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OBSERVATIONS ON THE PATHOGENESIS OF THIAMINE-DEFICIENCY ENCEPHALOPATHY IN THE CAT (FELIS DOMESTICUS)

A thesis

presented to the Faculty of the Graduate School of the University of Toronto in partial fulfillment of the requirements for the degree of Master of Veterinary Science.

by

John Nelson

March 1959.
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ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. D. L. T. Smith and Dr. J. D. Schroder for the facilities which made this study possible. He also wishes to thank Dr. K. V. Jubb for his unfailing interest and advice throughout the course of these investigations.

The author is indebted to Dr. L. H. Lord for the selection and supply of experimental animals, and to Professor E. V. Evans for the thiamine assays.

Thanks are due to Miss J. Zamin for skilled technical assistance.
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INTRODUCTION

The cerebral lesions of thiamine deficiency have been studied in a variety of species. Experimentally, thiamine-deficiency encephalopathy has been reproduced in the cat, dog, fox, monkey, pigeon and fish and published accounts of its natural occurrence in the fox, cat and man are available. In spite of this difference in species the lesions within the brain show a high degree of consistency in both character and distribution. Essentially, the cerebral lesions consist of focal haemorrhage and oedema restricted to the nuclear masses of the periventricular grey matter, and associated with a variable degree of neuronal degeneration.

Few attempts have been made to ascertain the mechanism of development of these lesions although many theories have been proposed. It is established that phosphorylated thiamine acts as a co-enzyme in pyruvate oxidation, and that in a deficiency of the vitamin normal carbohydrate metabolism is disrupted and pyruvate accumulates in the blood and tissues. The oxidation of pyruvate provides an almost exclusive source of energy for cerebral activities. The abnormal accumulation of pyruvic acid which occurs in thiamine deficiency were thought to be directly noxious to endothelia, or to be capable of causing nervous tissue damage, the results of which upset the
ionic balance on the cerebral side of the blood-brain-barrier, resulting in the production of oedema and haemorrhage. A direct anti-angiodegenerative effect has been attributed to vitamin B1.

In an attempt to throw more light on this problem, it was decided to investigate the development of the characteristic cerebral lesions of thiamine deficiency in cats, and to study in particular the role of the vascular elements and their supporting glia. These elements are presumed to form the anatomical basis of the functional blood-brain-barrier which is responsible for the selective transport between blood and brain. Because of this, experiments were conducted to determine if an altered cerebro-vascular permeability occurred in thiamine deficiency and led to the production of the focal oedema and haemorrhage.

In thallium poisoning in cats and dogs, cerebral lesions have been described which closely resemble those occurring in thiamine deficiency. These focal haemorrhages were confined to the cerebral grey matter and predominated in the periventricular regions. In thallium poisoning in man, similar haemorrhages described as "ring-bleeding" were reported but with less selectivity of distribution. Both conditions are characterised clinically by a peripheral "neuritis". In man, this "neuritis" has been attributed, in part, to a direct inhibition by thallium of the
co-enzymic junction of thiamine. Other reports have suggested that defective absorption of the B-group vitamins is caused by the toxic action of thallium on the gastro-intestinal mucosa. On the basis of these reports, experiments were undertaken to determine if thallotoxicosis in cats duplicated or exacerbated the cerebral lesions of thiamine deficiency, or antagonised the actions of the vitamin.

Finally, the author has observed sections of brain tissue from a cat known to have died of thallium poisoning, in which small haemorrhages were seen in the inferior colliculi and vestibular nuclei.
Cerebral lesions of thiamine deficiency appear to have been first described in the rat by Prickett (1). In this animal the cerebral lesions consisted of small focal haemorrhages associated with oedema and neuronal degeneration. These were bilaterally located in the cerebellar nuclei, vestibular nuclei, nucleus solitarius and in the abducens nuclei. The same author observed similar changes in the brains of thiamine-deficient pigeons, and these lesions were found in the vestibular and cerebellar nuclei and in the cerebral hemispheres and optic lobes.

Church (2) confirmed the findings of Prickett and agreed with him in his conclusion that the focal haemorrhage was of no significance in the production of the nervous signs shown by the pigeons, since these signs were promptly abolished by the administration of thiamine. As a possible explanation of the specific localization and production of the haemorrhages, he suggested that the disordered carbohydrate metabolism resulted in an increased concentration of pyruvic acid particularly in those areas showing signs of nervous tissue degeneration. The products of the tissue damage were presumed to result in a localized acidosis leading to the production of oedema and haemorrhage.

Among many other workers who have duplicated the experimental work on pigeons, Alexander (4) concluded that
the cerebral lesions in thiamine-deficient pigeons were analogous with those which occurred in alcoholic encephalopathy of man. He considered that the lesions in both conditions were due to a deficiency of thiamine, and ascribed to this vitamin a specific antiangiodegenerative function.

Campbell and Biggart (5) described the typical lesions of Wernicke's encephalopathy in man. These consisted of bilateral haemorrhagic and degenerative foci in the mamillary bodies, and in the trochlear, abducens, vagal, and vestibular nuclei. As regards the pathogenesis of these lesions, they considered that the vascular disturbance was so marked and so constant, and the associated neuronal damage so slight, that the vascular changes were the most important part of the process. They proposed three theories to explain the production and restricted localization of the haemorrhages. These were, that the vessels of the periventricular region of the brain possessed some structural peculiarities, or that an altered biochemical environment damaged the blood vessels directly, or indirectly by damage to the adjacent parenchyma. They maintained, however, that the vascular changes, whether primary or secondary, were responsible for the greater part of the parenchymal degeneration. Wernicke's encephalopathy was observed to occur in association with neoplasia of the gastrointestinal tract, intractable vomition of pregnancy, and various other diseases leading to cachexia. The authors
considered that a vitamin B-group deficiency could be the common etiological factor.

Experimental thiamine-deficiency encephalopathy has been produced in many animals. The lesions in the brains of pigeons have been described by Prickett (1), Church (2), Alexander (4), Swank and Swank and Bessey (13 A, B), Prados and Swank (14) and these workers are essentially in agreement as regards the nature and distribution of the lesions.

Evans et al (7) observed the cerebral lesions in foxes which died in an outbreak of Chastek paralysis. The condition was prevented by the daily administration of twenty-five milligrams thiamine. Their observations on the bilaterally symmetrical vascular lesions in the periventricular grey matter of the brain led them to conclude that the lesions were analogous in their pathological features to those reported to occur in dogs by Zimmerman (3), in chicks by Pappenheimer and Goettsch (28) and were associated with a deficiency of vitamin B₁. In recording the bilateral symmetry of the lesions, the authors observed that haemorrhages did not occur in areas devoid of nerve cells, and that the hypovitaminosis B₁ was probably responsible for the parenchymatous damage as well.

Alexander et al (8) in a comparative neurological study described as analogous Wernicke's encephalopathy in man, the cerebral lesions of Chastek paralysis in foxes and an encephalopathy of fish fed on a diet of raw fish.
Rinehart et al (9) in their study of experimental thiamine deficiency in the Rhesus monkey, produced bilateral cerebral lesions of focal haemorrhage and oedema associated with neuronal degenerative changes. These were located in the putamen, caudate nuclei, inferior colliculi, cerebellar vermis and in one or more of the cranial-nerve nuclei. They observed also that a localized oedema was the earliest detectable change, and that in his experiments no peripheral neuropathy was seen.

