A COMPARISON OF THE EFFECTS OF PYRIDOXINE AND PANTOTHENIC ACID DEFICIENCIES ON THE NERVOUS TISSUES OF SWINE*

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During the past 6 years, experiments have been reported from this laboratory on swine in which deficiencies in vitamins of the B complex have been produced. Of particular interest have been the studies on the relationship of vitamin deficiency to the integrity of nervous tissues. It was first shown that degeneration of the peripheral nerves, dorsal root ganglion cells, and posterior columns of the spinal cord could be produced when young swine were fed a basal diet (crude casein, sugar, lard, salts, and cod liver oil) supplemented only with crystalline thiamin and riboflavin (1). Although the inclusion of nicotinic acid in this diet led to some improvement in growth, the nerve, ganglion cell, and spinal cord changes continued to appear (2). When other vitamins of the B group became available, it was shown that good growth could be obtained and neural lesions could be prevented if choline, pyridoxine, and pantothenic acid were added to the basal diet already supplemented with thiamin, riboflavin, and nicotinic acid (3). Finally, it was established that lesions could be produced in sensory neurons when pyridoxine and/or pantothenic acid were excluded from the diet (3).

In the above experiments a system of grading was used to evaluate the degree of damage found microscopically in the peripheral nerves, spinal ganglia, and spinal cord. One plus (+) indicated myelin degeneration of the peripheral nerves only. When, in addition, chromatolysis or necrosis of the dorsal root ganglion cells was found, the severity was designated two plus (++). Myelin degeneration of the dorsal nerve roots in addition to the above led to classification of the lesions as three plus (+++) and when posterior column degeneration was also present the changes were regarded as being of maximum severity or four plus (++++). This classification was based on the concept that the degenerative lesions progressed centrally along the peripheral nerves to the ganglion cells, dorsal roots, and thence into the posterior columns.

In those experiments (3) in which nervous changes were produced by uncomplicated pyridoxine or pantothenic acid deficiency it became apparent that

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such a classification was not either entirely accurate or adequate, since a somewhat different morphological pattern appeared in animals deficient in one or the other of these two vitamins. For instance, chromatolysis of the dorsal root ganglion cells might be extensive in pantothenic acid-deficient animals even though myelin degeneration could not be demonstrated by suitable methods in the peripheral nerves. On the other hand extensive myelin degeneration was encountered in pyridoxine-deficient animals in the absence of chromatolysis of dorsal root ganglion cells.

The experiments to be reported here were undertaken to compare the effects of pyridoxine and pantothenic acid deficiency on the nervous tissues of swine. The demonstration that these vitamins may have specific actions on sensory neurons has obvious implications for some of the fundamental problems of neurophysiology and neuropathology.

Materials and Methods

Observations were made on 2 separate groups, totalling 45 swine of both sexes. One group (series I) was placed on the deficient diet at about 32 days of age, the other (series II) at about 20 days of age.

Full details of the experimental methods have been described elsewhere (3). When the pigs were received in the laboratory they were placed on the basal diet: Sheffield "new process" casein, 26.1 per cent; sucrose, 57.7 per cent; lard, 11 per cent; salt mixture, 5.2 per cent, and Mead's blended oil. Brewers' yeast was given until the experimental deficiency was begun, at which time crystalline vitamins were administered daily in the following amounts per kilo: thiamin hydrochloride, 0.52 mg.; riboflavin, 0.13 mg.; nicotinic acid, 1.20 mg.; choline chloride, 10.0 mg.; inositol, 0.1 mg. (series II) and para-aminobenzoic acid, 0.1 mg. (series II). Varying amounts of pyridoxine and/or calcium pantothenate were administered as shown in Tables I, II, and III.

