NEUROPATHOLOGY OF EXPERIMENTAL VITAMIN DEFICIENCY *

A REPORT OF FOUR SERIES OF DOGS MAINTAINED ON DIETS DEFICIENT IN THE B VITAMINS

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INTRODUCTION

Recently a number of workers have tried to produce lesions of the nervous system in animals with diets deficient only in the vitamin B complex or in one portion of it. The divergent results of these observations [1, 2, 3] may be due to several causes. Diets made up of natural foods vary in composition in a manner that is at present little understood; thus, only diets made up of artificial foods can be relied upon to produce the desired vitamin deficiency. Furthermore, different species of animals vary greatly in their capacity to live on a deficient diet without manifesting symptoms. The rat, for example, if development of polyneuritis is used as a criterion, has been shown to be quite resistant to the effects of a diet deficient in vitamin B. Even animals of the same species vary in their susceptibility to deficient diets. The type of food on which the animal has been living before the beginning of the experimental regimen may account to some extent for these individual differences. Finally, the length of time that an animal lives on the deficient diet is a consideration that has not been sufficiently stressed. When an animal is completely deprived of vitamin B, it loses its appetite and may die rapidly of starvation. Under these conditions, Woollard [4] and, later, Kon and Drummond,[5] have concluded that the minor nervous system lesions which they have been able to discover can be reproduced by inanition and cachexia alone. Their animals, however, appear to have

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been deprived of vitamin A at the same time that they were starved, and this may account for the lesions.[6]

Cowgill [7, 8] has done extensive work in the development of artificial diets, and has produced severe nervous symptoms in dogs fed on an artificial diet almost entirely deficient in the vitamin B complex, but presumably adequate in every other respect. Goldberger and others,[9] working with dogs, have been able to show that dried yeast contains two components, one of which is heat labile, the antineuritic factor, vitamin B_1 or F, and the other heat stable, which they considered the pellagra-preventing factor and which is now known as vitamin B_2 or G. Stern and Findlay [10] studied two series of rats fed on diets deficient in vitamins B_1 and B_2, respectively. They found early degeneration of the myelin in the peripheral nerves and chromatolytic changes in the ganglion cells of the spinal cord when vitamin B_1 was lacking, and only vacuolization with lipochrome deposits in the ventral horn cells of the spinal cord when there was a deficiency of vitamin B_2. Because of the resistance of rats, it is surprising that these authors were able to find so much evidence of pathological changes in their animals.

Gildea, Kattwinkel and Castle,[11] using a diet described by Cowgill [7] as deficient in the vitamin B complex, were able to reproduce the nervous system symptoms in dogs which Cowgill had reported as due to polyneuritis. They emphasized the fact that the symptoms suggested disturbance mainly of the central, rather than of the peripheral nervous system. In their first series of dogs maintained on the Cowgill diet, deficient in both vitamins B_1 and B_2, the affected animals were repeatedly treated with vitamin B concentrates * until they improved, and then were allowed to develop symptoms again. After a number of such therapeutic attempts the process became irreversible and large doses of the concentrate no longer brought about a recovery. Grinker,[12] working with rats, has questioned the severity of these symptoms, and the existence of a definite spastic paralysis. Cowgill [7] published, in 1921, pictures of dogs that had developed "paralysis" while subsisting on a diet of the same type as the one employed in these experiments. In Figure 1, pictures taken from a motion picture film of the dogs studied by Gildea and his associates are presented as a final answer to Grinker's

* Yeast vitamin B (Harris Laboratories); alcoholic extract of wheat embryo (courtesy of Eli Lilly and Company). See appendix.

criticism. These illustrations, which represent the condition of the animals when therapy with vitamin B concentrates was no longer able entirely to clear up the symptoms, are necessarily inferior to the projected film, but they show spasticity of the legs, with marked disturbances of equilibrium upon walking or running. Even in the last stages of the condition, when in some animals there was complete loss of motor power of the hind legs, the knee jerks remained active, as shown in Figure 1*C*.