Prados and Swank (14) discussing the thiamine-deficiency encephalopathy of pigeons and cats and the inter-relationship between the neuronal and vascular changes, support the views advanced by Church (2). These authors described neuronal degenerative changes in those nuclei in which haemorrhage would ultimately be expected to occur. Since focal haemorrhagic areas also contained visibly damaged neurons, they concluded that the initiation of vascular changes depended on pre-existing nervous-tissue damage. In their experiments on kittens, the neuronal changes were obviously advanced as seen from the following quotation. Referring to ganglion cells, "These changes consisted of chromatolysis, vacuolation, dissolution of the nucleus and finally disappearance of the cells, and silver stains indicated that the cell processes and cell bodies frequently degenerated simultaneously."

Jubb et al (15) produced cerebral lesions of
thiamine deficiency in cats and their findings differ from those of Prados and Swank (14) in one important respect. These workers consistently found that neuronal degeneration did not occur prior to the development of oedema and haemorrhage although many neurons ultimately did degenerate when involved in the vascular changes. After fixation of the brains of deeply anaesthetised cats by infusion, the histological appearance of this material was one in which the neurons retained their structural integrity, and in which degenerative Nissl changes were absent. They found themselves in agreement with the views held by Campbell and Biggart (5) in emphasizing the importance of the vascular lesions, although no attempt was made to explain their specific localization.

It is of interest to note that Follis et al (10) found no lesions in the central or peripheral nervous systems in their experimental thiamine-deficient pigs.

To the author's knowledge, no adequate account of cerebral lesions in association with thiamine deficiency exists regarding ruminants or horses.

While peripheral "neuritis" is frequently found in the more chronic forms of Beriberi in man, the position regarding its occurrence in animals deficient in vitamin B₁ is not clear. Prickett (1) records an absence of peripheral neuropathy in thiamine-deficient rats and this was confirmed by the work of Engel and Phillips (12) who also
noted its absence in chicks. Berry et al (11) found no peripheral nerve degeneration in cats, nor did avitaminosis B1 impair the regenerative capacity of injured nerves. Jubb et al (15) confirmed the results obtained by Berry. While Engel and Phillips (12) did not find peripheral nerve degeneration in chicks, Swank (13 B), Swank and Bessey (13 A) describe the occurrence of peripheral neuropathy in pigeons. Zimmerman (3) demonstrated its occurrence in thiamine-deficient dogs. Rinehart (9) found no involvement of peripheral nerves in the Rhesus monkey and Follis (10) recorded its absence in pigs.

The neuropathology of thallium poisoning has been investigated by many workers and the situation has been fully reviewed by Prick et al (16) in their book on thallium poisoning. It was established that thallium had an affinity for nervous tissue, and that in both animals and man it caused diffuse neuronal degeneration. The specific effects of thallium concerned the ganglion cells of the brain and cord. These cells were shrunken and elongated with pycnotic, eccentric nuclei, or swollen and showing a variety of Nissl changes. The neuronal swelling was associated with a peripheral or perinuclear lysis of Nissl substance and often the nuclei of these cells were swollen, pale and contained within an indistinct nuclear membrane. Ghost cells and tissue spaces indicating the complete lysis of neurons were frequently seen. These changes were without
specific localization. Of particular interest is the occurrence of cerebral haemorrhage in fatal thallium poisoning in man. This was described as "slight bleeding extended over the central nervous system". The haemorrhages were small and restricted largely to the perivascular spaces and for this reason were described by the authors as "ring-bleeding". They reported also an interstitial oedema of areas in the cerebral cortex and that the smaller arterial branches in these zones showed a contracted or closed lumen due to swelling of the endothelial cells. Referring to previous experimental thallium poisoning in rats the authors cite Mazel (17) who reported the presence of "diffuse clarifications" in brain sections stained with haematoxylin and eosin. This oedema was ascribed by Mazel to an increased capillary fragility caused by the direct toxic action of thallium.

Larson et al (18) reported the presence, in the brain of a dog accidentally poisoned with thallium, of petecchial haemorrhages of the basal ganglia and mid-brain, perivascular cuffing and areas of softening. They added that, to their knowledge, these findings were unusual.

Pile (19) described the clinical behaviour of cats accidentally poisoned by the ingestion of thallium, but unfortunately the description of the pathology of the central nervous system was not given in detail.

Peripheral neuropathy consisting of myelin
degeneration and fibre swelling and fragmentation is fairly consistently found. Prick (16) noted the frequency of its occurrence in man and cited Fraulini (20) and Mazel (17) who had earlier described its occurrence in the rat.
MATERIALS AND METHODS (GENERAL)

The selection of cats for experimental purposes was determined largely by their availability. Animals were rejected as unsuitable if immature, of advanced age, diseased, or if uncastrated males. Whenever possible young adults were selected. Each animal was examined clinically for the presence of disease, after which it was kept under observation for a few days before being placed on experiment. During this period they were fed a maintenance diet of a commercially prepared canned cat food. The same proprietary product, although in different batches, was used for all groups of experimental cats which required no specially treated diet. It was also the food which was rendered deficient in thiamine. Water, ad lib, was the only fluid provided.

To produce a diet sufficiently low in thiamine to precipitate an acute thiamine deficiency in cats, a commercial cat food, containing no fish or their by-products and of high animal protein content, was subjected to the following procedure.

Each fourteen-ounce tin of food was thoroughly mixed in a Waring Blender to a fine consistency. To this was added two grams of sodium bisulphite in ten millilitres water and remixed. The food so treated was pooled in a large flat tray and allowed to stand at room temperature
for twenty-four hours. The bulked sample was then autoclaved at twenty pounds pressure for three hours. After cooling, the contents were transferred to airtight containers and stored at -4°C. Random samples were presented for quantitative thiamine estimations. The highest figure obtained from these was 0.065 milligrams per pound. Several lower values presumably varied with alteration in the relative amounts of offals incorporated in the particular batch of food used.

Immediately prior to feeding the animals, vitamins were added to and mixed with the food once daily in amounts of 0.4 milligrams pyridoxine hydrochloride, 1.0 milligrams calcium pantothenate, 0.5 milligrams riboflavin and 1.0 milligrams niacin. With the onset of anorexia the vitamin supplement was administered intraperitoneally in equivalent amounts twice weekly.

Since the primary purpose of the investigation was to study the nervous system, the following method of tissue fixation was designed to minimize errors due to post mortem autolysis and to artefact.

The animals were destroyed by the intraperitoneal injection of nembutal. Within ten minutes after death both eyes were removed and placed in Zenker acetic-acid fixative solution. The entire cadaver was then infused with a ten per cent formalin solution through the left ventricle and aortic trunk. A small incision was made in the right
ventricle to allow free circulation of the fixative sol-
ation. Immobility of the tongue and lips of the cadaver was taken to indicate a sufficiency of nervous tissue fix-
ation. The brain was then removed and stored in ten per-
cent formalin. Twelve to twenty-four hours later the
spinal cord was removed together with the sciatic nerve
and brachial plexus. This delay was allowed in order to
avoid artefacts due to stretching or contusion which could
occur during their removal in an imperfectly fixed con-
dition. Exceptions to the prescribed method of fixation
occurred in five animals which died unobserved. These
were not included as valid material. The procedure was
modified for those animals prepared for vascular injection
mass techniques in that the blood vessels were washed out
with physiological saline before being infused with formalin.
All control animals were destroyed and their tissues fixed
by the procedure described. The fixed nervous tissues
were sliced and the presence or absence of gross lesions
noted.