When the pigs died or were killed complete autopsies were performed as promptly as possible. Blocks of all the tissues were fixed in 10 per cent neutral formalin. The spinal cord with its attached nerve roots and dorsal ganglia was removed and suspended in a tall vessel containing 10 per cent neutral formalin. Weights were attached to long segments of brachial and sciatic nerves which were then suspended in the same manner. The brain was preserved in 10 per cent neutral formalin. Blocks of bone and marrow were fixed in Zenker-formol.

All the soft tissues of representative animals were studied microscopically after imbedding blocks in paraffin, cutting, and staining with hematoxylin and eosin. Frozen sections were made from formalin-fixed, gelatin-imbedded blocks of brachial and sciatic nerves from the level of the elbow and knee and from the cervical and lumbar spinal cord of all animals; these were stained with Scharlach R. Paraffin sections of the spinal cord to include the nerve roots and dorsal ganglia were stained with methylene blue, Bodian's silver stain (4), and Weigert's myelin stain, modified by double imbedding (celloidin and paraffin). Sections of the brachial and sciatic nerves from the region of the elbow and knee, respectively, were stained by Bodian's method (4) and the modified Weigert method.

RESULTS

Pyridoxine Deficiency (Table I)

Observations during Life.—This group comprised 13 pigs. Six (series 1, pigs 7-28 to 7-32) were placed on the deficient diet at 32 to 38 days of age and

TABLE I

Pyridoxine Deficiency

Pig No.	Age when experiment began	Days on diet Manner of death*	Average daily weight gain	Ataxia first noted‡ Day of deficiency	Treatment: Amount pyridoxine given in γ/kg. and duration of deficiency diet when administered	Treatment: Effect on ataxia	Pathological findings: Peripheral nerves§ Myelin degeneration Br	Peripheral nerves§ Myelin degeneration Sc	Peripheral nerves§ Axis cylinder degeneration Br	Peripheral nerves§ Axis cylinder degeneration Sc	Dorsal root ganglion cells‖ Chromatolysis Cer	Dorsal root ganglion cells‖ Chromatolysis Lu	Dorsal root ganglion cells‖ Atrophy Cer	Dorsal root ganglion cells‖ Atrophy Lu	Dorsal roots‖ Myelin degeneration Cer	Dorsal roots‖ Myelin degeneration Lu	Dorsal roots‖ Axis cylinder degeneration Cer	Dorsal roots‖ Axis cylinder degeneration Lu	Spinal cord‖ Myelin degeneration Cer	Spinal cord‖ Myelin degeneration Lu
							Series I													
	days		*gm.*																	
7-30	34	50D	95	0	0	−	++	++	±	±	++	++	0	0	0	0	0	0	0	0
7-33	32	64D	109	Sp, 63	0	−	+++	+++	+	+	0	0	+	+	++	++	0	0	0	0
7-31	32	79D	76	0	10,66	0	++	++	+	±	0	0	+	+	0	0	0	0	0	0
7-28	38	150D	163	Sp, 28 Def, 63	10,66;20,81	0	++	++			0	0	+	+	0	0			0	0
7-29	34	171K	122	Def, 124	20,46;10,65; 20,81	0	++	++			0	0	+	+	0	0			0	0
7-32	32	177K	273	Sp, 32 Def, 82	10,66;20,82; 20,98–116; 40,117–127; 40,137–149; 100,150–162;500, 163–177	0	+	++			0	0	0	0	0	0			0	0
							Series II													
7-43	22	32K	180	Sp, 23	0	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7-46	21	55D	292	Sp, 41	0	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7-48	15	81D	30	Sp, 43 Def, 74	0	−	++	++	+	+	0	0	+	+	0	0	0	0	0	0
7-49	16	90D	158	Sp, 40 Def, 72	0	−	++	++	+	+	0	0	+	+	+	0	0	0	0	0
7-44	22	92D	228	Sp, 68 Def, 85	0	−	+++	+++	+	+	0	0	+	+	+	+	±	±	+	+
7-47	21	101D	105	Sp, 41 Def, 72	0	−	+++	++++	++	++	0	0	++	++	++	++	+	+	+	++
7-50	16	102K	110	Sp, 35 Def, 60	0	−	+++	+++	+	++	0	0	++	++	++	++	+	+	+	++