Gildea, Kattwinkel and Castle [11] reported that sections of the nervous systems of their dogs (Figs. 2, 3, 4 and 5) revealed evidence of myelin degeneration in the spinal cords, and in 3 out of 8 animals in the peripheral nerves. These lesions were first observed in the Weigert-Pal preparations. In order to confirm the findings, additional sections of the cords were stained with Spielmeyer's technique and corroboration of the lesions in the Weigert-stained sections was apparently obtained. Shortly thereafter Zimmerman and Burack [1] reported a study of the nervous systems of dogs that had rapidly developed symptoms on a similar Cowgill diet which was thought to be deficient in vitamins B_1 and B_2. They found demyelinization of the peripheral nerves, but were unable to find lesions in the spinal cord. Moreover, they observed some evidence of degeneration in the nerves of their control animals. They pointed out that the lesions in the Spielmeyer preparations of Gildea and his associates were probably artefacts. We have found Zimmerman to be correct in his criticism of some of the Spielmeyer-stained material, but we have been unable to discover any reason to question the lesions shown in Weigert-Pal preparations.

It is noteworthy that in their first series of experiments Zimmerman and Burack made no attempt to prolong the lives of the animals, or to create a state of chronic dietary deficiency. Recently they [13] have completed a study of the nervous system of 8 dogs which had been on a diet similar to the first one used by Gildea, Kattwinkel and Castle, except for the fact that they were given a B_1 concentrate (considered to be free of B_2) from the beginning of the experiment. In contrast to the first series of dogs studied by Zimmerman, these animals lived for as long as 300 days. In the 2 dogs of the series that died first, no changes in the spinal cord were observed. In the 6 animals that lived longer, the pathological changes consisted in a marked demyelinization of the peripheral nerves,

degeneration of the medullary sheaths, and replacement by gliosis of the dorsal columns of the spinal cord, particularly the fasciculi graciles. Degeneration of the medullary sheaths of the dorsal and often of the ventral nerve roots of the cord was found, and occasionally there were slight degenerative changes in many of the other fiber tracts of the cord. In contrast to their former observations it would appear that in this last series Zimmerman and Burack have reproduced in a thorough manner the *chronic* deprivation of vitamin B which was sought in our early experiments, and so have obtained corresponding pathological changes. Their observations, however, indicate that deficiency in the vitamin B_2 component may be responsible for the development of the lesions of the nervous system. In the light of our experiments and of the results of Zimmerman and Burack, the failure of Grinker and Kandel [3] to find evidence of lesions of the nervous system in rats deprived of the vitamin B complex seems probably to have been due either to the fact that these animals died in acute illness, or to the unsuitability of the species for this study.

Original Observations

Since 1928 we have made four attempts to throw light on the relation of vitamin B to lesions of the nervous system, especially of the spinal cord. The particular objective was to discover whether deficiency of vitamin B had a relation to those types of "combined system disease" encountered in pernicious anemia, in pellagra, and in chronic alcoholism. Thus, the first problem was to find out whether a disturbance of the central nervous system could be produced by deficiency of vitamin B, and whether the clinical symptoms of this syndrome could be accounted for by demonstrable pathological lesions. The present report confirms the fact that this objective was attained despite the criticisms of Zimmerman and of Grinker. The second problem was to discover whether vitamin B_1 was the deficiency involved, and whether inanition played a part in the production of the symptoms and lesions. Lastly, since the clinical condition was to a certain extent reversible, an attempt was made to determine whether or not the pathological lesions could be diminished by the use of a therapeutic agent. Observations were made throughout on the relation which peripheral neuritis bore symptomatically and pathologically to the syndrome.