Both paraffin and frozen sections of the nervous
tissues were prepared. The eyes were sectioned after em-
bedding in celloidin. The calottes trimmed from the eyes
were embedded in paraffin and sectioned. Sections were
stained routinely with Haematoxylin and Eosin. Selected
tissues were stained as required with Luxol-fast-blue for
normal and degenerating myelin, Oil-red-O for neutral fat,
Toluidine blue for Nissl substance, Holme's fibre-stain, Periodic Acid Schiff for basement membranes, Cajal's gold sublimate astrocyte stain, and Bielschowski's ammoniacal silver nitrate for axons. Heidenhain's azocarmine-aniline-blue was applied to eye sections. Benzidine stain for blood was also used.
THALLIUM POISONING IN CATS

It was apparent that the degree of thallotoxicosis might influence the occurrence and severity of the cerebral lesions which have been reported to resemble closely those of thiamine deficiency. Consequently, it was decided to produce both acute and chronic thallium poisoning in cats.

Materials and Methods:

Twelve animals were used in these experiments. Thallium, as a 0.1 per cent aqueous solution of thallous acetate, was added to the food.

The six cats in the acute toxicity experiments received the following doses of thallium. Two received two milligrams per kilogram of body weight, and the others were given four milligrams, six milligrams, eight milligrams, and sixteen milligrams respectively, per kilogram of body weight. The dose was given once daily.

Chronic thallium poisoning was produced by administering to each of three pairs of cats two milligrams, four milligrams, and six milligrams of thallous acetate daily, irrespective of the weight or age of the animals. At the first sign of malaise or anorexia the thallium administration was discontinued until the animals had made a clinical recovery. During this period of recovery the cats were fed on the standard maintenance diet. The toxic
agent was then reintroduced at the original dose rate. This process was repeated and continued until the animals succumbed to prolonged administration of thallium. Because of the method of administration and the variable appetites of the animals, particularly of those in the chronic toxicity experiments, it was impossible to estimate the amount of thallium ingested.

Observations were made on the permeability of the blood-brain-barrier in three cats showing advanced nervous signs of chronic thallium poisoning. This was done by injecting six millilitres of a one per cent aqueous solution of trypan blue intravenously. If this single injection did not produce an adequate blue colouration of the sclera after a period of one to two hours, a further six millilitres was injected intraperitoneally. The animals were destroyed at three and one-half hours, fourteen hours and twenty-four hours after the injection of the dye solution.

Two normal cats were maintained on the untreated food as controls for the thallium poisoning experiments.
RESULTS

Clinical Observations:

Chronic thallium poisoning in cats was associated with a recognizable pattern of behaviour. The initial onset of anorexia varied with the dose of thallium administered. In some animals it occurred about the thirty-second day of the experiment, and by the forty-ninth day all the cats in the chronic toxicity group were showing partial or total loss of appetite. Since it was the practice to discontinue the administration of thallium when loss of appetite first appeared and to reintroduce it when recovery was apparent, there followed in these animals succeeding periods of normal and reduced appetite of no fixed duration. Coincident with the repeated bouts of anorexia was a state of profound depression, the animal assuming a crouched position with hair erect, head low, eyes still and half closed. The cats showed no interest in their surroundings. Handling and examination of the cats in the initial stage of anorexia and depression met with no resistance. Similar treatment applied to the animals in the succeeding periods of depression provoked great irritability and resistance.

Loss of hair, beginning with longer coat hairs, was evident between the fourteenth and twenty-fifth days and was progressive. Initially the alopecia was not apparent but loss of hair was inferred from the accumulation of
hair in the cages and in the faeces. Later the coat became coarse and sparse especially on the tail, medial aspect of the hind limbs and on the face and head. Eventually, the effect was such that the cats appeared gaunt and long-legged, with disproportionately small heads and accentuated facial contours. This impression was produced partly by a great loss of bodily condition, and partly by the loss of the long coat hairs, the remaining ones being uniformly short. The skin was dry and inelastic. Frequently the epithelium of the nostrils and on the margins of the eyelids was dry, roughened and cracked. In two cats the latter lesions were complicated and encrusted with a catarrhal discharge. Moderately firm restraint applied to cats in the advanced stages of chronic thallium poisoning frequently resulted in complete denudation of hair at the areas were restraint was applied.

Nervous signs developed only after prolonged intermittent administration of thallium. Spasmodic flexion of a limb which was held in this position for some time was noted early in the course of the toxicity. Both hind and fore limbs were separately affected but this transient sign was not seen to occur in all of the animals. Hyperaesthesia was demonstrable before the advent of more definite nervous signs. This was shown by the unexpected violence of the resistance offered to normal handling and restraint. It appeared that simply holding the skin of the neck caused
an excessive amount of pain, as judged by the immediate furious response. This hyperaesthesia was present in cats which showed depression but no other clinical signs of nervous involvement.

An apparent stiffness of the hind limbs initiated a chain of distinct signs of nervous involvement. The hind limbs appeared to be over-extended. This gave the animal a stilted gait, and caused swaying motions of the hind quarters with the cat stationary. If the animal was allowed sufficient space in which to take more than a few steps, it was seen that hind limb flexion was also exaggerated giving the animal a peculiar high stepping action. The fore leg stance was widened but it was difficult to estimate if this was more than merely compensatory. The abnormalities of gait and posture increased in severity and were involved in an impairment of balance which supervened. At this stage, sudden movements of the head and neck were sufficient to unbalance the animal causing it to totter or fall to the side opposite to that in which the head was turned. Hyper-excitability developed shortly after the appearance of stiffness of the hind limbs. By moving a food bowl rapidly downwards and upwards the cat was seen to follow these movements by an exaggerated motion of the head and neck. If the animal's attention was attracted rapidly in an upward direction, the cat frequently showed a tendency towards dorsal flexion and upwards rotary movements of the
and a further inability to retain balance. Movement appeared to be a stimulus to hyper-excitability.

Paralysis eventually developed in three of the cats chronically poisoned and its actual onset was observed in two animals. These animals, on rising from the resting position, stretched, and in the act the hind limbs appeared to become fixed in a rigidly extended position with the tail strongly dorso-flexed. In attempting to regain balance the cats were forced to totter forwards on their fore limbs. One cat somersaulted into lateral recumbency, the other fell to the side. Loud, harsh mewing accompanied these actions. After a few futile attempts to rise, they lay still with the hind limbs in rigid extension, the tail similarly dorso-flexed, the back strongly arched. It required force to flex a hind limb or to straighten the tail, both of which returned to their original positions when released. The fore limbs were still capable of weak movements. A day or two later these became paralysed also, but were not rigid. Involvement of the head and neck did not occur, even after six days recumbency.

Dilatation of the pupil was seen late in the course of poisoning. This persisted until death. The paralysed cats frequently developed bilateral catarrhal conjunctivitis. An ophthalmoscopic examination was made on the eyes of one paralysed cat and no abnormalities were seen.
Even in the paralytic state a few of the animals continued to eat and drink when assisted to do so. The food had to be put on their lips since they could not see it, and apparently could not smell it. When water was given it was necessary to push the cat's nose into the water before it was aware of its presence.

