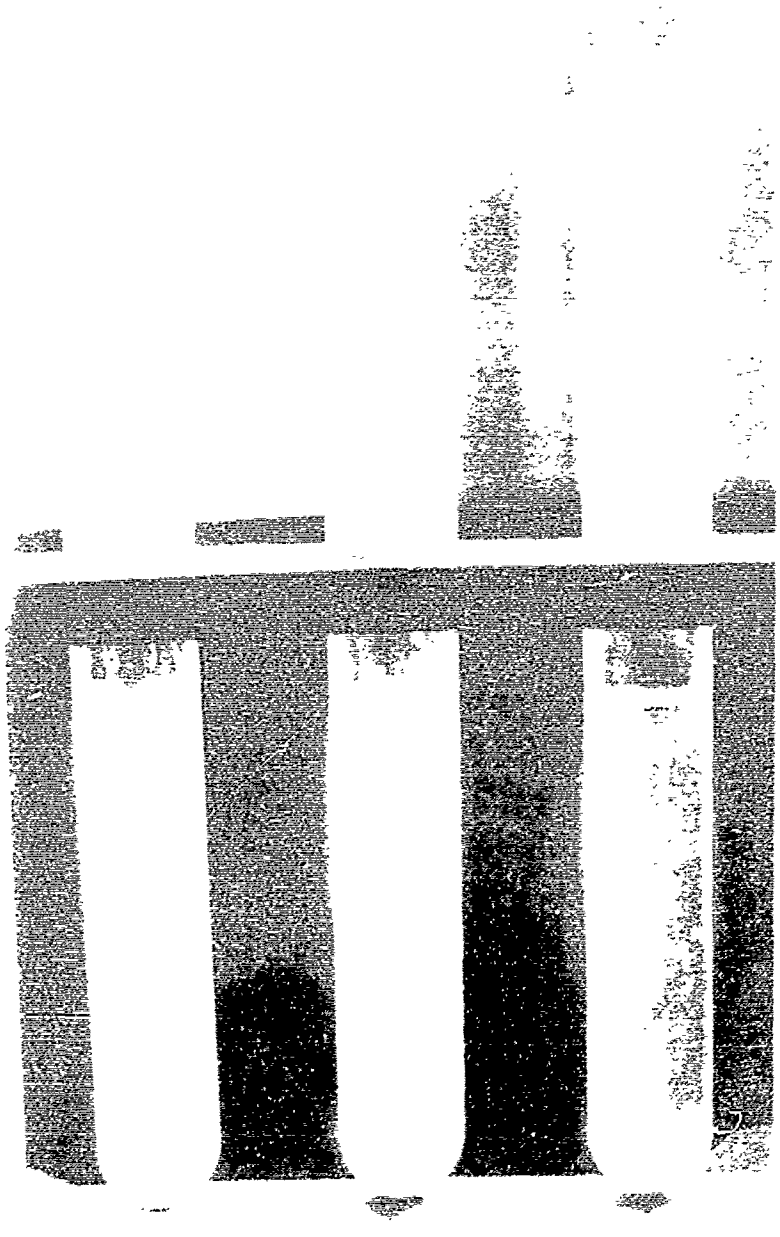
The spinal cords were very soft and difficult to handle at the end of the experiment. Consequently some damage was usually inflicted during the handling. Better results were obtained in this respect with the