Series I

Series I consisted of 8 dogs that were put on a diet devised by Cowgill [7] (Diet I)* and supposed to be deficient in the vitamin B complex. After approximately a month of this diet all of the dogs developed anorexia, listlessness or weakness. An effort was made to maintain a chronic condition in these dogs by giving them extract of wheat embryo or yeast when marked neurological signs, often including convulsions and coma, had appeared. Except when the disturbance was too far advanced, they responded within 24 hours with an initial stage of marked general improvement. Then followed a slower second stage of recovery lasting several days, during which residual spasticity or ataxia of the hind legs was gradually relieved, but with progressive difficulty in subsequent relapses. Thus in successive relapses, after the initial relief of convulsions and coma, the hind legs especially were stiff and weak, and in some animals finally became paralyzed. Although it is difficult to be certain of sensory symptoms in animals, the behavior of these dogs, their awkwardness, and the misplacement of their limbs certainly suggested that the perception of deep sensibility was abnormal (Fig. 1). In general their reflexes were normal or hyperactive, and they appeared to have neither loss of skin sensation nor tenderness over the nerve trunks. Convulsions occurred in all but Dog 1. Tetany and opisthotonos were observed. Death finally occurred following convulsions in Dogs 2, 3, 4, 5 and 7. Dogs 1, 6 and 8 died quietly. The animals lived from 2 to 8 months.

Autopsies were done as soon after death as possible. Grossly the findings were not remarkable. The alimentary mucosa was usually injected and occasionally there were minute hemorrhages. The brain and cord were usually somewhat hyperemic. Histological study of the central nervous system showed no definite cortical or cerebellar lesions in sections stained by the Weigert method, although in most cases Nissl stains showed that the cerebral nerve cells and the Purkinje cells were degenerating. Fat was present in varying amounts in the nerve cells and perivascular spaces in the cerebral cortices of all animals. The cords showed definite myelin lesions (Weigert) in Dogs 1, 2, 3, 5 and 6 and less clear-cut lesions in Dogs 7 and 8 (Figs. 2, 3, 4 and 5). The ventral horn cells were in

* For diets, see appendix.

poor condition (Nissl), and in some instances exhibited satellitosis. Fat was present in small amounts in the nerve cells of the spinal cords of Dogs 1, 2, 5, 6 and 7. The amount of fat in all of these sections was probably not significantly greater than in normal controls. Peripheral neuritis was considered present if more than 10 per cent of the fibers in a nerve trunk contained material stainable with scharlach R or with osmic acid by the Marchi technique. By this criterion Dogs 1, 3 and 8 had peripheral neuritis.

Series II

The second series of 6 dogs was kept on the same Cowgill diet, with the addition of autoclaved yeast (Diet II). It was thought that this diet was deficient only in vitamin B_1, the antineuritic vitamin. Within a month, as with Series I, the dogs showed loss of appetite, weakness and apathy. They did not seem to respond to vitamin treatment as well as the animals of Series I. This may have been due to the fact that not so much time was spent in tube feeding and other efforts to prolong life. In general they had progressive weakness and ataxia, followed by opisthotonos and convulsions with or without tetany. No paralysis was observed. The reflexes were always present, usually hyperactive, and the dogs exhibited no flaccidity or tenderness over the nerve trunks. They lived a much shorter time (an average of 2 to 3 months less) than the animals in Series I. All of them were dead within 6 to 14 weeks after the beginning of the experiment.

At autopsy the organs appeared to be normal in gross, except for the injection of vessels in the alimentary mucosa and in the brain and cord. Dogs 2 and 6 had opacity and erosion of the cornea. Dog 6 had an apparent increase in cerebrospinal fluid. Histological study of the central nervous system showed a questionable myelin lesion (Weigert) in the cortex of Dog 1, which was corroborated by a clearly positive fat stain. A definite lesion (Weigert) occurred in the cerebellum of Dog 6, as well as a lesion of the pyramidal tract in the medulla. Nerve cells in the cortex and cerebellum were found to be undergoing degenerative changes, but to a lesser degree than in Series I. Fat was present (scharlach R stain) in small amounts in the cortices of Dogs 1, 3 and 6, and in the cerebella of Dogs 2, 3 and 6. Definite lesions (Weigert) occurred in the cord of only 1 ani-

mal, Dog 6, which lived approximately 3 months (Fig. 6). The cresyl violet-stained section from the same cord showed gliosis. Practically no fat was found. Peripheral neuritis occurred in Dogs 2 and 6. In short, only 2 of the 6 animals had significant central nervous system lesions.