The duration of intoxication in these cats ranged from thirty-four to seventy days. One animal receiving two milligrams of thallium as an initial dosage rate succumbed twenty days before one receiving six milligrams per day, and the animal receiving four milligrams per day survived its partner by twenty-eight days. Since, as previously stated, the actual amount of thallium ingested by each animal was incalculable, no relationship between the initial dosage rate and the occurrence of the nervous signs can be deduced.

Characteristic behaviour patterns were not shown by the animals which were acutely poisoned. Total anorexia developed on the third to the fifth day of the experiment. The cats were moribund and were destroyed by the seventh to twelfth day. Vomition of clear tenacious mucus frequently occurred. The depilatory action of thallium was shown more by a looseness of the coat than by the small amount of hair which accumulated in the cages. Only one cat showed a mild degree of nervous involvement. This was manifested by an impaired ability to maintain balance, and a tendency to roll when placed on its side. No hyperaesthesia was shown and
the cats were not blind.
Neuropathology:

The fixed nervous tissues from both acute and chronic thallium poisoning in cats were inspected externally and internally for the presence of gross lesions. Macroscopic lesions were consistently absent.

A microscopic examination of the spinal cord showed the effects of thallium consisted of widespread neuroneal degenerative changes which were most readily perceptible in the neurons of the anterior horns and which decreased in severity cranially. The most striking feature of the histological study was an apparent paucity of neurons in the ventral horns of grey matter, the remaining cells being rather poorly stained. A closer study of this area revealed an actual loss of neurons, as evidenced by the tissue spaces containing pale fragments of disintegrated cells (Figure 1). The majority of the remaining cells showed various morphological abnormalities, and only occasionally was an apparently uninjured cell seen. It was apparent that the degenerative neuronal changes which led eventually to complete necrosis were not identical in every case. A study of the different degrees of degeneration shown by the individual neurons in an affected group suggested a probable sequence in the changes. In many cases the reaction of the cell to injury was a swelling of the cell body and a total lysis of Nissl material. The nucleus and nuclear membrane were still intact but barely distinguishable from
the cytoplasm. Ghost cells were found, in which the swollen nucleus was only faintly discernable or was absent, and consisted of a pale amorphous cytoplasm with no distinct margin. In other neurons the stage of necrosis and complete disappearance was preceded by a series of nuclear and Nissl changes (Figure 11). The least severe injury was shown by a perinuclear lysis of Nissl material and a margination of the remainder. This peripheral Nissl substance was coarsely clumped and intensely stained. The nucleus of these neurons showed little more than swelling and some loss of stain affinity. Distension of the nucleus with further fading and loss of outline (Figure 111), and the beginning of a lessened intensity of staining of the peripheral Nissl substance was taken to indicate a more advanced state of neuronal degeneration. Eventually the cell consisted of irregular, indistinct masses of Nissl substance only, marking the contours of the original neuron. The nucleus had disappeared. It was obvious that the Nissl changes did not follow a specific pattern. Neurons were seen in which the Nissl substance was scattered diffusely throughout the cell body, often obscuring the nucleus. It would appear that regressive changes in these cases consisted of nuclear swelling followed by dissolution or fragmentation of the nucleus without further alteration in the distribution of the Nissl corpuscles (Figure IV). Finally, the cell became a structureless mass of large clumps of Nissl substance.
unbounded by a visible cell-membrane. All of these morphological changes in neurons were seen to co-exist in an affected microscopic field.

The neuronal changes in the dorsal horns were never as severe as those described as occurring in the ventral horns. Total necrosis was not seen. While the majority of the neurons were involved the evidence of injury although consistent was not so dramatic. The reaction to injury was shown by a tendency to shrinkage of the cell-body with distortion of outline. The cells became narrow and uniformly densely stained. Often there was eccentricity of the nuclei which tended to assume an oval shape and to stain rather more deeply.

Within the brain similar neuronal degenerative changes were found showing no specific localization although most readily apparent in areas of greater neuron density. In general, the large neurons of the medulla, colliculi, pons and thalamus showed a severity of degeneration comparable with that which occurred in the ventral horns of the spinal cord. In the cerebrum and cerebellum the changes were more diffuse and restricted to distension, hyperchromatosis, nuclear condensation and eccentricity. In the brains of two cats the neurons of the medial vestibular nuclei, cochlear nuclei, oculomotor nuclei and red nuclei showed more extensive involvement and a greater severity of degeneration. In one of these,
this more severe involvement was associated with focal interstitial oedema of the vestibular nuclei, red nuclei and inferior olivary nuclei (Figure V). The oedema was bilateral and confined to the grey matter. In the other animal the oedema affected the dentate nucleus unilaterally. Focal haemorrhage did not occur.

The glial response was variable but never extensive. In the spinal cord, the gliosis was mild and diffuse, and in a few cases only was there a tendency for the astrocytes to be grouped into loose nodules (Figure VI). In the brain, the astrocytic nuclei appeared large and pale and some bi-nucleate forms were present. This was taken to indicate proliferation. Again, the gliosis was not marked.

Fragmentation of axis cylinders and myelin sheaths occurred in the peripheral nerves of all cats in the chronic poisoning experiments. These degenerative changes were advanced and extensive (Figure VII).

In the eye, as in other nervous tissues, thallium exerted its toxic effects on neurons, producing simple regressive changes. Although not entirely uniform, these changes were sufficiently so to suggest a probable sequence. The earliest changes observed in the ganglion cells consisted of swelling of the nucleolus within a nucleus of normal size but which stained rather more intensely. Perinuclear tigrolysis emphasized the peripherally clumped Nissl substance. There was no appreciable increase in the size of
the cells at this stage. More severe injury of the retinal ganglion cells was shown where the nucleus assumed an almost perfectly spherical outline and was larger than normal. This nucleus, now devoid of a nucleolus, showed an uniform, leaden intensity of staining of its nucleoplasm (Figure VIII A, B). In these cells chromatolysis was perinuclear. Other ganglion cells had elongated parallel with the surface of the retina and were devoid of a nucleus (Figure IX). Their cellular outlines were indistinct, partly by reason of the swelling of the cells, and partly by the diffuse disposition of the chromatin material. Outright necrosis of the ganglion cells of the retina was not observed, nor did their numbers decrease. The severity of the changes was greater towards the periphery of the retina. The spherical, swollen nuclei of the cells of the inner nuclear layer showed the same intense leaden staining of the nucleoplasm which was seen in the retinal ganglion cells. In this layer, however, the cell population appeared to be decreased. The rods and cones were apparently unaffected.

The results of the trypan blue injections in cats which were paralysed were entirely negative. No abnormal blue staining of nervous parenchyma was seen.
THIAMINE-DEFICIENCY ENCEPHALOPATHY IN CATS

Experiment 1.

Uncomplicated Thiamine Deficiency.

The object of this experiment was to produce a state of acute thiamine deficiency in cats in order to study the distribution and character of the lesions which occurred in the central nervous system.

Materials and Methods:

By killing the animals at various stages of the induced deficiency an attempt was made to obtain developmental stages of the typical lesion. No specialized techniques were employed other than those previously described under General Methods. The twenty-two animals which were used in this experiment were destroyed for examination of the lesions at the following clinical stages.