* D means died; K, sacrificed.
‡ Sp, suspicious; Def, definite.
§ Br, brachial; Sc, sciatic.
‖ Cer. cervical; Lu, lumbar.

received small amounts of pyridoxine at irregular intervals, while 7 (series II, pigs 7-43 to 7-50), which were started on the deficient diet at 16 to 22 days of age, received no supplementary pyridoxine at any time. In 2 animals of the latter group (7-49 and 7-50) the intake of iron was restricted.

The general course of the animals of series I has been described elsewhere

(5). Briefly, pyridoxine deficiency in these swine led to a disturbance in growth, severe anemia associated with a rise in serum iron concentration, hemosiderosis of the spleen, liver, and bone marrow, epileptiform seizures, fatty infiltration of the liver, and ataxia accompanied by lesions in the nervous tissues. The pigs of series II exhibited the same signs during life and at autopsy similar lesions were found, though to a lesser degree. The following discussion will center upon the nervous findings during life and in particular upon the pathological changes in the nervous tissues at autopsy.

Suspicious disturbances in gait were noted in animals of series II after they had been on the pyridoxine-deficient diet for 3 to 6 weeks. Similar changes appeared somewhat later in the swine of series I. Definite ataxia then appeared and by the 10th week was well marked in the animals that had received no supplementary pyridoxine. The ataxia first manifests itself as a slightly high lift of the hind legs together with a swaying of the hind quarters in walking. Often the hind legs twist in one direction and another. In walking the base becomes broad or the legs fold under with the result that the pig stumbles or falls. The fore and hind legs eventually are all involved. In severe cases the animal is completely incapacitated.

Pathological Findings.—Microscopically, lesions were noted in the nervous tissues of one animal (7-30) which had been on the deficient diet for 50 days. At this time, however, the appearance of morphological changes was not consistent as another pig (7-46) showed no lesions after 55 days on the deficient regimen. Nevertheless, after the 55th day all showed lesions in the nervous tissues so it can be assumed that pathological changes may be expected after animals have been on this deficient diet for 9 to 10 weeks. When the behavior during life was compared with the anatomical changes revealed by autopsy, it appeared that suspicious physiological disturbances were present before morphological alterations could be demonstrated (7-43 and 7-46). Definite pathological changes were found in the nervous tissues of all animals in which the ataxia was noted to be "definite."

The following description of the pathogenesis of the neural lesions is based on all the animals studied in this group.

Demyelinization of the peripheral nerves (brachial and sciatic) was the earliest morphological change (Fig. 1). The nerve fibers of larger diameters seemed to be the most affected. The degeneration was characterized by the appearance of small droplets of neutral fat in the frozen sections stained with Scharlach R and by the loss of the fine reticular structure of the myelin sheath in the Weigert preparations. In the latter sections clear areas appeared and dark staining droplets were found among the fibers. Axis cylinder stains showed questionable degeneration of these structures in the earliest period of the deficiency. Definite degeneration appeared, however, very soon and it is difficult to say whether myelin degeneration preceded axonal lesions or not (Fig. 2). In the early stages myelin degeneration was found only in the peripheral nerves and was not present in the dorsal roots until much later. In the early course of the deficiency chromatolytic changes and atrophy were not conspicuous in the dorsal root ganglion cells (Fig. 3). In only one animal (7-30) was chromatolysis noted.