Series III

Series III was designed to determine what relation inanition bore to the clinical and pathological conditions observed in Series I and II. Seven dogs (1 to 7) were placed on the original Cowgill diet, deficient in the vitamin B complex (Diet I). Three additional animals (2A, 3A and 4A) were given daily amounts of a similar diet with the addition of 4 per cent by weight of granulated unheated yeast (Diet II) equal in weight to the amount of the vitamin B-deficient food eaten the previous day by Dogs 2, 3 and 4, respectively. Dogs 4 and 4A were each given also 8 cc. of cod liver oil daily. No attempt was made to prolong life by treatment with vitamin B concentrates.

With the exception of Dog 3, all 7 of the animals that were on diets deficient in the vitamin B complex (Diet I) showed the symptoms described before — anorexia, spasticity and convulsions. In general they showed less ataxia than did the dogs of Series I and II, and their symptoms were less severe. They lived from 32 days to 4 months, and all but Dogs 1 and 5, which were found dead, were killed with chloroform. Dog 5 had had very severe symptoms and lived $3\frac{1}{2}$ months. Dog 3 showed no symptoms except mild loss of appetite. It was killed after $3\frac{1}{2}$ months, together with its control, Dog 3A. Because Dog 3 had not had much anorexia, Dog 3A had not had much inanition. Nevertheless, Dog 3A had been spastic, with exaggerated reflexes, for about a week before it was killed.

The autopsies were essentially negative in gross. Dogs 1 and 4 had corneal opacity and a purulent conjunctival discharge. Pneumonia was found in Dogs 2 and 4. In Dog 1 there was blood in the stomach and intestines. Histopathological changes in the cord were entirely absent except in Dog 5. Since this dog was found dead, and in rigor, the myelin change was probably the result of postmortem autolysis. Dogs 2, 3 and 4 showed definite peripheral neuritis, which is interesting in view of the fact that reflexes were absent in Dog 2. None of the animals showed tenderness over the nerve trunks.

Series IV

Series IV consisted of 10 dogs kept on the original Cowgill diet (Diet I) deficient presumably in the entire vitamin B complex. The experiment was designed to determine to what extent the pathological process was reversible. We hoped to demonstrate lesions in the animals that became sick and were allowed to die untreated, and either smaller lesions or none at all in the animals that were treated with tiki-tiki * during the acute phases of the illness, and with yeast (Diet II) every day during the following period. These dogs, unlike those of Series I, were not allowed to relapse repeatedly, but were treated as soon as convulsions appeared and continuously thereafter.

Four of the animals, Dogs 2, 3, 4 and 10, developed symptoms within 54 to 95 days after the beginning of the experiment. The symptoms, as before, were anorexia, spasticity, ataxia, paralysis, and finally convulsions. Therapy consisted in giving from 3 to 4 cc. of tiki-tiki in each case and in supplementing the diet from then on with 1 gm. of granulated yeast daily per pound of dog. The 4 dogs lived on this diet from 69 to 105 days, and all finally showed complete clinical recovery. They were killed with chloroform. The untreated animals, Dogs 1, 5, 7 and 8 became ill within 58 to 88 days and, with the exception of Dog 5, showed all the symptoms listed above. Dog 5 was extremely spastic after 67 days, but died without having convulsions. Dogs 7 and 8 were chloroformed. Dogs 6 and 9 showed no symptoms except a slight amount of anorexia.

The autopsies, as usual, were not remarkable upon gross inspection. Histological study showed no myelin change in any cord that we could not duplicate in our series of controls. Poliomyelopathy, consisting in disintegration of nerve cells, or in satellitosis with degenerative changes in most of the nerve cells, occurred in all of the dogs to a significantly greater degree than was found in our series of control animals. We could not demonstrate any less poliomyelopathy in the cured animals or in the symptomless dogs than in the dogs that died in acute illness. Fat stains were negative throughout. Peripheral neuritis was present only in Dog 4, and was minimal.

* See appendix.