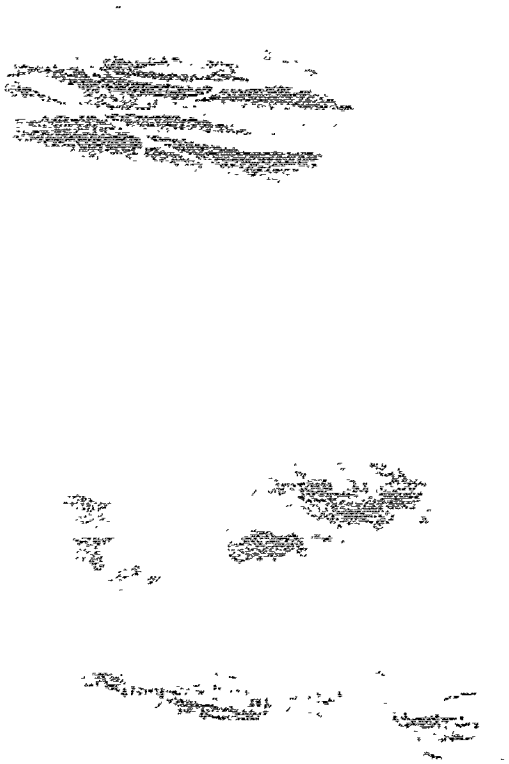
spinal cord of a dog. The spinal cord in this case was obtained from a dog killed by anesthesia. The spinal cord from the upper thoracic region to the lumbar region was used. It was carefully cut into six pieces of equall length and two portions were placed in each of three flasks and treated as described in the previous experiment. The titration values of the respective flasks are given in table XXII. After the experiment the pieces of spinal cord were again fixed in formalin and prepared for histological study. Cross sections of the dog's spinal cord are shown in plate 3. Here the protective influence of the vitamin is very distinct. Sample "B" which was placed in 0.85% saline with vitamin B_1 and enzyme shows little, if any, demyelination. Sample "A" on the other hand, shows very extensive demyelination. A human spinal cord, obtained at autopsy from a patient who died of pneumonia, was also used for this experiment to demonstrate the protective influence of vitamin B_1. Cross sections of the human spinal cord are shown in plate 4. In this case the tissue was stained with Morgan's stain and the demyelinated areas do not show up in such sharp contrast to the myelinated areas. However, a distinct marginal degeneratinn of the myelin can be seen in sample "A" of the illustration.

Table XXII.

The effect of vitamin on demyelination in vitro by pancreatic lipase.

Cord used.	Sample of cord in saline & enzyme	Sample of cord in saline & enzyme & vitamin	Control.
Rat.	2.38	1.85	0.05
"	2.22	0.95	0.04
"	3.04	1.46	0.04
"	2.96	1.42	0.05
"	3.16	1.38	0.04
"	2.87	0.98	0.05
"	2.98	0.98	0.05
"	3.10	1.20	0.05
"	2.68	1.04	0.05
Dog cord . .	6.10	3.10	0.05
Human cord.	15.10	8.30	1.90





IV. Disscussion

Most of the evidence to date points toward the belief that the lesion of multiple sclerosis are caused by a lipolytic process. This investigation bears that out, however, not from the point of view that there is always a demonstrable amount of lipase present in the spinal fluid. No lipase could be demonstrated in the spinal fluid of patients suffering from multiple sclerosis nor in normal spinal fluid. This is in agreement with the work of Sussner (87) Altmann (3) and others. There seems to be ample evidence that there is a lipase in the blood as shown by Brickner (15, 16 and 18), Crandall and Cherry (28) and Weil (96). However, Weil is not so ready to claim that the lipase present in the blood serum of multiple sclerosis patients is an abnormal lipase. It seems as though the lipase of the blood is not ordinarily diffusible into the cerebro-spinal fluid but it is not impossible that some pathological conditions can remove the"lipase barrier" between blood and spinal fluid. Even so, the amount of lipase in the would be small and under normal conditions a natural enzyme of the organism does not attack normal, living tissue. But in the case of multiple sclerosis, where gradual changes have been taking place over long periods

of time, changes which might render the myelin more liable to the action of a lipase, it is easily conceivable that the process of demyelination is a lipolytic hydrolysis. This is all the more reasonable if there is a lipase activating agent present in the cerebrospinal fluid of multiple sclerosis as this investigation shows. Crandall and Cherry and others claim that the demyelination of multiple sclerosis is associated with liver dysfunction. If, in this case, an abnormally large amount of lipase is present in the blood it might diffuse into the spinal fluid and with the presence of the activating agent cause an enzymatic hydrolysis of the myelin. That pancreatic lipase is capable of destroying myelin is shown in plates 2, 3, and 4 and in table XXII. Evidence has also been given by some investigators (74 and 84) that perivascular disorders which give rise to cerebral thrombosis may cause sclerosis. In this case the authors who show that demyelination takes place in areas surrounding a ruptured blood vessel in the brain thereby support the theory that demyelination is a lipolytic process, accomplished by the serum lipase .