As time went on, that is from the 11th to the 15th week in the animals of series II and through the 26th week in series I, myelin degeneration became more prominent in the peripheral nerves (Fig. 4). In addition, marked degeneration of the axis cylinders was also encountered (Fig. 5). As noted above no chromatolysis was ever observed in the dorsal root ganglion cells (with the exception of No. 7-30). The ganglia, however, were not normal, as atrophic cells were found, especially in the later stages (Fig. 6). In the ganglia of those animals showing the severest changes, necrotic cells were found, as well as foci where cells had already undergone phagocytosis. Such foci consisted of collections of mononuclear cells. In the swine showing extensive involvement of the peripheral nerves, demyelinization was present in the dorsal root fibers as well (Fig. 7), while in several axis cylinder degeneration was also observed. The ventral roots were normal (Fig. 7). In three animals (7-44, 7-47, and 7-50) degenerative changes were found in fibers in the dorsal columns of the spinal cord (Fig. 7). Such lesions were only marked, however, in one animal (7-47). No changes were detected in the nerve cells of gray matter of the spinal cord.

The brain in pyridoxine-deficient animals has been reported upon elsewhere (5). Inasmuch as the present experiments were designed primarily to explore differences in the reaction of the sensory neuron to pyridoxine or pantothenic acid deficiencies, the brains of the animals in this group will not be reported upon.

Effect of Treatment.—Few conclusions concerning the effect of treatment with pyridoxine could be drawn from this experiment. The majority of the animals (series II) were not treated; the remainder were treated inadequately. One animal (7-32) received the most extensive supplements of pyridoxine. Lesions, evidenced by free fat in the peripheral nerve fibers, were found. The only manifestation of any effect of treatment was found in the ganglion cells; these might have been expected to be atrophic if one judged by the findings in pigs 7-28 and 7-29, yet they were not. No improvement in the ataxia was detected.

Pantothenic Acid Deficiency (Table II)

Observations during Life.—This group comprised 18 pigs. Six (series I, pigs 7-34 to 7-39) were placed on the deficient diet at 32 to 38 days of age; all were given varying supplements of pantothenic acid from time to time. Twelve animals (series II, pigs 7-51 to 7-62) were placed on the deficient diet when 16 to 22 days old. Three received no pantothenic acid whatsoever while the remainder were given daily supplements of 50γ per kilo, which were augmented in some pigs by 1.5 mg. on one or more occasions.

The general course of these animals has been described elsewhere (6). Briefly, pantothenic acid deficiency in swine led to a striking disturbance in growth, loss of hair, cough and excessive nasal secretion, changes in the tongue, diarrhea associated with ulcerative colitis, and ataxia accompanied by lesions of the sensory neurons. The following discussion will center upon the nervous findings during life and in particular upon the pathological changes in the nervous tissues at autopsy.

One of the first signs of neurological involvement is a sudden lifting of one of the limbs from the ground as though it were painful. The gait reveals a