Discussion

Of the four groups of animals, Series I, given a diet deficient in the entire vitamin B complex, was the only group in which definitive myelin lesions were consistently found in the spinal cords. These dogs were the only ones in which repeated treatments with vitamin B concentrates were used to prolong life. Since in Series III and IV an exactly similar diet was given to 17 animals, but with no particular attempt to create a chronic deficiency, so that the animals died sooner, it is reasonable to conclude that a prolonged dietary deficiency is necessary in order to produce demyelinization and other lesions of the central nervous system in dogs. This fact very possibly explains the failure of Grinker and Zimmerman to confirm our original observations. As mentioned above, however, Zimmerman has recently found marked lesions in dogs which were fed with great care and persistence until they finally died at the end of about 300 days. These animals were given a similar diet but with the addition of a vitamin B_1 concentrate from the beginning of the experiment. Deficiency of vitamin B_1 is thus apparently not the basis of the morphological lesions.

In Series II deficiency of vitamin B_1 was found to produce severe disturbances, with a clinical picture including convulsions and coma. As in Series I, and as had previously been observed by Cowgill,[7] the administration of vitamin B concentrates had a rapidly beneficial effect. This result is in agreement with the belief of Findlay,[14] Kinnersley and Peters,[15] and Gavrilescu and Peters [16, 17] that the syndrome produced by vitamin B_1 deficiency must, in part at least, be accounted for by a "functional" disturbance of the central nervous system, *i.e.* by one not demonstrable by our *present-day histological methods*.

In Series I and II we were not able to demonstrate lesions (Weigert) in the cortex, although in 1 animal (Dog 6, Series II) we found a definitive myelin change (Weigert) in the cerebellum. The presence of fat in the cerebral cortices, both in nerve cells and in scavenger spaces, of all the dogs of Series I and in 3 of the dogs of Series II leads us to believe that there were definite lesions present. The condition was probably not sufficiently advanced to produce myelin lesions. Cord lesions, which occurred in similar areas in a number of sections, were demonstrated by the Weigert-Pal technique in 7 of

the 8 dogs of Series I but in only 1 of the 6 dogs of Series II (Figs. 2, 3, 4, 5 and 6). It was impossible to check these lesions with any other stain. The fat-stained sections were invariably negative, and the Nissl-stained sections showed only minimal changes. Mucicarmine stains were done on both series and showed nothing abnormal. Marchi stains unfortunately were not done.

In Series III and IV, despite acute symptoms, little or no morphological evidence of myelin lesions was observed. Because of the symptoms of Dogs 3 and 3A (Series III) we conclude that either inanition may produce a small part of the clinical syndrome (spasticity), or else a diet that has an adequate vitamin content for most animals may be deficient for certain individuals. In Dogs 2, 3 and 4 of this series histological peripheral neuritis is shown to bear an entirely inconstant relation to the clinical symptoms, having been found in the 3 animals that exhibited, respectively, flaccidity, no symptoms and spasticity.

Conclusions

1. Seventeen dogs given a diet deficient in vitamin B (Cowgill) developed signs of acute disturbance of the central nervous system and died without treatment with vitamin B concentrates. Only minimal histological changes were found in the central nervous system.

2. Eight dogs given a similar diet, but whose acute neurological signs were repeatedly and temporarily relieved with vitamin B concentrates, developed gradually a residual degree of spastic ataxia and eventually motor paralysis, with reflexes present. Definitive histological lesions of the central nervous system were found in all but 1 animal.

3. Nissl stains of the cerebral and Purkinje cells and of the ventral horn cells revealed evidence of degeneration. Weigert-Pal stains of the spinal cords showed definite losses of myelin in 7 dogs. The peripheral nerves of 3 dogs showed an increase of material staining with scharlach R or with the Marchi technique.

4. The results of observations on the effect of partial starvation, of supplements of cod liver oil, and of therapy with dried yeast on morphological changes in the central nervous system were rendered inconclusive, probably because the basic deficiency was not sufficiently prolonged to produce morphological changes in the nervous system of any of the animals in such experiments.

