PHYSIOLOGICAL STUDIES OF ACUTE THIAMIN DEFICIENCY IN THE CAT

By

Guy M. Everett

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
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INTRODUCTION AND HISTORICAL BACKGROUND

The study of the vitamins as a branch of experimental science may be considered to have stemmed largely from the classical work of Sir F. G. Hopkins (1912) who in a series of carefully controlled experiments showed that diets of purified fats, carbohydrates, proteins and minerals were inadequate to maintain normal health and growth in young rats. The addition of "astonishingly" small quantities of milk resulted in a return of growth. From this finding it was apparent that certain substances in minute quantities were essential for adequate nutrition. Hopkins emphasized the physiological importance of these nutritional constituents and proposed the term "accessory factors" for them. In the same year Funk (1912) reviewed the literature on nutritional diseases and proposed the term "vitamines" for these special nutritional substances.

Following the recognition of the vitamins there was a period of separation and fractionation of these substances into various groups according to their physical and chemical properties. At this time such terms as "Fat-Soluble Fraction", Water-Soluble Fraction", "Heat stable" and"Heat labile" were used to describe the fraction being studied. From such work it soon became apparent that there were many vitamins of diverse chemical properties and playing diverse rôles in the body economy.

The most recent major advance in the field has been the isolation and chemical synthesis of these substances. During the past ten years all of the important known vitamins have been obtained in crystalline form. With these materials at hand it has become possible

to determine more precisely what part each of these substances plays in biochemical and physiological processes.

The development of our knowledge of the vitamin B-complex has a history similar to that of other vitamins. It was first designated as "Water-Soluble-B" by McCollum and Davis (1915). Later it was separated into a heat stable and a heat labile fraction. The latter fraction was shown to contain the anti-beriberi factor. The separation and determination of the chemical structure of the B-complex vitamins has been a difficult task because of its many members. At the present time at least seven compounds have been recognized as true vitamins belonging to the B-complex; they are: Thiamin, Riboflavin, Pyridoxin, Pantothenic acid, Nicotinic acid, Inositol and p-Amino-benzoic acid. Of these Thiamin has received the greatest attention. Since its synthesis by Williams and Cline (1936), hundreds of papers have appeared in regard to its possible biochemical and physiological significance. As in the case of other vitamins, thiamin in excess has little or no effect on normal animals. Its function must be largely determined through the study of animals depleted of thiamin. The most commonly observed signs in thiamin deficient animals are anorexia and vomiting in the early stage with cardiac and nervous disorders appearing later. The administration of the vitamin brings about a rapid recovery. Clinical studies have shown that thiamin deficiency is associated with beriberi and nutritional polyneuritis.

The importance of thiamin in nutrition is now well recognized, the work of Peters (1936) and others having shown that this vitamin is essential for the proper oxidation of carbohydrate intermediates, particularly pyruvic acid. Yet the mechanism of its physiological

action still remains obscure. The experiments herein reported were undertaken with the idea of producing and studying specific thiamin deficiency in the cat in the hope of throwing more light on this question. The work is divided into four parts. The first is concerned with production and experimental verification of specific thiamin deficiency. The second deals with biochemical studies on blood and tissues. The third is devoted to work on electrocardiography. The fourth outlines the observations and analysis of the neurological disorders which occur in acute thiamin deficient cats.

DEVELOPMENT OF SPECIFIC THIAMIN DEFICIENCY IN THE CAT, A CRITIQUE ON DIET

One of the weakest points in much of the physiological and biochemical work on thiamin deficient animals has been the inadequate control of vitamin supplements, particularly other members of the B-complex group. In the older studies the diets have been fortified with autoclaved brewer's yeast which supposedly contains sufficient quantities of the B-complex vitamins other than thiamin. However, the possible destruction of other vitamins in the autoclaving process, particularly thermolabile pyridoxine and pantothenic acid, cannot be excluded. A further criticism arises from the anorexia which gradually develops in thiamin deficient animals and greatly increases the difficulty of supplying adequate vitamin intake if only the addition of autoclaved yeast is relied upon. Forced feeding is not tenable as it usually produces vomiting in such animals. From such considerations the possibility of multiple deficiencies arising with such dietary regimes cannot be easily dismissed. To avoid these criticisms the

the more recent experiments have used supplements of pure crystalline vitamins, administered either orally or parenterally, independent of the diet.

Materials and Dietary Régime

The studies in this and subsequent sections were made on a group of 85 cats. These animals were chosen from a large stock colony. Only adult males in good health and nutritional state were selected. Their weights ranged from 2.75 to 4.5 kilograms. The animals were kept in individual cages in a steam heated room at an average temperature of approximately 25° C. The cages were cleaned each morning and fresh water was placed in the drinking cups. The cats were weighed and examined once a day, usually in the late afternoon just before feeding.

The chief component of the diet consisted of a canned dog food[1] containing ground rabbit meat and bone, wheat, rolled oats, soy bean flour and carrots. Charcoal and codliver oil were added. The analysis of this food as given by the manufacturer is:

Crude protein	(min.)	12%
Crude fat	(min.)	3%
Crude fiber	(max.)	2%

Thiamin was destroyed by autoclaving the sealed cans at 15 pounds pressure for five hours. Under the same conditions brewer's yeast was also autoclaved in open glass dishes. In addition a solution containing pyridoxin hydrochloride, riboflavin and calcium pantothenate[2] was prepared fresh every week and kept in the refrigerator at 5° C.

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[1] Wild-Life Rabbit Meat Dog Food prepared by General Laboratories, Inc., Des Moines, Iowa.

[2] We wish to thank Dr. D. F. Robertson and Mr. L. J. Ruland of the Merck Company for generous supplies of crystalline vitamins.

The completed diet was mixed fresh each day and has the following composition:

(Amounts per cat per day)

Autoclaved canned dog food	225 grams
Autoclaved brewer's yeast	0.5 gram
Pyridoxine HCl	0.4 mg.
Calcium pantothenate	1.0 mg.
Riboflavin	0.5 mg.

Once a week 5 cc. of cod liver oil were added.

Since the vitamin requirements of the cat have not been determined the quantities of crystalline vitamin supplements are arbitrary and represent approximately double the requirements determined for the dog by Schafer, et al (1941).

Once a week the animals on diet were injected intraperitoneally with the same quantities of crystalline vitamins as given in the food. Also, 5 mg. of alpha tochopherol were given orally or injected.

As anorexia developed the amount of canned food was reduced proportionately while the yeast and other supplements remained unchanged.

EXPERIMENTAL EVIDENCE FOR SPECIFIC THIAMIN DEFICIENCY

In Figure 1, Curve A,is shown the typical gradual weight loss which occurs in cats on thiamin deficient diet. The loss per day was greatest in the second week and then became fairly constant, a decrease of 30 to 50 grams a day being found in most cases. The total weight loss in 75 per cent of the animals studied was of the order of 20 to 30 per cent of the original body weight. About 10 per cent of the cats showed decreases of only 10 per cent. In another 15 per cent weight losses of 35 per cent or more were found. The only other obvious signs of deficiency during this first period, lasting