TABLE II

Pantothenic Acid Deficiency

Pig No.	Age when experiment began	Days on diet Manner of death*	Average daily weight gain	Ataxia first noted‡ Day of deficiency	Treatment: Amount pantothenic acid given in γ/kg. and duration of deficiency diet when administered	Treatment: Effect on ataxia§	Pathological findings: Peripheral nerves‖ Myelin degeneration Br	Peripheral nerves‖ Myelin degeneration Sc	Peripheral nerves‖ Axis cylinder degeneration Br	Peripheral nerves‖ Axis cylinder degeneration Sc	Dorsal root ganglion cells** Chromatolysis Cer	Dorsal root ganglion cells** Chromatolysis Lu	Dorsal root ganglion cells** Atrophy Cer	Dorsal root ganglion cells** Atrophy Lu	Dorsal roots** Myelin degeneration Cer	Dorsal roots** Myelin degeneration Lu	Dorsal roots** Axis cylinder degeneration Cer	Dorsal roots** Axis cylinder degeneration Lu	Spinal cord** Myelin degeneration Cer	Spinal cord** Myelin degeneration Lu	Spinal cord** Ganglion cell degeneration Cer	Spinal cord** Ganglion cell degeneration Lu
	days		*gm.*																			
Series I																						
7–37	32	34D	−19	—	40,18–33	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0
7–38	32	64D	59	Def, 59	80–320,18–64	0	++	++	±	±	+++	+++	0	0	0	0	0	0	0	0	0	0
7–36	32	181K	137	Sp, 55 Def,100	20–80,18–111;500–1500,111–181	Sl Imp	0	0			0	+	0	0	0	0			0	0	0	0
7–34	38	190K	20	Sp, 55 Def,169	80–50,50–181	?	0	0			+	++	0	0	0	0			0	0	0	0
7–39	32	198K	92	Sp, 96 Def,128	640–60,18–181	?	0	0			+	+	0	0	0	0			0	0	0	0
7–35	34	364D	83	Sp, 82 Def,114	10–60,18–169;500, 169–272	Imp	0	0	0	0	+++	+++	+	+	0	0	0	0	0	0	0	0
Series II																						
7–51	22	18K	100	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7–54	21	19K	225	0	50,1–19	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7–52	19	32K	116	Sp, 25	0	—	0	0	0	0	+	++	0	0	0	0	0	0	0	0	0	0
7–53	20	44D	51	Sp, 23 Def, 41	0	—	0	0	0	0	+++	+++	+	+	0	0	0	0	0	+	+	
7–55	22	31K	190	0	50,1–31	—	0	0	0	0	++	+++	0	0	0	0	0	0	0	0	0	0
7–57	21	41D	200	Sp, 37 Def, 40	50,1–41	—	0	0	0	0	++	+++	0	0	0	0	0	0	0	0	0	0
7–61	16	49D	86	Sp, 32 Def, 41	50,1–49; 1500,41	0	++	++	0	0	+++	+++	+	+	0	0	0	0	0	0	0	0
7–58	17	54D	118	Sp, 41 Def, 53	50,1–54; 1500,41	0	++	+++	+	+	++	++	++	++	0	0	0	0	0	+	+	
7–56	20	58D	150	Sp, 33 Def, 42	50,1–58; 1500, 42	0	++	++	+	+	++	++	+	+	0	0	0	0	0	0	0	0
7–59	16	66D	91	Sp, 34 Def, 45	50,1–66; 1500,39, 54;64	0	++	++	+	+	+	+	0	0	0	0	0	0	0	0	0	+
7–62	21	99D	90	Def, 32	50,1–99; 1500,41, 56,66	0	++	++	+	+	+++	+++	0	0	++	++	+	+	+	+	+	+
7–60	16	117K	113	Sp, 47 Def, 60	50,1–117; 1500,41; 56,66	0	++	++	+	+	+	+	+	+	0	0	0	0	0	0	0	0

* D means died; K, sacrificed.

‡ Sp, suspicious; Def, definite.

§ Sl, slight; Imp, improvement.

‖ Br, brachial; Sc, sciatic.

** Cer, cervical; Lu, lumbar.

broadening base and a jerky, almost military "goose step" appears. The twisting of the legs seen in pyridoxine deficiency is usually absent. Eventually the pig is unable to stand or walk. Since the animals in series I were given varying supplements of calcium pantothenate, they will not be included in gauging the time of onset of the ataxia. Suspicious disturbances in gait were observed during the 4th week in animals receiving no supplements of the vitamin (7-52 and 7-53). On the average similar signs appeared a little later in the pigs whose diet had been supplemented with 50γ of calcium pantothenate. Definite ataxia appeared in these animals after the 5th week.

Pathological Findings.—Morphological changes were observed in 2 animals (7-37 and 7-55) in which no signs of ataxia had appeared. On the other hand no lesions could be detected in the 2 remaining swine which failed to exhibit abnormalities in gait (7-51 and 7-54). Lesions were found in the rest of the animals, all of which had ataxia of varying degree. The observations in the early stages of the deficiency would indicate that pathological changes may precede any objective evidence of abnormal gait.