APPENDIX

Diet I [7]

20 gm. of this diet were given per kilo of body weight

	gm.
Commercial casein water-washed grade	6.3
Cane sugar	4.5
Butter fat	1.1
Lard	2.8
Bone ash	0.4
Salt mixture, Karr [15] *	0.2
Water	4.7

* Sodium chloride, 10 gm.; calcium lactate, 4 gm.; magnesium citrate, 4 gm.; iron citrate, 1 gm.; Lugol's solution, few drops.

Diet II

Basal diet as above, with the addition of 4 per cent by weight of autoclaved Fleischmann's yeast

. . . . . .

Washed and purified casein from A. H. Thomas Company, and Adler Company, Philadelphia, Pa.
Dried yeast, Fleischmann Yeast Company, Boston, Mass.
Bone ash, Howe and French, Boston, Mass.
Yeast vitamin B extract, Harris Laboratories, Tuckahoe, N. Y.
Alcoholic extract of wheat embryo, Eli Lilly and Company, Indianapolis, Ind., supplied by Dr. G. H. A. Clowes.
Tiki-tiki, alcoholic extract of rice polishings, Bureau of Science, Manila, P. I.

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DESCRIPTION OF PLATES

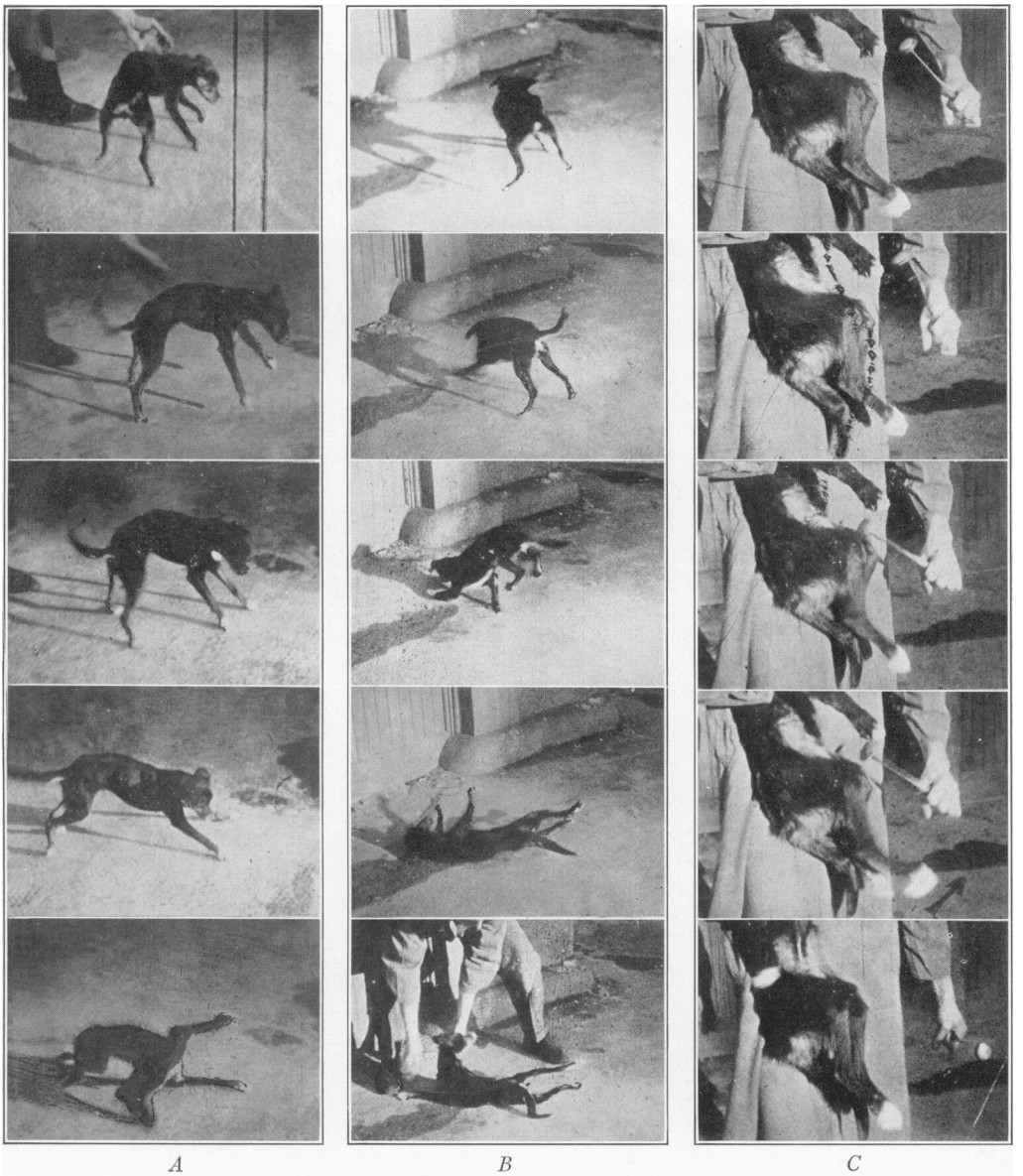
PLATE 92