1. Two animals were destroyed when anorexia was total but prior to the development of nervous signs.

2. Three animals were destroyed when ataxia, disturbance of balance, partial loss of righting reflexes and pupillary dilatation were present, but in the absence of inducible convulsions.

3. Six cats were destroyed when convulsive seizures
could be strongly indicated or momentarily induced by suspending the cat, head-downwards, and swaying gently.

4. Nine of the remaining animals were allowed to proceed to the advanced critical state and were then destroyed in a moribund condition.

5. Two cats were observed to reach the early critical stages of convulsive activity and then treated with an intraperitoneal injection of two milligrams of thiamine hydrochloride. They were then replaced on the deficient diet and destroyed at the advanced critical stage. Each group of cats contained one normal animal on the untreated diet.
RESULTS

Clinical Observations:

All animals fed the low-thiamine diet behaved in like manner. This specific clinical syndrome was divided into two stages.

The induction phase was non-specific and consisted of a progressive decrease in food consumption with a corresponding decline in weight. Animals at this stage continued to be moderately active and alert, and the daily introduction of fresh, treated food was attended with great interest. This initial interest in food was not lost until just prior to the critical phase. The average period on the low-thiamine food required to produce total anorexia was thirty-two days, but it must be added that there was considerable variation in the behaviour of the individual cats during that period. Some animals showed a simple, progressive decline in food consumption while others showed totally irregular appetites.

The onset of the convulsive stage was preceded by a period of up to six days when a variety of mild nervous signs were exhibited. Dilatation of the pupil was apparent shortly before the appearance of total anorexia, the dilatation increasing, and the pupillary light response decreasing towards the onset of convulsions. Allowed freedom at this stage, the subject appeared to lack
confidence in its ability to jump down from even low objects. Righting reflexes were still present although noticeably sluggish. Occasionally, some posterior stiffness was seen early in the course of the deficiency, and immediately prior to the convulsive stage posterior weakness with swaying of the hind quarters was observed. This was not ascribed to nervous causes, but rather to inanition and dehydration. Depression did not occur. In fact, all the cats under experiment became progressively more docile, greatly facilitating handling and examination which they appeared to appreciate. They retained an alert and interested appearance until the sudden onset of the critical phase. Signs of impending spontaneous convulsions were elicited twenty-four to thirty-six hours before their spontaneous occurrence by suspending cats, head-downwards, and swinging in a short pendulum motion. Normal cats under these conditions react with a strong dorsal flexion of the head, while the experimental animals showed mild ventro-flexion of the head with flattening of one or both ears, and a ventral flexion of the vertebral column.

Spontaneous convulsive seizures were identical in all of the groups subjected to the experimental diet. When observed in the cage, the animal maintained a low, crouched position with the head and neck extended. The cat then cautiously advanced to the edge of the open door and immediately on lowering its head to look downwards, a
convulsion was precipitated. It was characterized by a sudden extensor thrust of the fore limbs, partial protrusion of the claws followed by a violent ventro-flexion of the head until the chin was pressed to the sternum with the forehead thrust on the floor of the cage. This posture was maintained for five to ten seconds, and if the seizure occurred while the animal was allowed its freedom, it was succeeded by a compulsory rapid backwards progression with the body in a crouched position, hind limbs rigidly flexed, and the fore limbs in an extended position. Then followed a short period of relaxation when the animal slowly raised its head and resumed the standing position. Ataxia was now advanced. If untreated, the convulsive episodes became more frequent, with a rapid progression to the irreversible phase of continual crying, semi-coma, maintained extensor tonus and death.

One notable exception to the characteristic behaviour was seen in a cat which did not develop loss of balance and convulsions. This cat showed no signs of nervous involvement and died quietly after thirty-nine days on the thiamine-deficient diet. At necropsy a bilateral suppurative inflammation of the bulla tympanica was found. The brain did not show the focal haemorrhage characteristic of thiamine deficiency in cats.

Animals in the preconvulsive and early convulsive stages reacted dramatically to a curative injection of
thiamine hydrochloride. Within a period of thirty to sixty minutes following such treatment the animal appeared and reacted as normal, showing only mild weakness and ataxia.

Ophthalmoscopic examinations made at the early critical stage showed dilatation and turgidity of the retinal vessels. No haemorrhages were observed.

Spontaneous convulsions occurred after an average of thirty-two days on the experimental diet.
Neuropathology:

In all cases the gross lesions observed were confined to the central nervous system and were considered to be specific for thiamine-deficiency encephalopathy. Parenchymatous tissues showed only the non-specific changes of malnutrition and dehydration. The brain, spinal cord, peripheral nerves and eyes were examined and, of these, the brain alone showed the characteristic lesions.

The macroscopic lesion consisted of small, focal haemorrhages. In a few cases, these foci were single and unilateral, sometimes multiple and of unequal distribution, but in the majority of cases the lesion was typically bilateral, focal and symmetrical. With a few exceptions the site of the lesions was in the periventricular grey matter. In all cases the inferior colliculi were involved and the focal areas of haemorrhage were clearly confined to the grey matter. Apart from the invariable and typical involvement of the inferior colliculi (Figure X), macroscopic haemorrhage also occurred in the superior colliculi, medial vestibular nuclei, in the lateral geniculate bodies, in the habenula, the small pyramidal layer of the cerebral cortex, the grey matter of the cerebellum. In two cases, pial haemorrhage occurred. It would appear that the haemorrhages showed no specific pattern of distribution with the exception of their invariable occurrence in the inferior colliculi. The medial vestibular nuclei were involved in
approximately twenty-five per cent of the brains examined.

Histologically the typical advanced lesion consisted of focal haemorrhage, oedema, endothelial proliferation, gliosis and neuronal degeneration.

The haemorrhages were never of great magnitude and showed little tendency to infiltrate the surrounding tissues. In most cases these extravasations were confined to the interstices of the vascular wall and the perivascular spaces, producing a ring or collar of erythrocytes around the vessel of origin (Figure XI A, B). Vascular dilatation was generalized throughout the lesions, but the haemorrhage appeared to involve the medium-sized vessels and capillaries only. Haemorrhage from capillaries was much less frequent. Endothelial swelling and proliferation together with a varicose dilatation of the blood vessels produced the impression of greatly increased vascularity of the affected region. A closer examination of the vascular wall showed the endothelial and adventitial nuclei to be increased in number and poorly stained. The endothelial nuclei in particular showed a tendency to be unevenly grouped (Figure XII). Numerous small eosinophilic droplets were observed between the endothelial and adventitial elements in the distended perivascular spaces and within the cytoplasm of adjacent astrocytes. These Periodic Acid Schiff-positive and phloxine-negative droplets were thought to be serum proteins. Perivascular and interstitial oedema
was always present (Figure Xlll A, B). It would appear that the degree of oedema depended largely on the acuteness of the development of haemorrhage in any particular lesion since the number and intensity of the haemorrhages was inversely proportional to the extent of the oedema. Serial sections of haemorrhagic lesions failed to reveal an actual rupture of the vascular wall.

The glial response in these lesions was one of diffuse astrocytic hypertrophy and hyperplasia (Figure XLV A, B). Sections stained by the Cajal's gold sublimate showed that as well as an increase in the numbers of astrocytes, the cell bodies and vascular feet were increased in thickness. In spite of the perivascular oedema, it was seen that the vascular end-feet of the astrocytes still maintained contact with the vessel wall.