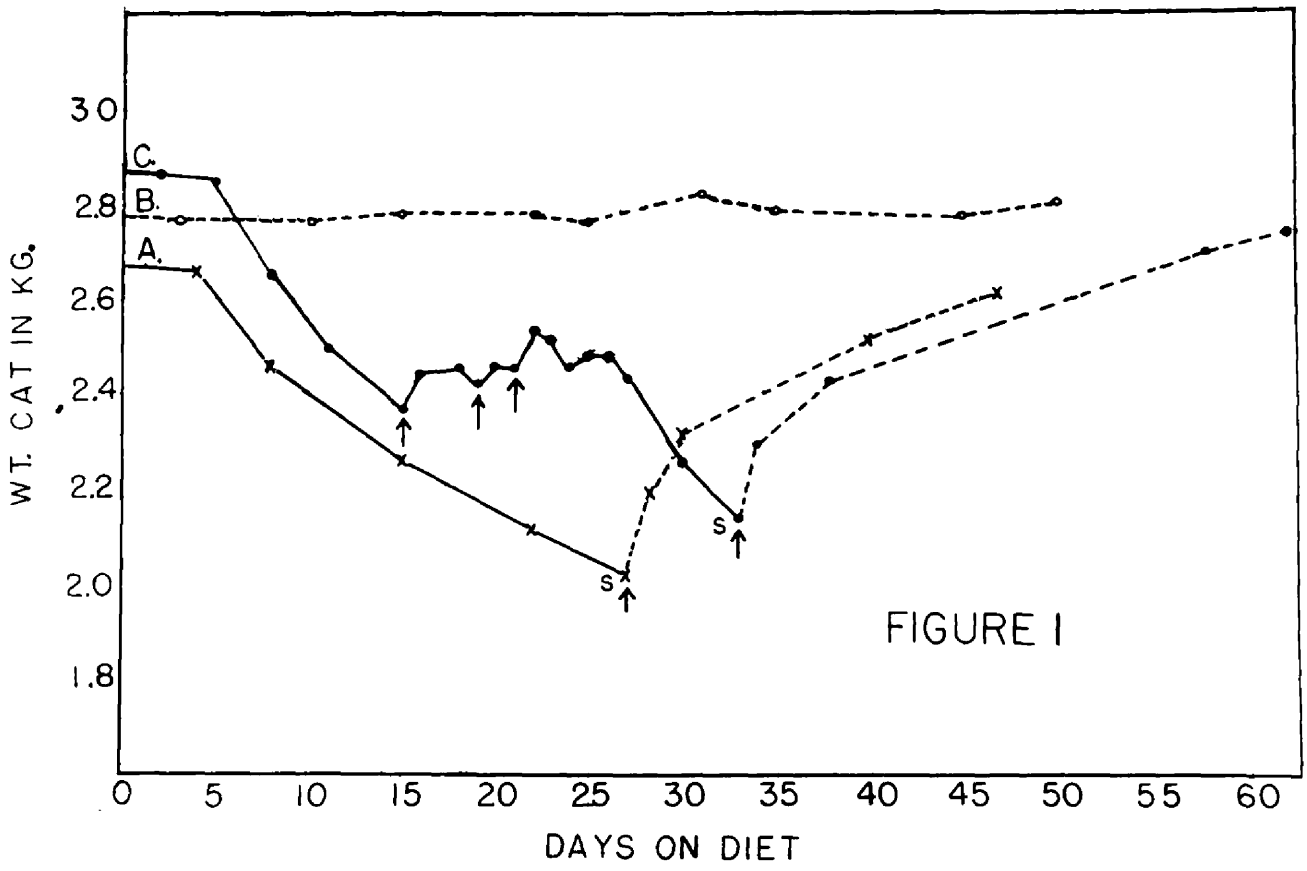

Figure 1. Weight curves of cats. Solid line, thiamin deficient diet. Broken line, diet plus 0.5 mg. of thiamin per day.

Curve A. Cat No. 10 on diet. Convulsive seizures appeared at S.
Curve B. Cat No. 3 on diet plus thiamin.
Curve C. Cat No. 7 on diet. Single injections of 0.5 mg. thiamin at arrows.

about three weeks, were anorexia and vomiting. Slight ataxia was observed in the hind limbs during the third and fourth week, inaugurating the onset of the critical stage characterized by abnormal posture, ataxia, asynergia, dysmetria and short tonic convulsive seizures. The critical stage usually appeared in 25 to 35 days from the beginning of the diet. Prostration and death followed a few days later.

To check the adequacy of the diet a series of cats were placed on the thiamin deficient régime with the addition of 0.5 mg. of thiamin per day. A typical case is shown in Figure 1, Curve B. In every instance the animal maintained its original body weight or gained, and showed no neurological disorders after six weeks to three months on diet. Thus the diet was complete except for thiamin if judged by its ability to maintain the animals in good health.

The anorexia and concomitant weight loss were clearly the result of thiamin deficiency as shown by the following experiment. A group of animals on diet, allowed to develop anorexia, were injected with 0.5 mg. of thiamin intramuscularly. Appetite returned within an hour and with no further thiamin the animals ate normally for a few days and then gradually developed anorexia again. A case typical of the whole series is shown in Figure 1, Curve C. Each time thiamin was injected the animal's weight increased due to the increased food consumption and probably better utilization, after which the weight curve levels off and falls with the decline of appetite. Essentially similar results were obtained when the thiamin was given orally.

The convulsive seizures and other neurological disorders which appeared in the critical stage were also clearly the result of thiamin deficiency. In 40 convulsive cats the injection of 1.0 mg. of thiamin

brought about a rapid dramatic recovery. Convulsions disappeared within an hour and the following day the animal was essentially normal. The injection of calcium pantothenate, pyridoxine HCl, riboflavin, alpha tochopherol, choline, nicotinic acid, either alone or in combinations, into critical stage animals brought about no improvement, the animal dying if thiamin was not given. The administration of glucose likewise showed no curative action. Particular attention was given in these studies to pyridoxine and pantothenic acid because a deficiency of these vitamins may give rise to disorders somewhat similar to those found in thiamin deficiency. Wintrobe, Stein, Miller, Folis, Najjar and Humphreys (1942) have reported epileptiform seizures in pyridoxine deficient pigs and also nervous disorders due to pantothenic acid deficiency. In the same animal on thiamin deficient diet no neurological signs other than anorexia and vomiting were observed (Wintrobe, et al., 1942). In the cat, however, the situation was clearly different for the dietary regime used, and the experiments reported here seem to limit the observed effects strictly to a specific lack of thiamin. Since the course of thiamin deficiency in the present studies developed rapidly and ended with death in a period of 30 to 40 days, it may be properly considered an acute deficiency in distinction to the chronic type studied in some animals where symptoms appear after 60 to 200 days on diet.

BIOCHEMICAL STUDIES ON ACUTE THIAMIN DEFICIENT CATS

Many attempts have been made to find a specific biochemical change which would reflect or perhaps be the causative agent of the disorders observed in thiamin deficiency. The results for the most part have

been inconclusive. A review of the literature by Williams and Spies (1939) led them to conclude: "The biochemical upset associated with lack of thiamin is widespread and profound but intangible as to precise cause. The evidence is confused and often contradictory."

The present work reports studies on the blood chemistry and tissue metabolism of acute thiamin deficient cats.

Blood Studies

Large cats weighing 3.5 to 4.0 kilograms were used in these studies. The animals were injected with 0.04 gram of nembutal per kilogram prior to withdrawing 10 to 15 cc. of blood from the jugular vein. In order to check on the possible changes due to the barbiturate three cats were killed by a blow on the head and blood was taken from jugular or vena cava. Data on blood sugar and electrolytes obtained from the group did not differ significantly from the values found for nembutalized animals. All filtrates and serum were prepared immediately after taking the blood.

Serum Potassium: Increases in the size of the adrenal glands of thiamin deficient animals have been observed by a number of investigators. Sure (1938) has reported a fifty per cent enlargement of the adrenals in deficient rats when compared to paired feeding controls. In acute thiamin deficient dogs, Goodsell (1941a) found a true hypertrophy of the adrenal cortex, using normal animals for comparison. Further, Goodsell (1941b), using the Bitterling bioassay method, found an increased amount of steroid compounds in extracts of the hypertrophied cortex. This suggests that in the thiamin deficient animal there may be disturbances in the secretion of adrenal cortical hormones. The extensive work of Zwemer and Truszkowski (1937) on cats has shown

the importance of the adrenal cortical hormone in the control of normal distribution of potassium. Adrenalectomy results in abnormally high serum potassium values, while injection of adrenal cortical hormone into normal animals results in lowering the serum potassium. Thus the serum potassium reflects the functional condition of the adrenal cortex. This approach has been adopted in the present study of acute thiamin deficient cats.

The serum potassium of one cc. samples was determined by the modified platinic chloride method as given by Consolazio and Talbott (1938). In all instances duplicate determinations were made on each sample. The method under optimum conditions may have an accuracy of $\pm$ 2 per cent.

From a consideration of the individual cases as presented in Table I it is apparent that no significant change occurs in serum potassium during the development of thiamin deficiency, including the critical stage. In only one instance (Cat No. 35) does there appear to be a significant change, in the direction of a decreased potassium. When this value (16.4 mg. %) is compared with the lowest normal value (15.0 mg. %) it does not appear to be abnormally low. A summary of the data is included in Table II. A comparison of the normal values obtained in this study with those reported by Zwemer and Truszkowski (1937) shows an excellent agreement. In 19 cats they found a maximum value of 23.1 mg. %, a minimum of 14 mg. %, with an over-all average of 19.3 mg. %. In animals 12 days after adrenalectomy they observed values as high as 46.1 mg. %. By injecting adrenal cortical extract into normal cats they could reduce the serum potassium by 20 per cent.