FIG. 1. Single exposures taken at short intervals from a motion picture film of Dogs 2, 5 and 6 of Series I. The pictures demonstrate the spasticity of the legs, with marked loss of equilibrium on walking or running, and the retention of the knee jerk with complete motor paralysis in the final stages of the condition.

(*A*) Dog 5 with wide base of hind legs and spasticity. Note that the dog is jumping clear of the ground in the second picture, and after landing on rigidly extended legs attempts to run, but falls over in a rigid attitude.

(*B*) Dog 5 demonstrates particularly well the spasticity of the hind legs, which are shown lifted completely off the ground in the third picture, before falling.

(*C*) Dog 6 in the final stage, with complete motor paralysis of the hind legs. Note, however, that the knee jerk is present, causing blur in the fourth picture.



A B C

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PLATE 93

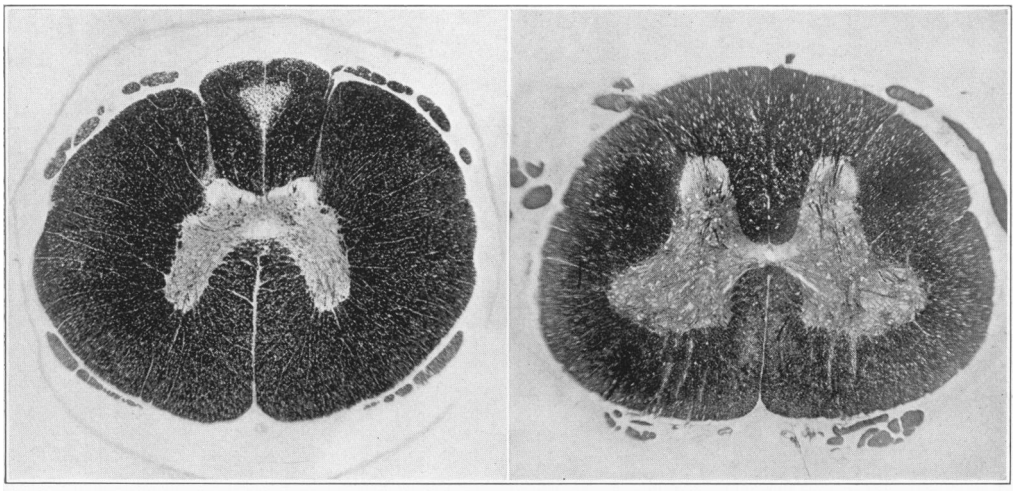
FIGS. 2, 3, 4 and 5. Photomicrographs of sections from the cords of 4 dogs of Series I which died after 4 to 6 months on a diet deficient in the vitamin B complex. Weigert-Pal stain. × 10.

FIG. 2 (Dog I, Series I). Shows a symmetrical, circumscribed loss of myelin in the dorsal columns.