The following description of the pathogenesis of the nervous lesions is based upon observations on all the animals in this group.

The earliest morphological change was found in the dorsal root ganglion cells. These cells exhibited classical signs of chromatolysis (Fig. 9). The Nissl bodies became finer and finally dissolved leaving a homogeneous ground substance. This process in most instances began in the center of the cell and proceeded peripherally. No cell type seemed affected more than any other; both large and small ganglion cells were equally involved. In many cells the nucleus was displaced from the center to the periphery where it assumed an oval or flattened shape. These cells then became necrotic and their remnants were removed by macrophages. Other cells were observed which had become atrophic. Such cells were not as numerous, however, as those seen in pyridoxine-deficient animals. During the early stages of the deficiency when changes were observed in the ganglion cells no alterations in the peripheral nerves or dorsal roots could be demonstrated (Fig. 8). Later, however, that is from the 49th day on (series II) with increasing changes in the dorsal ganglia (Fig. 10) loss of myelin was found in the peripheral nerves of all animals (Fig. 11). Axis cylinder degeneration was present as well (Fig. 12). In only one pig (7-62) were similar areas of demyelinization encountered in the dorsal roots (Fig. 13). This animal was the only one in the entire group to exhibit lesions in the white matter of the spinal cord; here fibers that had lost their myelin sheaths were found in the dorsal columns (Fig. 13). Changes in the ganglion cells of the spinal cord were found in 4 swine (7-53, 7-58, 7-59, and 7-62). Chromatolytic cells were present in the anterior horns and in the intermediate gray matter as well (Fig. 14). It might be mentioned that collections of minute, heavily staining bodies just outside the membrane of the anterior horn cells which have been reported in a previous publication (2) were not observed.

Effect of Treatment.—In one animal of series I (7-35) sufficient supplements of pantothenic acid were given to diminish the signs of ataxia. Marked chromatolytic changes were found in the dorsal root ganglion cells of this animal. No demyelinization of the peripheral nerves could be demonstrated. The influence of the treatment on the lesions of this pig is not at all clear.

In one other animal (7-36) slight improvement was noted. Too little calcium pantothenate was given the other animals in the group and the changes noted were too slight to draw any conclusions.

TABLE III

Pyridoxine and Pantothenic Acid Deficiency Combined

Pig No.	Age when experiment began	Days on diet Manner of death*	Average daily weight gain	Ataxia first noted‡ Day of deficiency	Treatment: Amount vitamin given in γ/kg. and duration of deficiency diet when administered§	Treatment: Effect on ataxia	Pathological findings: Peripheral nerves‖: Myelin degeneration: Br	Peripheral nerves‖: Myelin degeneration: Sc	Peripheral nerves‖: Axis cylinder degeneration: Br	Peripheral nerves‖: Axis cylinder degeneration: Sc	Dorsal root ganglion cells**: Chromatolysis: Cer	Dorsal root ganglion cells**: Chromatolysis: Lu	Dorsal root ganglion cells**: Atrophy: Cer	Dorsal root ganglion cells**: Atrophy: Lu	Dorsal roots**: Myelin degeneration: Cer	Dorsal roots**: Myelin degeneration: Lu	Dorsal roots**: Axis cylinder degeneration: Cer	Dorsal roots**: Axis cylinder degeneration: Lu	Spinal cord**: Myelin degeneration: Cer	Spinal cord**: Myelin degeneration: Lu
	days		*gm.*																	
7-21	36	15D	53	0	0	0	0	0	0	0	+	+	+	+	0	0	0	0	0	0
7-20	36	63D	14	0	Pan.59,26-63	0	+	+	0	0	++	++	+	+	0	0	0	0	0	0
7-24	33	90D	60	Sp, 63	Pan.59,25-80	0	+	+			+	+	+	+	0	0			0	0
7-19	38	146D	18	Sp,110	Pan.54,26-146	0	++	++			+	+	+	+	0	0			0	0
7-22	36	149D	17	Sp, 90 Def,121	Pan.52,26-152	0	+++	+++			+	+	++	++	0	+			0	+
7-23	34	171D	29	Sp, 73 Def, 94	Pan.57,26-171	0	++	++	+	+	+	++	+	+	+	+			+	+
7-25	32	193K	53	Sp, 90 Def,104	Pan.54,26-167;500, 167-193; Pyr.200, 167-193	0	+	+			+	+	+	+	+	+			+	+
7-18	38	210K	41	Sl, 82 Def, 206	Pan.54,26-164;500, 164-210	0	++	++			+	+	++	++	0	0			0	0