TABLE I

Serum Potassium in Normal and Thiamin Deficient Animals

Values in Mg. Per Cent

Cat No.	Normal	Thiamin Deficient Diet		
		2nd week	3rd week	Critical stage
32	20.7	--	--	20.9
40	22.0	--	--	20.8
30	18.4	18.3	--	--
34	15.0	16.0	--	--
31	19.3	20.2	--	--
35	20.4	--	20.2	16.4
37	18.5	--	19.1	18.6
41	22.5	--	--	21.5
52	--	--	--	23.8
38	17.4	--	18.1*	--

*Normal diet, restricted feeding.
Weight loss 28%.

TABLE II

Summary of Blood Studies on Acute Thiamin Deficient Cats

		Normal	Thiamin Deficient Diet			Paired feeding*
			Second week	Third week	Critical stage	
Serum K (Mg. %)	Maximum	28.2	20.2	20.2	23.8	
	Minimum	15.0	16.0	19.1	16.4	
	Average	21.3	18.2	19.6	20.3	18.1
	No. cats	27	3	2	6	1
Serum Cl (Mg. %)	Maximum	712			730	
	Minimum	600			665	
	Average	674			688	704
	No. cats	8			7	1
Serum glucose (Mg. %)	Maximum	150	133	120	138	
	Minimum	87	90	92	79	
	Average	138	124	116	118	96
	No. cats	10	5	4	6	1
Hematocrit	Maximum	39			39	
	Minimum	26			28	
	Average	31			32	34
	No. cats	11			11	1

* Cat on restricted diet, weight loss 28%.

In the light of their experiments the slight changes found in the present study may be considered to represent normal variations. The fact that serum potassium levels are normal throughout the development of thiamin deficiency in the cat may be considered as strong evidence that the functional integrity of the adrenal cortex is maintained.

Serum Chloride. In conjunction with the work on serum potassium, studies on serum chloride were made. They were determined by the method of Schales and Schales (1941), using diphenylcarbazone as indicator. The data obtained are summarized in Table II. A comparison of the normal values with those found in the critical stage shows no significant change in chloride occurs during thiamin deficiency.

Blood Sugar. The importance of thiamin in intermediate carbohydrate metabolism is well established (Peters, 1929). However, its possible function in determining blood sugar level remains somewhat vague. Sure and Smith (1932) reported a decrease of blood sugar in thiamin deficient rats. Lewinson (1937) found a lowered blood sugar in the early stages of deficiency in the dog with a rise later. In the pigeon hyperglycemia persists throughout the period of vitamin depletion (Lewinson, 1937). If these findings are correct, widely diverse responses in different species are indicated. However, the changes are in all cases too small to be considered the direct cause of the nervous disorders observed.

In the present study serum glucose was determined on cadmium sulphate filtrates. Glucose was estimated by the colorimetric method of Boettiger (1943). It is based on the formation of a blue complex when a solution of diphenylamine in glacial acetic acid is heated in

the presence of glucose. The unknowns were compared against standards using an Evelyn photoelectric colorimeter. As used in these experiments the method was accurate to within ±10 mg. %.

The maximum, minimum and average values of serum glucose obtained on cats in various stages of deficiency are shown in Table II. The serum glucose tends to decrease during the development of thiamin deficiency. However, the change is relatively small and of doubtful significance in the light of the wide variation of normal values in different cats and the lowered glucose found in paired feeding animals.

Hematocrit. The two cc. samples of blood to be used for hematocrit determinations were placed in small bottles containing 8 mg. of ammonium oxalate and 12 mg. of potassium oxalate to prevent clotting. The clinical hematocrit tubes were then filled and centrifuged for 30 minutes at 1200 revolutions per minute, after which the level of the cells was read.

Hematocrit values were determined on 11 normal cats and on 11 animals in the critical stage. From the data summarized in Table II it is clear that thiamin deficiency has little effect on the relative proportions of erythrocytes and plasma since the hematocrit does not change significantly.

From these studies on blood constituents in the acute thiamin deficient cat it is clear that no striking changes occur in serum electrolytes, serum sugar or hematocrit. Apparently the physiological mechanisms involved in maintaining these materials at normal levels are not markedly affected by a deficiency of thiamin.

Studies of the Catatorulin Effect

It has been shown by Peters (1938) that minced brain tissue of thiamin deficient pigeons has a lower oxygen consumption in the presence of pyruvate than normal. Upon the addition of minute amounts of thiamin to the deficient tissue the oxygen consumption increases (catatorulin effect), while in normal tissue thiamin has no effect. The reduced ability of brain tissue to oxidize pyruvic acid due to a depletion of thiamin is spoken of as the "biochemical lesion" of thiamin deficiency (Peters, 1936). A critical examination of data on catatorulin effects reveals that large consistent increases are demonstrable only in chronic deficient pigeons. The changes observed in the rat (O'Brien and Peters, 1935) are so small as to be of doubtful significance.

An attempt was made to demonstrate the catatorulin effect in the cerebral cortex of three thiamin deficient cats (convulsive stage). The animals were killed by a blow at the base of the skull and the brain tissue was removed immediately thereafter. Weighed portions of cortex were minced and suspended in phosphate buffered Ringer-Locke, pH 7.5. Oxygen consumption of the tissue was determined using Warburg manometers. The details of the method outlined by Peters (1938) were followed precisely. In the ten determinations made there was no increase in oxygen consumption upon the addition of thiamin. This negative result indicates the absence of a metabolic lesion in the cortex. Such a conclusion is given support by the normal cortical function observed during thiamin deficiency. Perhaps demonstrable metabolic changes occur in the brain stem since this appears to be the central

locus of the nervous disorders.

Bisulfite Binding Substances

As a result of the incomplete oxidations of pyruvic acid and associated carbohydrate intermediates, increased quantities of these substances are found in the tissues and body fluids of thiamin deficient animals. Kinnersley and Peters (1929) reported large amounts of lactic acid in the brain tissue of avitaminotic pigeons. Banerji and Harris (1939) found that the bisulfite binding substances (pyruvic acid, lactic acid, etc.) in urine increased progressively during the development of thiamin deficiency in the rat. Abnormally large amounts of bisulfite binding substances were found in the blood of thiamin deficient rats and pigeons (Thompson and Johnson, 1935).

As a further check on possible metabolic disturbances in the acute thiamin deficient cat, determinations of blood and urine bisulfite binding substances have been made. The method outlined by Banerji and Harris (1939) was used. No consistent change during the development of thiamin deficiency was found in the bisulfite binding substances of the urine. Wide variations from day to day occurred in both normal and deficient cats. From 6 to 38 mg. of bisulfite binding substances were excreted per day.

Blood bisulfite binding substances were determined on trichloracetic acid filtrates. In five determinations no bisulfite binding substances could be demonstrated in the blood.

From these preliminary experiments it seems unlikely that any profound change in carbohydrate metabolism occurs in acute thiamin deficient cats.

ELECTROCARDIOGRAPHIC STUDIES ON THIAMIN DEFICIENT CATS

Introduction

Heart pathology and dysfunction have long been associated with beriberi in human beings. Clinical studies by Weiss and Wilkins (1937) on beriberi patients showed tachycardia and T wave changes in the electrocardiogram. Animal experiments have given somewhat diverse results in different species. Carter and Drury (1929) reported bradycardia and heart block in rice fed pigeons. A study of thiamin deficient rats by Birch and Harris (1934) revealed marked bradycardia appearing after three weeks on diet. For the same species Weiss, Haynes and Zoll (1938) found changes in the size and form of the QRS complex and T wave. Tachycardia was found in some of the chronic thiamin deficient dogs studied by Swank, Porter and Yeomans (1941) and changes in the T wave were noted. They also observed circulatory collapse in some animals and autopsies revealed enlarged edematous hearts.