Neuronal degeneration always accompanied the focal haemorrhage and oedema. In these experiments there was total and uniform involvement of the neurons in the affected nuclear groups. The changes consisted of a swelling or shrinking of the cell body and condensation of the nuclei. The whole was uniformly deeply stained with haematoxylin. Necrosis of neurons was not observed. Under these experimental conditions, an examination of the peripheral nerves showed little more than a swelling of the myelin at the nodes of Ranvier. Demyelination and fibre fragmentation was not seen.
An examination of the brains from two cats which were sacrificed when early nervous involvement was first detected, showed a total absence of vascular changes and of oedema. Astrocytic gliosis was not seen. Diffuse neuronal changes similar to those found in typical hemorrhagic lesions were seen, apparently of the same non-specific nature.

At a more advanced clinical stage sections of brain from these cats showed, in addition to the previously described changes, that the blood vessels were congested but not markedly dilated. The congestion of the capillaries made these structures more readily visible. The perivascular spaces appeared to be a little larger than previously. Swelling and proliferation of astrocytes was now obvious, but endothelial proliferation could only be inferred from the apparent density of capillary-sized blood vessels.
Experiment II

Combined Thiamine Deficiency and Thallium Poisoning.

In order to elucidate a postulated antagonism of action between thallium and vitamin B₁, it was decided to superimpose thallium poisoning on the course of thiamine deficiency in cats.

Materials and Methods:

Five cats were rendered thiamine deficient to the stage of reduced appetite and then four milligrams of thallous acetate in solution was added to the food each day. This was continued until the cats developed total anorexia. The total amount of thallium administered to any one animal did not exceed twenty milligrams.
RESULTS

Two of the five cats in this experiment died unobserved and were discarded as unsuitable material for histological examination. All of these animals reached a stage of convulsions within twenty-four hours of the thirty-seventh day of experiment. It was noted that in this group the time taken to reach that stage exceeded by five days the average time taken by uncomplicated thiamine-deficient cats.

In most of the cats the pre-convulsive behaviour was marked by a stiff-legged, awkward gait, and a tendency to hyper-excitability. These signs varied in severity with each animal. The pupils were widely dilated. Convulsions, typical of those which occurred in uncomplicated thiamine deficiency in cats, succeeded these signs. A state of rigid paralysis of the extended hind limbs and tail followed the onset of convulsions rather rapidly. Again, this was not invariable, and, in fact, only two of the five cats showed this rigid position distinctly, although all showed degrees of resemblance to the paralysis of chronic thallium poisoning. It was also seen that the paralytic rigidity of the hind limbs did not persist for more than twenty-four hours. Shortly before destruction in semi-coma all limbs were flaccid.

Histologically, the lesions of the central nervous
system differed from those of uncomplicated thiamine deficiency by a severe degree of neuronal degeneration only. The neuronal degenerative changes were diffuse and of greater severity than was expected in animals on so small a dose of thallium. Otherwise, the neuronal regressive changes were identical with those found in chronic thallium intoxication. Extensive and severe involvement of the ganglion cells in the anterior horns of the spinal cord was seen in all three cats examined.

The severity of the focal haemorrhages and their anatomical distribution did not differ from those which characterized the cerebral lesions of uncomplicated thiamine deficiency.
Experiment III (Part 1).

Observations on Cerebro-vascular Permeability in Thiamine Deficiency

It was observed from Experiment I that interstitial oedema invariably accompanied the focal haemorrhage in the periventricular nuclei in the brains of cats suffering from a deficiency of vitamin B₁. The intention of the following stage by stage investigation was to determine if this was due to an altered cerebro-vascular permeability and, if so, to determine at which clinical stage it was first detectable.

Materials and Methods:

In order to provide a visual indication of increased permeability intra-vital staining by means of the acid-dye trypan blue was chosen as the most suitable method. A one per cent aqueous solution of the dye was freshly prepared for each group of experimental animals and injected into the cephalic vein in six millilitre amounts. Distinct blue colouration of the sclera was taken to indicate a sufficiency of staining, and if this was not achieved by a single intravenous dose, a further six millilitres was given intraperitoneally.

The animals in this series of experiments were rendered thiamine deficient by the methods described under
the heading "Materials and Methods (General)", and investigations were made into the permeability of the blood-brain-barrier to trypan blue at the following clinical states.

**Stage I:**

When the animal was showing pupillary dilatation, ataxia and sluggish righting reflexes, but not exhibiting spontaneous or inducible convulsions.

**Stage II:**

When, in addition to the clinical signs of stage I, the imminence of convulsions could be indicated by a momentary ventral flexion of the head and neck when the cat was suspended by the hips.

**Stage III:**

At that point when convulsions could be first induced but did not yet occur spontaneously.

The dye solution was injected at the above stages and the animals destroyed one to two hours later. Thereafter they were subjected to the specified post mortem procedure. After fixation the brains were sliced through the nuclei known to be frequent sites of haemorrhage and examined macroscopically for the presence of parenchymal
staining. Sections for microscopic examination were also made from these areas.

Two cats in normal health and maintained on an adequate diet received the aqueous trypan blue in similar amounts and by the same routes of administration as applied to the experimental cats. These were destroyed and used as controls for the dye-injection technique.
No abnormal penetration of the trypan blue into the parenchyma of the brain was seen in those cats injected with the dye and destroyed before the onset of inducible convulsions. All of the five cats injected and destroyed in stage III showed parenchymal staining of the brain in those nuclei which would ultimately become sites of focal haemorrhage. Only in three of these brains was the staining totally uncomplicated by haemorrhage in any part of the brain. In the remaining two brains in which simultaneous focal haemorrhage complicated the parenchymal staining, some nuclei were found to be already blue without co-existing haemorrhage, and in others the intensity of the trypan blue staining was not related to the site of the co-existing haemorrhage within the affected nucleus. All of the three brains with abnormal staining of the periventricular grey matter and a total lack of macroscopic or microscopic haemorrhage showed pigmentation of the inferior colliculi (Figure XV). In one, staining was also observed in the vestibular nuclei and in the other, the lateral geniculate bodies were involved. This abnormal parenchymal staining was bilaterally symmetrical and sharply confined to the involved nuclear masses.

When these stained areas were examined microscopically the vascular changes were striking. The blood
vessels were congested and dilated and surrounded by enlarged perivascular spaces. The oedema radiated from these vessels separating fibre bundles and surrounding the neurons. Many of the ganglion cells showed a tendency to be shrunken or swollen, more darkly stained than normal but still structurally intact. In many cases the axons of these cells appeared swollen. In the interstices of the adventitial cells and fibres of the blood vessels small eosinophilic droplets were seen.

Endothelial and adventitial hyperplasia was seen but it was not marked. Astrocytic gliosis, however, was obvious and diffuse.

In those animals in which haemorrhage complicated the oedema, the haemorrhages were typically restricted to the oedematously separated adventitial tissue and the perivascular spaces.
Experiment III (Part II).

Effect of Vitamin B₁ on the Restoration of the Blood-Brain-barrier.

The preceding experiment demonstrated an increased cerebro-vascular permeability in thiamine-deficient cats and related its onset to the clinical signs shown by the animal. It was decided, therefore, to investigate the effect of a curative injection of thiamine given at the stage of inducible convulsions.