FIG. 3 (Dog 7, Series I). Shows a more diffuse but definite loss of myelin without symmetrical distribution except in the uncrossed pyramidal tracts along the anterior fissure.

FIG. 4 (Dog 6, Series I); and FIG. 5 (Dog 2, Series I). Show definite but irregularly distributed loss of myelin.

FIG. 6. Photomicrographs of two sections from the same block of the cord of Dog 6, Series II. This dog had lived for 3 months on a diet deficient in vitamin B_1 and died in convulsions. Both sections show a loss of myelin in the same area. The same lesion was observed in fourteen other sections from the same block. Weigert-Pal stain. × 10.



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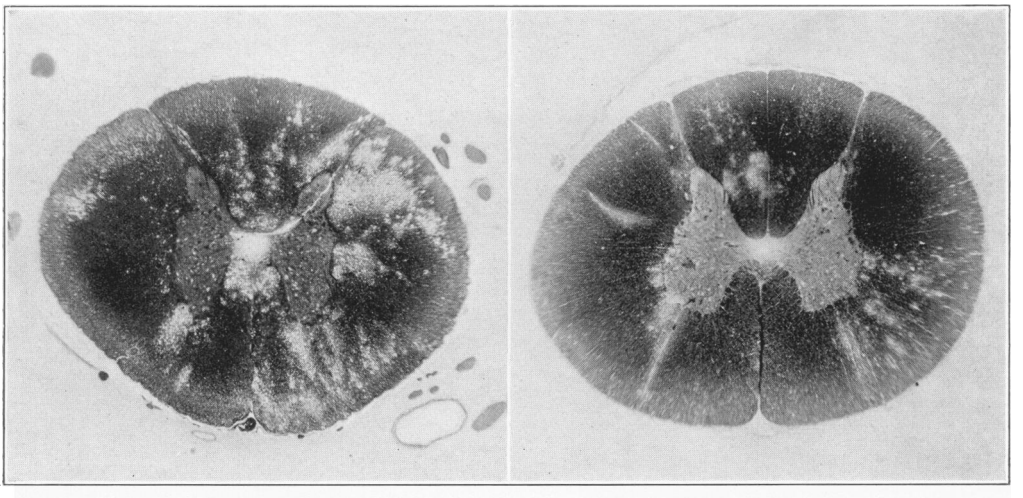

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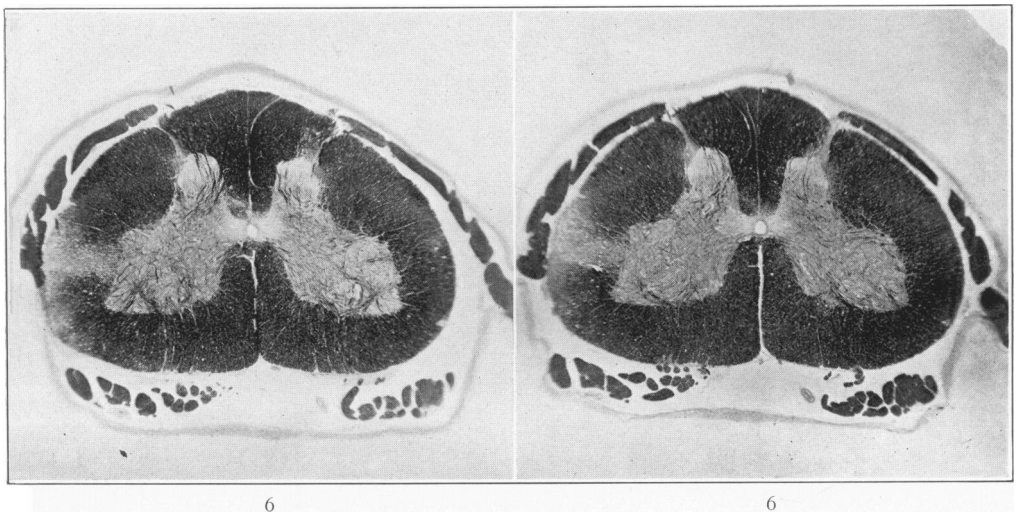

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