Experimental Results

Electrocardiographic studies have been made on 25 cats, records being obtained on animals in all stages of thiamin deficiency. A consideration of heart rates revealed rather wide variations (100 to 200 beats per minute) in both normal and deficient cats. A number of such cases showed no consistent trend in either the direction of a bradycardia or a tachycardia during the development of thiamin deficiency. However, in these instances where sinus irregularities occur the overall rate was 110 per minute in the critical stage, and the day following thiamin injection it was 143. For cat No. 61 (Fig. 2, C and D) the rate in the critical stage was approximately 100, and

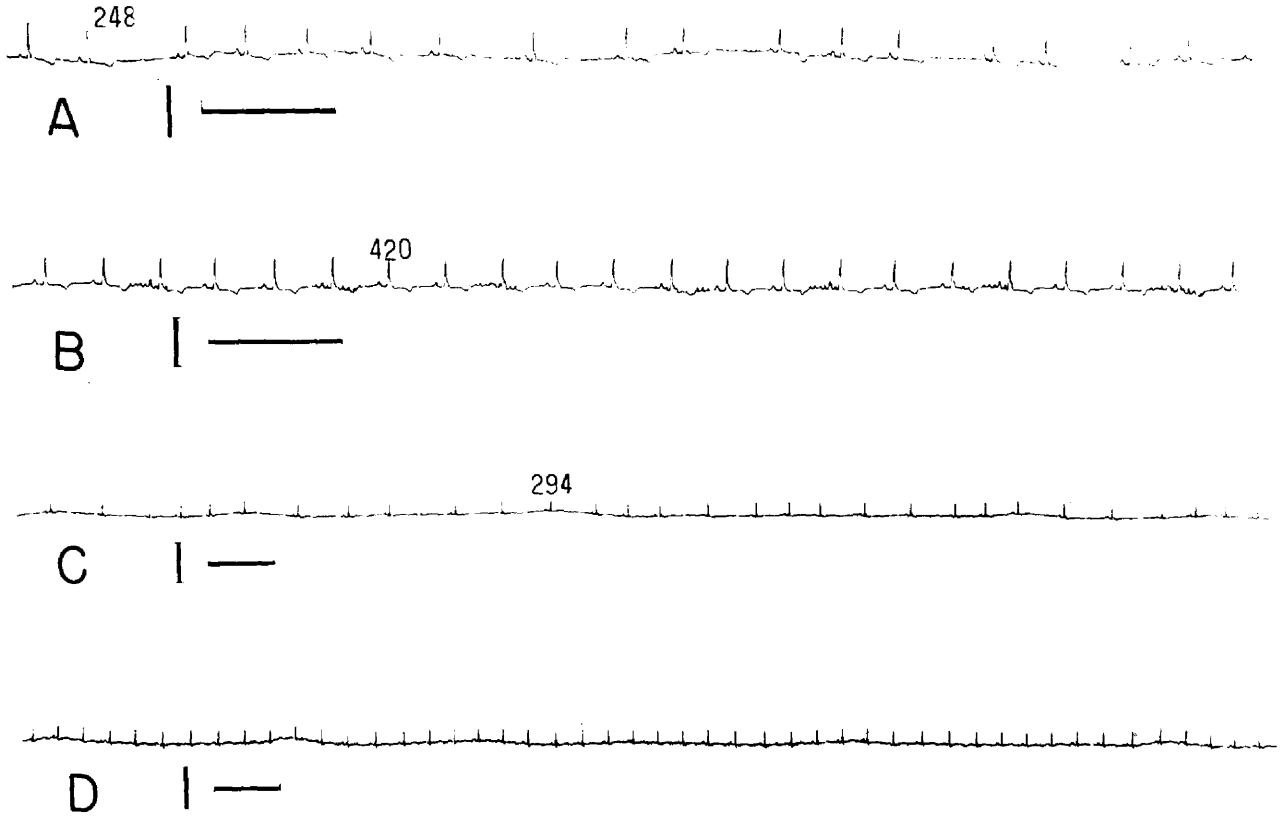

FIGURE 2

Figure 2. Electrocardiograms showing sinus irregularity in thiamin deficient cats. Vertical dashes, 1 millivolt. Horizontal dashes, 1 second.

A. Cat #40 EKG Lead III, 3/23/43. Critical stage. Resting sinus irregularity.
B. Cat #40 EKG Lead III, 3/23/43. 24 hours after one mg. thiamin intramuscularly. Regular sinus beat.
C. Cat #61,EKG Lead III, 3/22/43. Critical stage. Resting sinus irregularity.
D. Cat #61 EKG Lead II, 3/23/43. 24 hours after one mg. thiamin intramuscularly. Regular sinus beat.

the day after thiamin it had risen to 151.

With regard to the wave size and form of the electrocardiogram, it was found that striking changes may occur due to slight differences in the position of the cat or of the electrodes on the paws. Therefore conclusions based on variations in the size and form of the QRS complex or T waves do not seem justified. Further, it is doubtful if the relatively slight changes reported by Weiss, et al.(1938) for the rat, and by Swank, et al. (1941), for the dog are significant.

Twelve cats on diet, 7 of which were in the critical stage, have shown irregularities in heart rate due to discontinuous sinus arrest. Records of two such cases are shown in Fig. 2, A and C. If these be compared with normal records of the same animals a day after thiamin was given (Fig. 1, B and D), it is seen that the fast beats during the deficiency are approximately of normal duration, while the delayed beats are almost twice as long. These irregularities were not correlated with respiratory rate. It appears as if the pacemaker gave rise to a number of beats at the normal rate and then produced a series of long delayed beats with a subsequent return to the normal rate of discharge, the cycle then repeating itself. No irregularities have been observed in the records of 20 normal or recovered animals.

Electrocardiograms taken during and immediately after convulsive seizures have revealed escaped ventricular beats. Such a record is shown in Fig. 3, B to E. Immediately following the seizure the heart rate is increased. Five seconds later sinus arrest suddenly appears and a series of escaped ventricular beats (of right bundle branch origin) occur. This is followed by a true bradycardia and finally a return to the typical irregularities previously discussed.