One might suggest that the protein content of the spinal fluid used in these experiments produced a more complete emulsification of the olive oil which was used

as the substrate in testing the activating power of the spinal fluid. This objection is easily over ruled by the fact that in all the cases of multiple sclerosis studied the spinal fluid contained a normal or a sub-normal amount of protein. The normal protein content of the spinal fluid is 45 mgs%. In one case of multiple sclerosis the protein content of the spinal fluid was as low as 30 mgs % but the increase in the amount of hydrolysis amounted to 5% more than in the controls. Another case of multiple sclerosis with a nofmal spinal fluid protein content, there was an increase in hydrolysis of 6%. On the other hand it was noted that in the case of other diseases where there was an elevated spinal fluid protein of 100 mgs % or more, the spinal fluid caused an increase in hydrolysis of only 2 to 3 %. When the spinal fluid of multiple sclerosis is added to a lipase system there is an increase of 33.89 mg of oleic acid. In contrast to this the spinal fluid of other diseases caused an increase of 14.73 mgs of oleic acid . In other words there is more than twice the amount of hydrolysis due to the presence of the spinal fluid of multiple sclerosis than that caused by the spinal fluid of other diseases. This is indeed a significant difference when one considers that the limit of error in those titration experiments

amounts to 0.20 cc of N/20 NaOH. In terms of milligrams of oleic acid this would make the experimental error plus or minus 2.82 mgs.

The inhibitive effect of vitamin B_1 on the action of pancreatic lipase and esterase is very striking. Glick has recently demonstrated the inhibition of choline esterase by vitamin B_1 (108) and this work is in harmony with his results. In these experiments it was not possible to maintain an optimum pH for the pancreatic lipase because the vitamin is too unstable in an alkaline solution. The initial pH in these experiments ranged from 6.35 to 7 as determined on the pH electrometer.

In the experiments on demyelination in vitro, the smallest amount of vitamin necessary to prevent the demyelination was 30 international units (100 gamma). This is a much higher concentration than is found in the spinal fluid where it is present to the extent of one to two gamma per 100 cc. But it must be remembered that the concentration of the enzyme used in these experiments was also much higher than could appear in the spinal fluid during diseases such as multiple sclerosis. The objection might be raised that the normal concentration of the vitamin in the spinal fluid is much too low to prevent demyelination according to this study. But

it must be remembered that the concentratin of the myelolytic substances in the spinal fluid is very small and the process of demyelination as it occurs in multiple sclerosis is an exceedingly slow process; one which takes place over a period of three or more years. If an individual has a deficient supply of vitamin B_1 during this time it is possible for the process of demyelination to take place.

Space does not permit going into detail on the therapeutic use of the vitamins but the literature abounds with publications on the necessity of the vitamin B complex for the normal health and functioning of the nervous system. No authors, however, have presented facts which reveal the manner in which vitamin B_1 prevents the symptoms caused by demyelination. It is clear from the results of this investigation that the vitamin prevents demyelination by inhibiting the action of the myelolytic substance present in the nervous system. Another striking fact is the similarity between multiple sclerosis and pernicious anemia. The necessity of a proper vitamin B supply is well known.

The alkalinity of the spinal fluid is a factor which should not be over looked. Katzenelbogen found in the case of multiple sclerosis an increase in the alkalinity

of the spinal fluid. This would be conducive to the destruction of the vitamin present in the spinal fluid. A study of experimental demyelination under different conditions of alkalinity or acidity might reveal some interesting facts on this subject. In fact this investigation provokes a number of interesting questions to investigate and the subject of this thesis is by no means a closed issue.

V. Summary and Conclusions.

In this work a study was made of the spinal fluid of multiple sclerosis. Because of the importance of vitamin B_1 in the central nervous system the effect of the vitamin on the activity of pancreatic lipase and esterase was studied. The results of the experiments led to the following conclusions:

1- There is present in the spinal fluid of multiple sclerosis a substance which activates pancreatic lipase.

2- Vitamin B_1 in amounts as low as 10 gamma inhibits the actinn of pancreatic lipase and esterase. This was demonstrated by using olive oil, ethyl butyrate and lecithin respectively as substrates.

3- Vitamin B_1 prevents demyelination of spinal cords in vitro by pancreatic lipase.

Finis

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