Materials and Methods:

Fourteen cats were used. These were rendered thiamine deficient to stage III of the preceding experiment; that is, when convulsions were first induced. At that point, the cats were given an intraperitoneal injection of two milligrams of thiamine hydrochloride in solution. At varying intervals thereafter, when the cats were showing a clinical recovery, six millilitres of an one per cent trypan blue solution was injected into the cephalic vein. Some of the larger animals received an additional six millilitres intraperitoneally. After three hours, the cats were destroyed, their tissues fixed by the specified method and the brains examined grossly for the presence or absence of abnormal parenchymal staining.
The results presented in Table 1 show that an altered cerebro-vascular permeability was associated with focal haemorrhage in the brains of those animals destroyed up to five and one-half hours after the vitamin $B_1$ was injected. Over that period no defect in the blood-brain-barrier was demonstrated and haemorrhage was absent.

**Table 1**

<table>
<thead>
<tr>
<th>Cats No.</th>
<th>Time between Vitamin B$_1$ and dye injections</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Parenchymal staining</td>
</tr>
<tr>
<td>1.</td>
<td>1 hr. 30 mins.</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>3 hrs. 30 mins.</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>3 hrs. 50 mins.</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>5 hrs. 30 mins.</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>7 hrs.</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>8 hrs. 20 mins.</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>9 hrs. 30 mins.</td>
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Experiment IV.
The Origin of the Haemorrhages in Thiamine Deficiency.

Since the type of blood vessel involved in the characteristic focal extravasations which characterize thiamine-deficiency encephalopathy in cats is not clearly identified, it was decided to investigate this aspect of the problem.

Materials and Methods:

Eight cats were allowed to proceed to the advanced convulsive stage of thiamine deficiency. Thirty minutes before receiving a lethal dose of nembutal, they were injected intramuscularly with one millilitre of heparin to prevent intravascular coagulation. The blood vessels of the brain were then flushed with physiological saline and infused with formalin. A small area of the cranium was removed and the dura mater incised to expose the cerebrum. The arteries were injected via the carotid trunk with a suspension of red formulated latex until the finer vessels of the pia mater were seen to be filled. One hour later the head was removed from the cadaver and immersed in a ten per cent solution of formalin. The following day, the brain was removed from the cranium and sliced across the inferior colliculi, since this area was the most consistent site of haemorrhage. The inferior colliculi were removed
as a block and frozen sections were made of the area of haemorrhage. These sections were examined unstained and when stained with benzidine for extravasated erythrocytes.
RESULTS

Of the eight cats used in the experiment, five were discarded for lack of sufficient haemorrhage or failure of the latex injection. Frozen benzidine-stained sections of brains which were successfully injected showed that the haemorrhage originated from vessels which were not filled with latex. Haemorrhages occurred in vessels easily recognizable as capillaries, and in larger, thin-walled vessels devoid of latex. These latter vessels were taken to be venules.
While the neuropathological findings in the present series of experiments in thallium poisoning do not differ in essence from those described by other workers in this field, a few discrepancies and details merit discussion.

In view of the frequency of petechiation in the brains of humans and animals in thallium poisoning, as described by Prick et al (16) and Larson et al (18), the absence of haemorrhage in the brains of the cats in the present experiments is remarkable. It should be noted that the human cases, and the canine case of thallotoxicosis described by Larson were accidental, and it was not known what previous nutritional or disease states might have complicated the picture. As regards the focal haemorrhage of the inferior colliculi in one cat which was reported to have died of thallium poisoning, it is possible that this animal was deficient in thiamine prior to ingestion of the thallium.

The bilateral oedema of the vestibular nuclei which was observed in the brain of one chronically poisoned cat bears some resemblance to the focal symmetrical lesions of thiamine deficiency. Since, however, the involved nuclei showed neither endothelial proliferation nor haemorrhage, but a markedly greater severity of neuronal damage, the
similarity is considered to be mainly in the location of the oedema. Prick (16) observed oedema associated with endothelial changes in the frontal lobes in thallium poisoning. This author also cited the results obtained by Mazel (17) who observed focal cerebral oedema in experimental thallotoxicosis in rats sufficiently frequently to suggest that this change was the result of an increased capillary fragility. In order to establish that the oedema observed in the present experiments was not the result of excessive pressure of infusion at fixation, two normal cats were destroyed and the infusion performed with excessive force; subsequent histologic examination of the brains from these two cats revealed no interstitial oedema. There is no explanation for the bilateral symmetry or the localization of this isolated instance of cerebral oedema in the present series of thallium-poisoning experiments.

The peripheral neuropathy of thallium poisoning was discussed by Prick et al (16). They suggested that it could be due, in part, to a thiamine deficiency which could arise from decreased absorption of vitamins as a result of direct toxic action on the gastro-intestinal mucosa. Furthermore, they suggested that thallium had a "direct disturbing" effect on the pyruvate oxidase system of enzymes in which phosphorylated thiamine participates, resulting in a local increase in pyruvic acid in peripheral nerves. That other metals do indeed interfere with the
pyruvate oxidase system was shown by Peters et al (22) who demonstrated that many compounds of arsenic inhibit these enzymes by interacting with the essential thiol group. Thompson et al (23) showed that compounds of antimony, gold and mercury acted in the same way, their activity being associated with increased levels of pyruvic acid in the blood. Presumably thallium is capable of the same interference, but this should not imply a direct cause-and-effect relationship between peripheral neuropathy and an increased endogenous pyruvate: in thiamine deficiency there is an increase in levels of circulating pyruvate but, in cats at least, no peripheral neuropathy.

The retinal lesions described in these experiments in thallium poisoning are especially interesting since, as far as the author is aware, the nuclear alterations have not previously been described in neural cells in any condition. Blindness was consistently present in animals which were chronically poisoned but did not occur in those which died acutely. The nuclear changes in the retinal ganglion cells did not follow the general pattern of degeneration shown by the neurons of the brain and cord. It is probable that the injury to the ganglion cells of the retina was responsible for the blindness rather than the inconstant and mild optic neuropathy.

In the combined thallotoxicosis and thiamine-deficiency experiments there appears to be no adequate
explanation for the longer time taken by these cats to reach the convulsive stage characteristic of thiamine deficiency. It may be that, as with arsenic, lead and mercury, the toxic effects of thallium on nervous tissues are preceded by an increased nervous irritability. This stimulation could conceivably offset the generalized nervous depression of thiamine deficiency and so delay the onset of convulsions. Similarly, the severity of the neuronal degeneration seen in the brains of these cats, which was equalled only by those produced after prolonged thallium poisoning, may have been the result of an increased susceptibility following the devitalizing effects of thiamine deficiency.

Haemorrhages did occur in the brains of the cats in the combined experiments, but in these instances their distribution was typical of thiamine deficiency and there is no evidence that they were modified in any way by concurrent thallium poisoning.