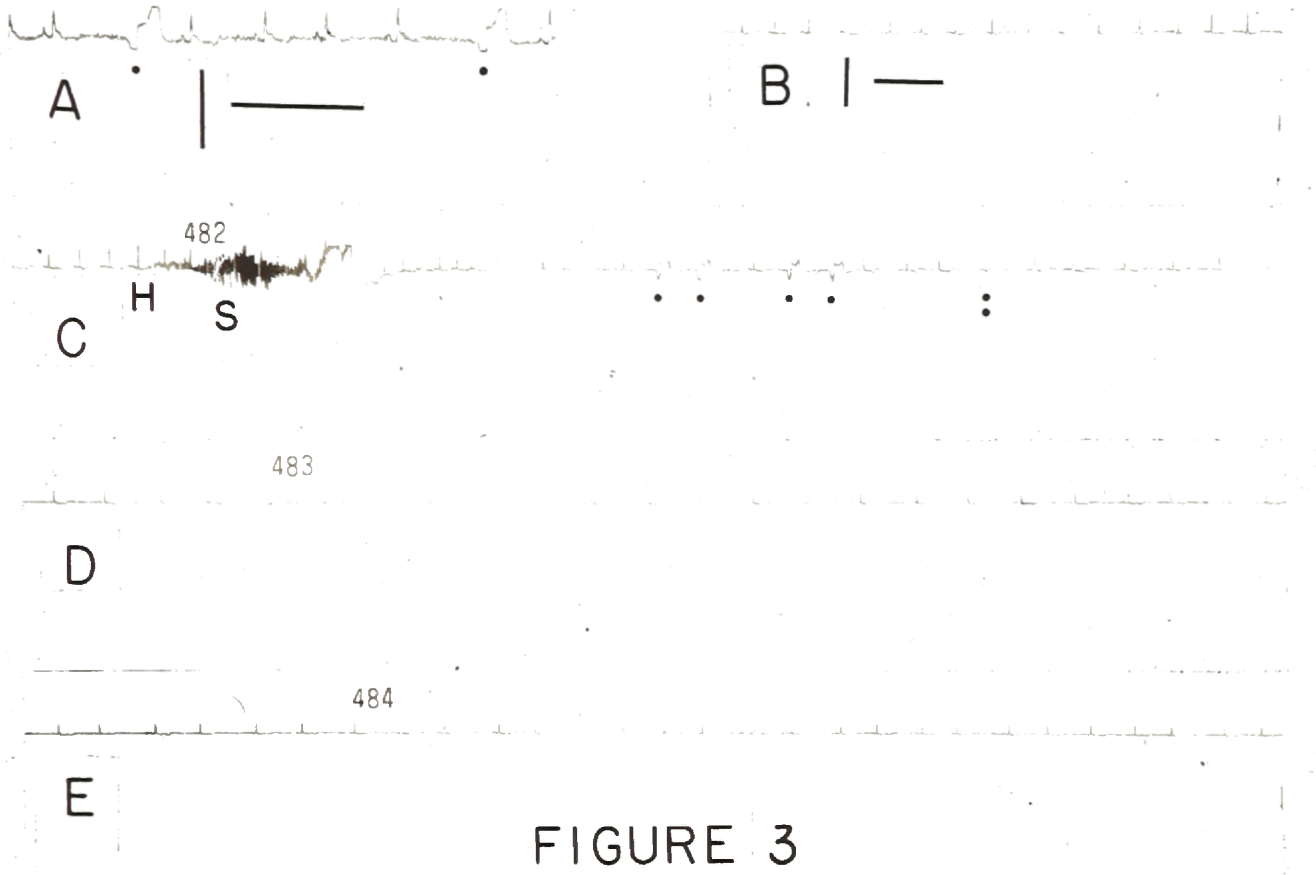

FIGURE 3

Figure 3. Electrocardiogram showing ventricular escaped beats following convulsive seizures. Vertical dashes, 1 millivolt. Horizontal dashes, 1 second.

A. Ventricular escaped beats beginning 5 seconds following convulsion precipitated by handling.
B, C, D, E. Continuous record.
B. Animal resting
C. Handled at H, short convulsive seizure appearing at S. Note initial cardiac acceleration followed by sinus arrest. Ventricular escaped beats are indicated by a dot. (New focus at semicolon dots.)
D. Period of true bradycardia.
E. Recovery toward initial rate with sinus irregularity persisting.

Two out of three thiamin deficient animals on which studies of cardiovascular reflexes were made showed irregularities of heart rate in blood pressure recordings. Autopsies of animals in the terminal stages of deficiency revealed no gross cardiac damage or enlargement.

In order to determine whether the sinus arrest results from increased vagal tone or from changes in the sinus node itself, one animal, showing heart irregularities, was atropinized. The dosage was sufficient to completely paralyze the vagi. Another record was then taken. The heart rate showed a two-fold increase and all signs of heart irregularity due to sinus arrest had disappeared. Thus the sinus arrest must be of vagal origin.

Discussion

From these findings it is concluded that the most prominent sign of cardiac disorder in acute thiamin deficient cats is sinus arrest of vagal origin producing arrhythmia and a type of bradycardia. The bradycardia observed in thiamin deficient pigeons is also of vagal origin according to Carter and Drury (1929). This is not the case, however, in the rat since Drury, Harris and Maudsley (1930) found no increase in rate upon atropinization or cutting the vagi; in this case a sinus origin is indicated.

OBSERVATIONS ON THE BEHAVIOR AND NEUROPHYSIOLOGY OF ACUTE THIAMIN DEFICIENT CATS

Introduction

The nervous manifestations of thiamin deficiency may be divided into acute reversible functional disorders and chronic degenerative lesions. The latter group have received the most attention with some attempt to correlate the nerve tissue damage with the functional disorders observed. Swank (1940) studied the degenerative changes which occur in the peripheral nerves of chronic thiamin deficient pigeons. The extent of the lesion paralleled the severity of paralysis observed at the time the bird was killed. From a detailed histological examination of the peripheral and central nervous system of chronic deficient rats Prickett (1934) found no abnormal changes in peripheral nerves, but observed lesions in the medulla and pons. No paralysis was seen in these rats, but marked ataxia and disturbances of posture were present. At best such studies can tell little about the reversible functional disorders where actual tissue damage is ruled out by the rapid recovery when thiamin is administered. Brief descriptions of the actue signs of thiamin deficiency have been reported for a number of species. In all cases anorexia is the first sign of deficiency. After 3 to 4 weeks on diet ataxia and convulsions suddenly appear in the rat (Prickett, 1934), the cat (Odom and McEachern, 1942), and the pigeon (Swank, 1940). The dog shows ataxia and spasticity of the extremities but does not exhibit convulsive behavior (Swank, Porter and Yeomans, 1941). In contrast to these findings are those of Wintrobe, Stein, Miller, Follis, Najjar and Humphreys (1942) who found no nervous disorders other than anorexia and vomiting in either acute

or chronic thiamin deficient pigs. Apparently the only work which goes beyond a description of general behavior in thiamin deficiency is that of Church (1935), who studied reflex activities in deficient rats and found changes which indicated functional disorders in the brain stem.

The present paper reports a similar but more complete study on the behavior and reflex activities of acute thiamin deficient cats.

General Behavior

In presenting a detailed description of the course of thiamin deficiency in the cat it must be borne in mind that slight variations exist when comparing one animal with another. The description which follows is a composite of the salient features which were most often observed in the series of 50 cats studied. The course of thiamin deficiency in the cat may be considered to occur in three stages.

1. Induction Stage. The first change in the behavior of the thiamin deficient cat is a gradual loss of appetite which usually begins in the second week on diet, with complete anorexia by the fourth week. Vomiting may also occur during this period. Other than the decline in food consumption the general behavior of the animal is normal. It shows interest in food and salivates, but little or nothing is actually eaten. Walking is normal until the end of the third or fourth week, when a slight ataxia is often present in the hind limbs. Within a day the animal passes from this period of essentially normal behavior into the critical stage.

2. Critical Stage. This is characterized by a host of nervous disorders. Abnormal posture, ataxia and pupil dilatation are constant features, with short tonic convulsive seizures in 80 per cent

of the cases. During this period the cat moves with great difficulty, showing marked ataxia,dysmetria and asynergia. The animal walks on a broad base with a staggering, swaying gait. Walking in small circles is commonly observed. In most cases the head is ventroflexed at all times and may show a twist to one side (See Fig. 4, A to D). By the third day the animal is barely able to crawl about and loses its balance easily.

When present the most dramatic episodes of the critical stage are sudden convulsive seizures which may occur spontaneously at any time in the walking or resting animal. There are no preliminary signs. Suddenly the head and neck ventroflex maximally, the fore and hind limbs are extended and abducted violently, and the body tends to flex. Such tonic seizures last from 3 to 5 seconds and terminate as suddenly as they begin. In a few cases convulsions lasting for a minute were observed, with both tonic and clonic components present.

Pupillary dilatation is always maximal during and immediately following seizures (See Fig. 5 and 6). Even when resting the pupil size is approximately double that of a normal animal with illumination of the same intensity.

The early period of the critical stage is often associated with considerable general activity of the animal. One of the characteristic signs is loud crying, which may continue for hours in some cases and always occurs following seizures. There seems to be little or no impairment of higher cortical functions as judged from general behavior. The cat still shows interest in food, will come to the observer when called, and will follow. The animal while walking may lean heavily against any support placed against the body. If the support be quickly