* D means died; K, sacrificed.

‡ Sl, slight; Sp, suspicious; Def, definite.

§ Pantothenic acid given in average amounts and time periods are shown. Pyr. refers to pyridoxine.

‖ Br, brachial; Sc, sciatic.

**Cer, cervical; Lu, lumbar.

Pyridoxine and Pantothenic Acid Deficiency Combined (Table III)

Observations during Life.—This group consisted of 8 animals. All but one (7-21) were given varying amounts of calcium pantothenate. Only one (7-25) received any pyridoxine. The duration of the deficiency varied from 15 to 210 days. In the 2 animals coming to autopsy at 15 and 63 days, no disturbances in gait could be detected. In the third animal dying at 90 days and in the rest from then on ataxia was present.

Pathological Findings.—Lesions were found in all the animals of this group. The changes were of the same morphological character as those observed when either pyridoxine or pantothenic acid were lacking. At 15 days chromatolysis and atrophy of the dorsal root ganglion cells were present though demyelinization could not be detected. After the 63rd day demyelinization of the peripheral nerves was present in all animals and loss of myelin was found in the dorsal roots of some. Several of those living longest also exhibited demyelinization in the posterior columns of the spinal cord.

Controls

Six animals served as controls. All were given pyridoxine and pantothenic acid in adequate amounts. Their total food intake was restricted, however, to control the factor of inanition. No disturbances in gait were noted. Microscopic examination of the nervous tissues showed no lesions.

DISCUSSION

These experiments confirm previous studies (3) from this laboratory and show that diets containing inadequate amounts of pyridoxine or pantothenic acid or both lead to degeneration of the sensory neurons of swine. A different morphological pattern has been observed in the two groups, however, especially when the pathogenesis of the lesions was studied during the early stages. Myelin degeneration of the peripheral portion of the sensory nerve appeared to be the initial change in pyridoxine-deficient animals. Axis cylinder degeneration almost immediately became apparent as well. Chromatolysis of the dorsal root ganglion cells was extremely inconspicuous, either early or late in the course of the deficiency. Atrophy, followed by necrosis and neuronophagia, was observed without supervening chromatolytic phenomena. In contrast, the initial morphological change found in animals whose diet was deficient in pantothenic acid has been pronounced chromatolysis of the dorsal root ganglion cells. Atrophy of these cells was not prominent. Only later could evidence of myelin and axis cylinder degeneration be detected in the peripheral nerves and dorsal roots where it was never as extensive as that observed in the pyridoxine-deficient pigs. The nervous tissues of animals deficient in both vitamins revealed changes similar to those found when pyridoxine or pantothenic acid deficiency was present alone.

Before commenting on the possible significance of these findings it would seem desirable to review briefly the commoner evidences of neuron degeneration.

It is well known that the neuron can exhibit several reversible or irreversible morphological changes as a result of injury. The cell body may show chromatolysis and/or atrophy followed by necrosis; pigment and/or lipoid may be found in cells which ordinarily do not contain these substances. Then, too, damaged cells may assume unusual sizes or shapes. The axon may degenerate; the myelin sheath may

break down into less complex lipoid constituents. A number of these morphologic alterations were observed in our experimental animals: chromatolysis, atrophy, and necrosis of the cell body, as well as myelin and axon degeneration. In order to interpret these changes some mention should be made of their nature.