In the experiments on uncomplicated thiamine deficiency the absence of distinct neuronal injury at any stage in the development of the typical lesion is in accord with the findings of Campbell and Biggart (5) and Jubb et al (15), but is in direct contrast with the results reported by Prados and Swank (14). The latter authors agree with Church (2) in proposing that the focal cerebral oedema and haemorrhage which characterized vitamin B1 deficiency was invariably accompanied by obvious neuronal
degeneration. They considered that this neural damage preceded and was responsible for the exudative vascular changes. The assumption that the level of pyruvic acid in nervous tissue would parallel the severity of neuronal injury lead them to propose a possible mechanism for the focal nature of the vascular lesions. This was that the nervous damage in the periventricular nuclei coincided with a marked local pyruvic acid concentration leading to acidosis, vaso-dilatation and haemorrhage. It is difficult to reconcile their statement that the severity of haemorrhage was directly proportional to the degree of neuronal degeneration since in the present experiments haemorrhages were not associated with such changes. Furthermore, there is a local increase in permeability of the blood-brain-barrier in these nuclei before haemorrhages occur and this ought to grant freer diffusion of the pyruvic acid from these nuclei to the blood. In their experiments, Prados and Swank (14) found only small haemorrhages in the brain of one of their thirteen thiamine-deficient cats, yet all showed advanced neuronal degeneration. That the products of this neural injury do not, per se, result in the production of oedema and haemorrhage was seen from the results of the thallium poisoning experiments in which neuronal degeneration was severe, and the haemorrhage was absent. The type of neuronal change seen in the present thiamine deficiency experiments conforms more to that described by Ferraro et
al (24) in their acute inanition experiments in cats, in that the neuronal changes were diffuse in distribution.

Astrogliosis occurred in these experiments prior to oedema or vascular changes. That changes in the astrocytes do contribute to the vascular lesions is probable, and can be inferred from their intimate association with the vascular endothelium of capillaries and venules as shown by Farquhar et al (25) and Dempsey et al (26) in their electron microscopic studies. Hicks (21) has demonstrated that astrocyte proliferation is adequately stimulated only by astrocyte injury. In this respect it is notable that astrogliosis was well-marked shortly before the occurrence of oedema and haemorrhage, indicating that functional injury to the astrocytes precedes the vascular lesions.

The remarkable feature of the experiments on the blood-brain-barrier of thiamine-deficient cats is the invariable inter-relationship between the occurrence of oedema and trypan blue staining, and the onset of inducible convulsions. The onset of oedema corresponds to the period at which permeability to trypan blue develops. Since the time between the imminence of convulsions, when an altered permeability had not yet developed, and their induction varied only from four to fourteen hours, this defect occurred acutely. It must be emphasized, however, that the vital-dye method of investigating cerebro-vascular permeability lacks sensitivity by reason of the particle size of the dye, and
the difficulty of detecting minor degrees of staining of the parenchyma. Despite these technical limitations, the onset of an increased cerebro-vascular permeability, while possibly less acute in development, is clearly related to the convulsive state. More sensitive methods would be required to elucidate the relationship between cerebro-vascular permeability and the occurrence of the earlier signs of central involvement. That either the oedema, per se, or its pyruvate content is responsible for the convulsions seems unlikely, since the injection of thiamine promptly produces a complete remission of nervous signs without relieving the oedema or producing an immediate fall in the elevated levels of pyruvic acid. It seems probable that thiamine, or a derivative of thiamine, participates in some other enzyme system within nervous tissue, possibly in relation to the transmission of impulses. Evidence that thiamine might possess a separate neurophysiological function was supplied by Sebrell and Harris (29) who cite the experimental work of DeJong (30) on thiamine-deficient pigeons. DeJong proved that acute polyneuritis developed before the rise of blood pyruvate and disappeared, following the administration of thiamine, before the pyruvate level returned to normal. Wooley and White (27) demonstrated that by using the anti-thiamine, pyri-thiamine, the nervous signs of thiamine deficiency in white mice were unaccompanied by pyruvate accumulation. Eusebi and Cerecedo (31) proceeded
to demonstrate in mice that nervous signs were not attributable to the disordered carbohydrate metabolism of thiamine deficiency. These authors, unfortunately did not publish a description of the neuropathology. That convulsions are not necessarily secondary to the cerebro-vascular lesions in thiamine deficiency is suggested by the work of Jasper and Erickson (6) who investigated the relationship between experimentally induced epileptiform discharge and the changes in blood flow, pH and polarisation. These workers concluded that electrically induced excessive neuronal activity resulted in increased acidity of the blood, an increased flow and vascular dilatation, and that the initiation of the epileptiform discharge was usually primary to pH and blood flow changes.

The principal weakness of the results of the second part of the cerebro-vascular permeability experiment was the need to identify the onset of increased permeability only by the clinical behaviour of the animal. By injecting two different coloured dyes, one when convulsions were first induced, and the other at the specified time after the injection of thiamine, it was hoped to avoid this inaccuracy by providing visual evidence that altered permeability had, in fact, occurred. Congo red in a one per cent aqueous solution was used. This dye resembles trypan blue in being unable to cross a normal blood-brain-barrier. However, it proved impossible to determine the
faint pink staining of Congo red when obscured by that of trypan blue. A soluble ferrous-oxide was substituted for the Congo red solution. It was hoped that having noted the presence or absence of the trypan blue staining, the onset of oedema could be identified by the Prussian blue reaction of the sliced brain tissue when immersed in a solution of potassium ferrocyanide. Since neither blue staining proved to be intense, the necessity of distinguishing between the two shades of blue was abandoned as impracticable. Furthermore, the individual response to the curative administration of two milligrams of thiamine could not be estimated, either in its adequacy or rapidity of action. It follows then, from the tabulated results, in the case of cats one, two, three, and four, that the parenchymal staining could have been due to haemorrhage which developed before or after the vitamin B₁ injection. Similarly the results in the case of cats five, six and seven are invalid since it was not definitely established that a defect in the blood-brain-barrier had developed before the administration of thiamine.

The vascular changes observed in the present series of experiments have been shown in Experiment IV to occur in capillaries and venules. The congestion which was seen in the earlier stages of development of the lesion was part of a generalized vascular congestion of the brain. It has been shown that the sudden dilation and exudative changes which occurred were preceded by changes in the
Since the blood vessels affected do not have basement membranes as peri-capillary sheaths (Dempsey et al. (26)), it would seem possible that the statement made by Broman et al. (32) that the intima is the controlling factor in cerebro-vascular permeability, is open to question. The localization of the lesions of thiamine deficiency is still unexplained.
SUMMARY AND CONCLUSIONS

The experimental production of the encephalopathies of thallium poisoning and thiamine deficiency in cats has been described together with the associated clinical behaviour and neuropathology. Observations on the permeability of the blood-brain-barrier to trypan blue were made in both conditions. The type of vessels involved in the cerebral vascular changes of thiamine deficiency were investigated.

The results obtained from these experiments are considered to support the following conclusions:

1. That thallium poisoning in cats does not resemble thiamine deficiency in that animal, in clinical behaviour or in neuropathology.

2. That concurrent thallium poisoning in cats does not influence the distribution or severity of the vascular lesions of thiamine deficiency.

3. That the blindness associated with chronic thallium poisoning in cats is due to retinal ganglion cell degeneration of an unusual type.

4. That the vascular lesions in thiamine deficiency are preceded by functional injury of astrocytes.

5. That distinct morphological changes in the neurons of the periventricular nuclei do not occur in thiamine-deficiency encephalopathy in the cat.
6. That a focal increased cerebro-vascular permeability precedes the development of oedema and haemorrhage of thiamine deficiency.

7. That the increased cerebro-vascular permeability coincides with the passage of trypan blue to the brain parenchyma and with the onset of convulsions.

8. That the focal haemorrhages of thiamine-deficiency encephalopathy in cats derives from capillaries and venules.

9. That peripheral neuropathy is not a feature of thiamine deficiency in cats.
REFERENCES


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