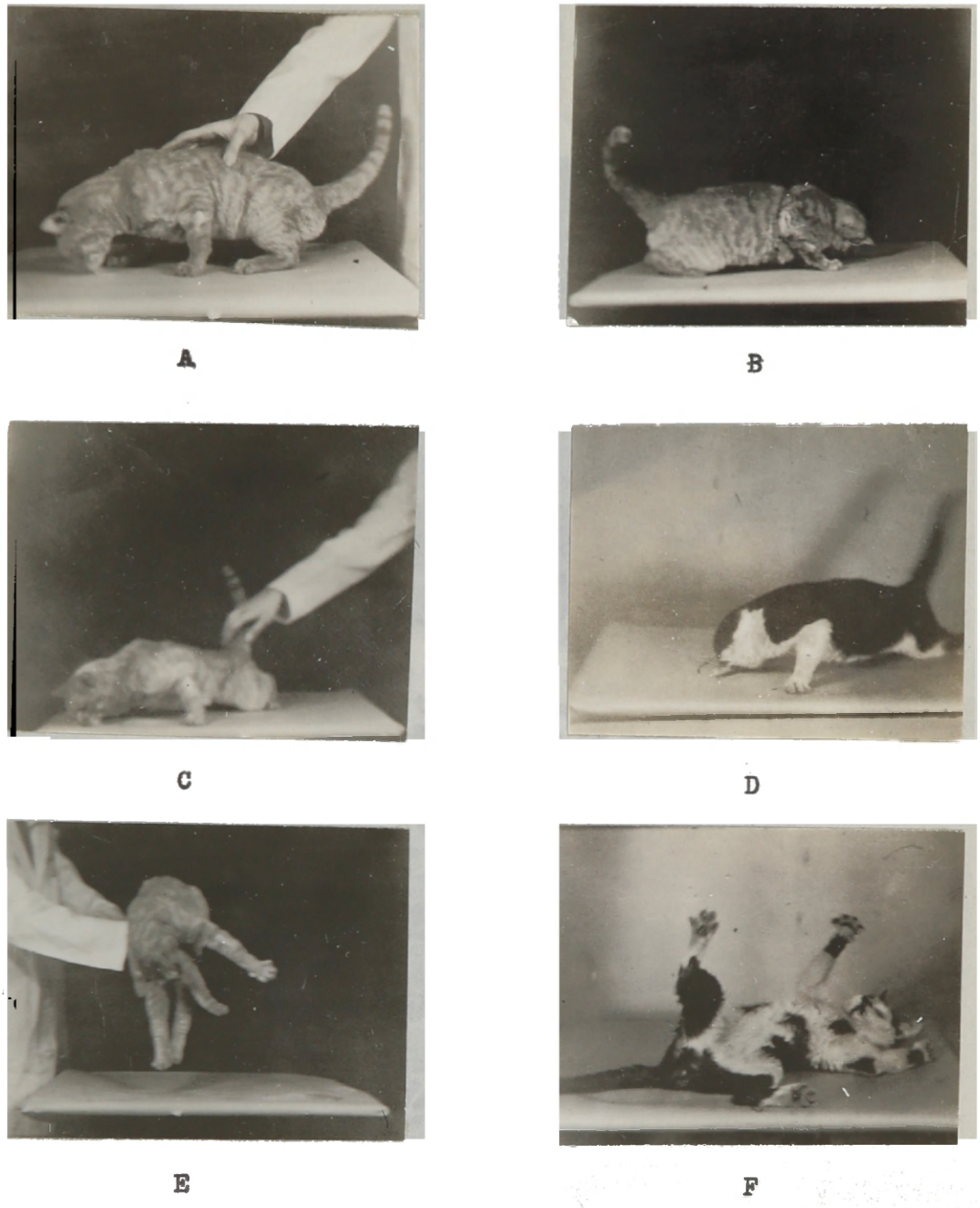

Figure 4. Photographs of thiamin deficient cats in the critical stage. A, B, C and D show the typical abnormal head and body posture. E shows the extension of the limbs, body twist and head ventroflexion which occur when the animal is lifted. F shows the extension and abduction of the limbs just after a convulsive seizure.

Figure 5. Cat No. 20 in the critical stage, showing pupillary dilatation in bright light.

Figure 6. Cat No. 20 two days after injection of thiamin and return to normal diet. Pupils show normal constriction in bright light.

withdrawn, the cat falls over. When the animal is picked up, marked spastic tone in the limb and body muscles is noted. Limb abduction and powerful movements of the trunk are commonly observed in carrying such animals(See Fig. 4, E). Cats in the critical stage are unable to jump from one level to another. The head and neck ventroflex and the animal turns half a somersault, landing on its back.

It has been mentioned that seizures appear spontaneously in the resting animal. They may also be precipitated by external stimuli such as loud noises or bright lights. By far the most effective method is to suddenly change the animal's orientation or position. Thus, turning the cat upside down and then placing it back on its feet will almost invariably produce a seizure.

The marked neurological disturbances observed in the early period of the critical stage must be considered reversible functional disorders since all signs of the deficiency disappear within a day following the injection of thiamin. Cats given thiamin during the late critical stage may show some functional impairment, particularly ataxia in the hind limbs, for several weeks.

3. Terminal Stage. The activities of the critical stage gradually lessen until the animal becomes weak and unable to walk. At this time it enters the terminal stage. In some cases extensor tone appears in the limbs; the animals seem comatose and are apparently functionally decorticate. All attempts to recover animals from the terminal stage with thiamin failed. It is therefore considered a period of irreversible changes which lead to death within a day or two. The period from the onset of the terminal stage to death is 1 to 3 days.

Study of Reflex Action in Thiamin Deficient Cats

The present series of experiments on the reflex activities of cats in the critical stage were conducted with the idea of localizing more precisely the site of the functional nervous disorders observed. Extensive use has been made here of the monograph by Fulton (1938) which contains an admirable summary of the reflex activities of the cat and the neural pathways involved.

1. Spinal Reflexes. These reflexes are relatively simple. Their presence indicates the integrity of the spinal reflex arc and associated end organs.

Flexor Reflex. The flexor withdrawal of the limb from painful stimuli was present in all stages. Even animals in the last terminal stages showed a feeble response.

Extensor Reflex. The knee jerk was essentially normal in all stages, indicating the proper function of the proprioceptors in the patellar tendon and associated pathways.

2. Postural Reflexes. The proper maintenance of orientation of the head and body when an animal is in motion or at rest is achieved by a number of complex reflex activities grouped under postural reactions. The general behavior of animals in the critical stage was indicative of functional disturbances of these reflexes, and a detailed study was therefore undertaken.

Tonic Labyrinthine Reflexes. If a blindfolded normal animal is lifted by the hind limbs, the animal dorsiflexes the head and neck bringing the head into a plane as nearly parallel with the floor as possible. This reflex arises from stimulation of the otolithic maculae of the labyrinth which respond to changes in the orientation of the

head. Animals in the critical stage do not show the normal reaction, the head remaining ventroflexed (See Fig. 7). Further, when the normal animal is held in the air supine the head tends to turn, while cats in the critical stage when held supine show spastic head ventroflexion which remains unchanged no matter in what position the animal is held (See Fig. 8). These abnormal reactions are indicative of impaired tonic labyrinthine reflexes.

Righting Reflexes. The righting reactions of the cat are developed to an exceptional degree in regard to their speed and accuracy. If a normal animal be held supine a few feet from the floor and is then released suddenly, righting occurs and the cat lands easily on its feet. In startling contrast is the almost complete loss of body righting in air observed in the critical stage. When such an animal is held in the supine position the limbs are usually extended and abducted and and the head ventroflexed. Upon releasing the cat this position remains "frozen" throughout the fall. However, when the back of the animal contacts the surface righting occurs immediately. Righting in the air involves five types of reflexes arising (1) in the labyrinth, (2) from the body to the head, (3) from the neck, (4) from the body to the body, and (5) from the eyes. In the blindfolded animal the whole sequence is initiated by the reflex turning of the head resulting from the stimulation of the macular portion of the labyrinth.

Body Righting Reflexes Acting on the Head. When a blindfolded labyrinthectomized cat is laid on its side, the head is brought up and the body assumes the normal resting postion. This righting is initiated by the unequal stimulation of the two sides of the body. Cats in the critical stage when laid on their side are able to right

Figure 7. Cat in the critical stage, showing head ventroflexion and loss of head righting.

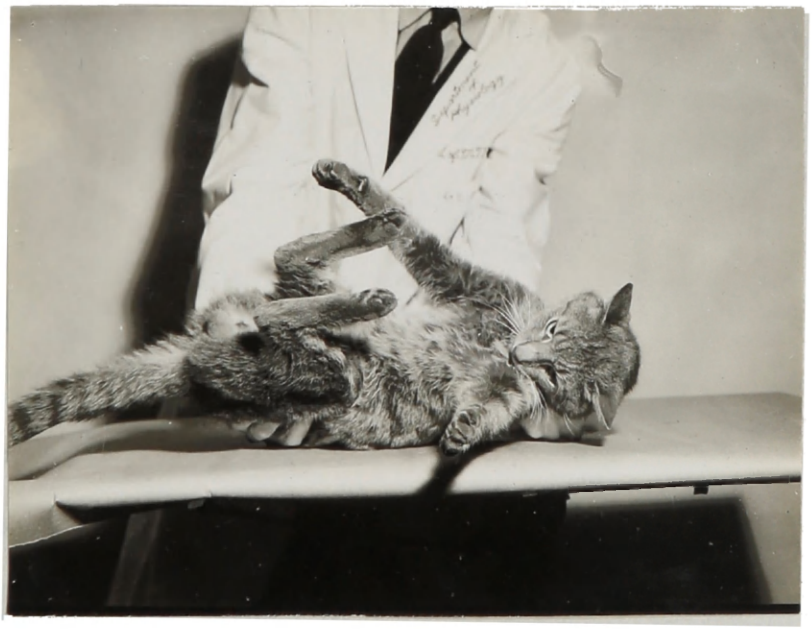

Figure 8. Cat in the critical stage, showing limb extension and abduction and head ventroflexion.

themselves. The same reflex apparently accounts for the righting which occurs when the animal falls on its back. This is the only righting reflex studied which remains functional.

Vestibulo-ocular Reflex (Nystagmus). In order to obtain more specific information with regard to the functional condition of the semi-circular canals and associated reflex pathways, a study of postrotatory nystagmus was made on a series of 10 cats during the course of thiamin deficiency. The experiments were conducted with a specially constructed revolving table. The animal was placed with the head over the center of rotation. Considerable care was taken in centering the head, which was fixed securely and in a horizontal plane (See Fig. 9). An electric motor turned the table at 120 revolutions per minute. The animals were rotated for one minute. The motor was then stopped and ten seconds later the revolving table was brought to a halt. Nystagmus time and the number of excursions of the eyes were then taken. Duplicate runs on the same animal checked within a second and variation from day to day in normal controls was never greater than three seconds.

A consideration of the data presented in Table III reveals the wide variation in nystagmus time in different cats. It is necessary therefore to study each case separately in determining the possible effects of thiamin deficiency. During the induction stage in the cat the nystagmus time tends to decrease slightly, but the change is so small as to be of doubtful significance. When the animal reaches the critical stage a striking decrease in the duration of nystagmus occurs. In contrast to these findings are those of Church (1935) who found nystagmus time became progressively greater during the develop-

ment of deficiency in the rat up to the time of the onset of disburbances of equilibrium and then decreased precipitously. These rats were on a diet in which autoclaved yeast was used as the only source of complex vitamins and therefore the possibility of other vitamin deficiencies occurring cannot be overlooked.

The depression of the vestibulo-ocular reflexes during the cortical stage is a reversible phenomenon, for after recovering the cat by injecting thiamin nystagmus time increases toward normal and the ocular movements are of the large normal type.

Hopping and Placing Reflexes. These postural reactions have been studied in the cat by Bard (1933) and shown to have a strict cortical localization in the cerebrum. The hopping reflex is tested by holding the blindfolded animal suspended with only one forelimb on the table. The body is then moved forward. The normal cat hops to bring the limb into proper position to support the body. To test the placing reflexes, the blindfolded animal is held so that the edge of the table contacts the anterior surface of the forelimbs. The normal cat immediately lifts the limbs, placing the paws on the table surface. These two reflexes are present in cats on diet up and including the critical stage. The presence of these reactions is evidence of the functional integrity of the cerebral cortex.

3. Cerebellar Function. Special attention was directed to the study of limb and body movements when the animals were walking. These observations revealed some of the typical signs associated with cerebellar lesions, namely, ataxia, asynergia and dysmetria. The ataxia is seen as an awkward placement of the limbs. The asynergia involves the lack of integration of movements of the fore and hind limbs, and the

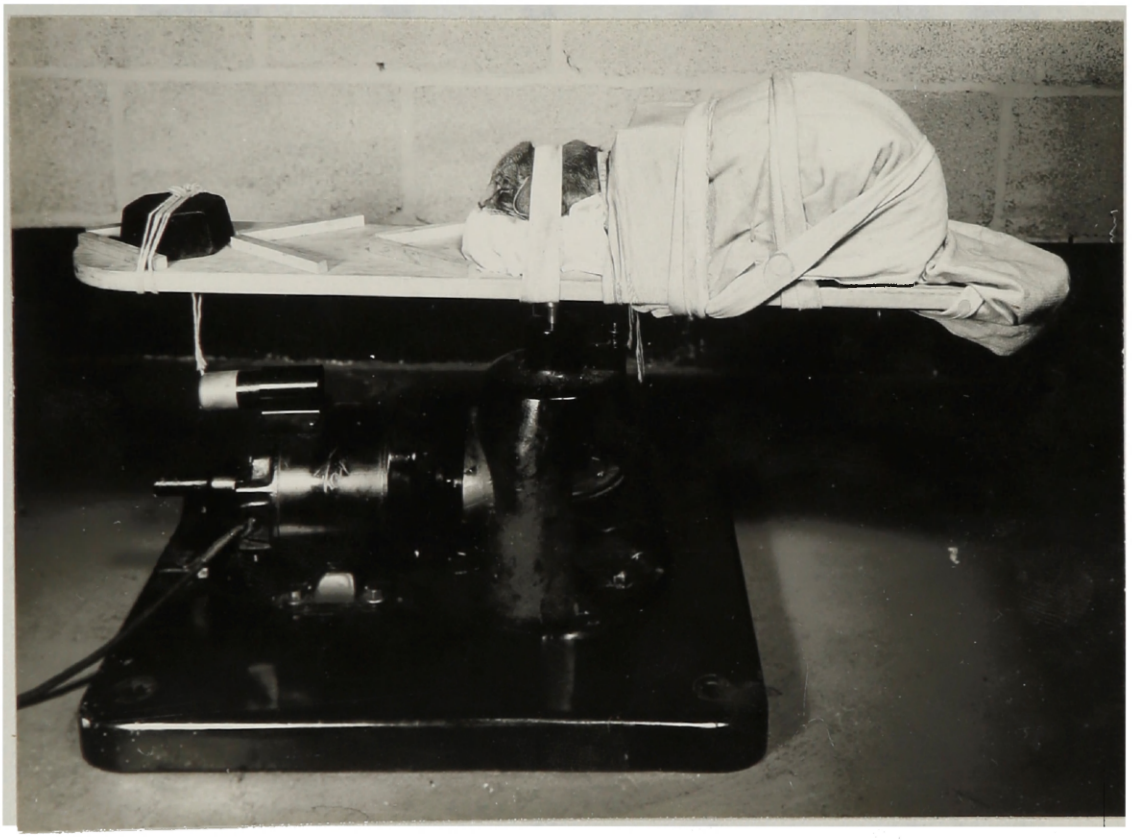

Figure 9. Cat in position on the nystagmus table.

TABLE III

Postrotatory Nystagmus Time and Characteristics in Normal and Thiamin Deficient Cats

Cat No.	Condition or stage	Days on Diet	Nystagmus time Seconds	Number of Excursions	Type of Excursion
56	Normal	--	36	98	Normal, large
	Induction	4	37	97	Normal, large
	Induction	14	32	97	Normal, large
	Critical	28	16	26	Erratic, small
	2 days post B_1	--	19	41	Normal, large
65	Normal	--	22	60	Normal, large
	Induction	4	23	65	Normal, large
	Induction	14	22	60	Normal, large
	Critical	31	5	4	Erratic, small
40	Normal	---	22	30	Normal, large
	Induction	19	17	39	Normal, large
	Critical	29	4	4	Erratic, small
	2 days post B_1	--	24	40	Normal, large
50	Normal	--	36	--	Normal, large
	Induction	16	35	--	Normal, large
	Induction	22	34	--	Normal, large
	Critical	30	20	--	Erratic, small
	Critical	31	16	--	Erratic, small
	1 day post B_1	--	22	26	Normal, large
58	Normal	--	29	--	Normal, large
	Induction	14	27	70	Normal, large
	Critical	27	2	3	Erratic, small
51	Induction	24	32	--	Normal, large
	Induction	30	32	--	Normal, large
	Critical	31	9	--	Erratic, small
	1 day post B_1	--	12	--	Normal, large
62	Normal	--	26	--	Normal, large
	Induction	12	20	--	Normal, large
	Critical	32	7	10	Erratic, small
	2 days post B_1	--	17	26	Normal, large
61	Induction	5	12	28	Normal, large
	Induction	14	12	27	Normal, large
	Critical	27	8	9	Erratic, small
	2 days post B_1	--	12	24	Normal, large
66	Induction	14	30	105	Normal, large
	Critical	32	16	20	Erratic, small
32	Normal	--	18	--	Normal, large
	Induction	5	17	40	Normal, large
	Induction	14	17	34	Normal, large
	Critical	31	0	0	None

* Number of days after injection of one mg. of thiamin and return to normal diet.

dysmetria is seen when the animal lifts the limbs abnormally high in walking. These three conditions result in a staggering, weaving gait with the animal often losing its balance and falling over. Taken together ataxia, asynergia and dysmetria are strong evidence of cerebellar dysfunction.

4. Cerebrocortical Function. It has been mentioned that the general behavior of the thiamin deficient cat indicates little impairment of cortical function. The ability of the animal to follow an object with the eyes shows that sight is present as does also the avoidance of obstacles when walking about. The light reflex is present but pupillary constriction is less than normal. For a given light intensity, the pupils of a deficient cat in the critical stage are approximately twice the size of those of a normal animal. This reflex can be impaired at levels below the cortex and therefore does not necessarily indicate cortical involvement. That the animal hears is indicated by the presence of startle to loud noises and the turning of the ears to less intense sounds.

Discussion and Analysis

The localization of functional nervous disorders from observations of general behavior and reflex studies depends upon deductions drawn from the available knowledge of reflex pathways and associated synaptic centers. It is beyond the scope of this paper to review all of the anatomical and physiological evidence upon which the analysis of the neurological manifestations herein reported is based. As has been mentioned, much of this information is to be found in the monograph by Fulton (1938).

The presence of normal flexor response and knee jerk make it

highly improbable that any of the observed signs of thiamin deficiency are due to impairment of the sensory or motor end organs and associated nerve fibers. Further, the wide spread nature of the disorders and their almost simultaneous appearance direct attention to the central nervous system as the site of these functional disturbances.

The cerebral cortex does not appear to be involved since general behavior, sight and hearing are normal.

It is in the postural mechanisms that the most striking abnormalities are observed. This directs attention to the centers and pathways concerned in these reactions. Other than the simple spinal reflexes the reactions studied involve complex pathways with synapses in several nuclei. Thus impairment at any point along the route may modify the response observed. The abnormal posture and loss of righting in air most probably result from the functional impairment of the reflexes arising in the otolithic maculae. The first synapse in this pathway may occur in the vestibular nuclei located in the medulla oblongata. From here secondary neurons apparently carry impulses to the reticular substance at the level of the red nuclei in the midbrain (mesencephalon) where righting reflexes are integrated (Ingram, Ranson and Barris, 1934).

The decreased nystagmus time observed indicates a disturbance in the vestibulo-ocular reflex pathway. This arises in the cristae of the semicircular canals with fibers passing to the vestibular nuclei. Second neurons pass to the nuclei of the third, fourth and sixth nerves lying in the midbrain and pons. From here the third order motor neurons pass to the extra-ocular muscles.

The cerebellum has numerous afferent and efferent pathways. On

the efferent side one of the most important is the cerebello-rubrospinal tract which involves synapses in the red nuclei of the midbrain. Its functional importance is indicated by the work of Ingram and Ranson (1932) who found that destruction of the red nuclei of the cat leads to ataxia, asynergia and dysmetria, signs similar to those observed following ablation of the cerebellum, and also similar to those observed in the present study.

A constant accompaniment of the critical stage is the pupillary dilatation which is at all times greater than normal and becomes maximal during and immediately after convulsions. Release of adrenalin during the seizures may be involved in the dilatation seen at this time. However, a disorder in the reflex pathway seems more probable since the pupil dilatation becomes progressively greater and finally maximal in the terminal stage when convulsions no longer occur. The light reflex has its origin in the retina, the afferent fibers passing to the pretectal region of the midbrain where they synapse with secondary neurons. These carry impulses to the Edinger-Westphal portion of the nucleus of the third nerve. Tonic activity of the nucleus causes pupillary constriction, while its destruction results in pupil dilatation and a complete absence of response to light.

From these considerations it is seen that the functional nervous disorders observed in the thiamin deficient cat involve reflex pathways which have important synaptic centers in the midbrain. Functional depression of the nuclear masses in the upper portion of the brain stem would therefore account for most of the observed signs. Whether direct impairment of the cerebellum and vestibular nuclei also occurs cannot be determined. From his studies on beriberi rats Church (1935)

described a similar locus to account for the neurological disorders which are in many respects similar to those seen in the cat. Prickett (1934) described marked cellular damage in the region of the vestibular nuclei and cerebellum of chronic thiamin deficient rats. A possible biochemical correlation with these findings is the report of increased quantities of lactic acid particularly in the brain stem of thiamin deficient pigeons (Kinnersley and Peters, 1930). Perhaps in the cat this region would also show a catatolurin effect in contrast to the negative result found for the cerebral cortex.

It is more difficult to locate the origin of the convulsive seizures. Generally, it is assumed that these result from cortical activity. However, the short duration of the convulsions in the thiamin deficient cat, lasting for only a few seconds in most cases, militates against cortical origin. Cannon and Rosenbluth (1942) in a study of the character of cortical discharge found activity lasting for a minute or more. Possibly then the seizures observed in the critical stage animals may have a subcortical origin, perhaps in the midbrain itself.

Since the onset of nervous disorders is very sudden it appears that the deficiency builds up to a critical point beyond which normal function is no longer possible. The simultaneous appearance of the signs of the critical stage is indicative of a localized functional lesion. The anorexia and vomiting which occur much earlier must involve a different mechanism, possibly a disturbance of tone or absorptive capacity of the gastro-intestinal tract.

The onset of the critical period is one of dysfunction not entirely depressive in character. The hypertonia, restlessness and seizure are

more indicative of hyperexcitability or release phenomena. As the animal progresses into the late critical stage this excitability gives way rapidly to general depression and finally the irreversible changes associated with the terminal stage.

SUMMARY AND CONCLUSIONS

1. A dietary regime, having as a unique feature the use of crystalline vitamin supplements given both orally and intraperitoneally, has been shown to meet the requirements for the production of specific thiamin deficiency. Thus, the diet was shown to maintain the animal in normal health if thiamin was added. Further, the disorders arising while the animals were on diet, namely, anorexia in the early period and postural disturbances and convulsions in the critical stage, were specifically aleviated within a period of a day after the injection of thiamin. Injection of other B complex vitamins had no effect.

2. A study of serum electrolytes, serum glucose and hematocrit during the course of thiamin deficiency in the cat revealed no significant change from normal. The maintenance of a normal serum potassium is considered indicative of the functional integrity of the adrenal cortex. The mechanisms which control the concentration of the other blood constituents studied are also apparently not impaired by thiamin deficiency.

No catatorulin effect was found in the cerebral cortex of critical stage cats. Further, no consistent change in the bisulfite binding substances of blood and urine occurred during the development of the deficiency. These negative results indicate that the biochemical changes in intermediate carbohydrate metabolism of acute thiamin de-

ficient cats are either very small or are not demonstrable by these techniques.

3. Electrocardiographic studies revealed that arrhythmia and a type of bradycardia, resulting from sinus arrest, occur in fifty per cent of the thiamin deficient animals in the critical stage. The sinus arrest is of vagal origin as shown by the disappearance of irregularities when the cat is atropinized.

4. The development of thiamin deficiency in the cat may be divided into three stages: (1) Induction stage, characterized by the development of anorexia, (2) critical stage, characterized by the sudden appearance of many neurological disorders, particularly of the postural mechanisms, and usually short tonic convulsive seizures. The animal becomes progressively weaker and passes into (3) the terminal stage where the animal is prostrate and cannot be recovered; death follows within a day or two.

A detailed study of the reflex activities of cats in the critical stage revealed impairment of (1) labyrinthine righting reactions as shown by the abnormal head position and loss of righting in air, (2) vestibulo-ocular reflex as indicated by the decreased nystagmus time, (3) pupillary light reflex as revealed by the increase in pupil size. Dysfunction of the cerebellum was indicated by the presence of asynergia, ataxia and dysmetria.

An analysis of the reflex pathways involved in the reactions which showed impairment revealed that all of them have synaptic centers in the midbrain. This is proposed as the probable locus of the functional lesion giving rise to the observed nervous disorders.

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