Chromatolysis may be produced in a number of ways: injury of axons by mechanical, electrical, or thermal trauma; certain infections, particularly those of virus etiology; a wide variety of chemical poisons; anoxia; fatigue; metabolic disturbances and many other causal factors could be mentioned. Chromatolysis may be either a reversible or an irreversible change and is morphological evidence of damage to neurons. Certain factors have been stated to influence the course of chromatolysis: the type and age of the animal; the distance from the central nervous system at which an axon is injured and the structure and function of the neuron (7). The morphological change may be primary, that is due to damage of the cell body itself, or secondary, due to injury to the myelin sheath and/or axon. Recent experiments reported by Gersh and Bodian (8) have placed the morphological phenomena observed in chromatolytic cells on a physicochemical basis.

Cells which are injured or killed need not exhibit the classical stages of chromatolysis, however. Atrophy may occur in the absence of chromatolysis and may in turn be followed by necrosis. In nerves myelin degeneration may either be primary, that is due to initial damage of the lipoid material making up the myelin sheath, or secondary, as a result of damage to either its axon or neuron. In like manner, axon degeneration can be classed as primary, due to direct injury or secondary as a result of damage to the neuron or its myelin sheath.

From this brief review of some of the pathological changes which may be encountered in neurons and based on the lesions which have been observed in our experimental animals, an orderly sequence of events need not be expected when neurons are injured in different ways. Quite the contrary, any portion of the neuron may be involved; it is likely that whichever portion is affected depends upon whatever phase of neuronal metabolism is disturbed.

The morphological changes described in this report would seem to indicate that the primary site of injury to the neuron in pyridoxine-deficient animals is in its peripheral process (myelin sheath and axon). In contrast, the initial locus of damage in pantothenic acid-deficient animals would seem to be in the cell body itself. It must clearly be borne in mind that such a conclusion is based entirely on morphological data. However, the hypothesis is an intriguing one and helps explain the differences in the pathologic pictures. Moreover, it has a basis of fact if the pathogenesis of some common neurological diseases is considered.

The "primary" diseases of the nervous system can be divided pathologically into two types: myelinoclastic and polioclastic (9). In the first group myelin degeneration is the first manifestation of the disease, followed later by axonal and cellular degeneration. Multiple sclerosis, Schilder's disease, and the vaccinial type of encephalitis are myelinoclastic diseases. In the second group

the first discernible changes are found in the neuron itself; axonal and myelin degeneration follow. Poliomyelitis, rabies, epidemic encephalitis, herpes simplex in rabbits, and damage resulting from anoxia are examples of the polioclastic disease type. According to this classification pyridoxine deficiency would fall into the myelinoclastic group while the lesions occurring in pantothenic acid-deficient swine would fall among the polioclastic diseases. Further detailed anatomical studies as well as physiological observations must be made in pigs and other animals placed on similar deficient diets before the primary point of injury to the sensory neuron can be stated with absolute certainty.

The course of the degeneration of the nerve fibers was not specifically studied in these experiments. However, since degeneration of the sensory nerve roots and dorsal columns was not seen in animals deficient in either vitamin unless well marked changes were found in the peripheral nerves, it would seem that the latter, especially in pyridoxine deficiency, are the most vunerable. This is also of interest in view of the difference in the reaction of dorsal root ganglion cells to section of their central or peripheral processes. Hare and Hinsey (10) have shown that chromatolysis does not follow section of the central process. Whether nerve degeneration which is so prominent as an early manifestation of pyridoxine deficiency begins at the very periphery of the nerve fiber and extends centrally is an important question to settle, especially in relation to Wallerian degeneration which is said to take a centrifugal course, that is the central portion degenerates more rapidly than the parts more peripheral (11). Studies of sensory nerve endings, especially the muscle receptors, in relation to the morphological alterations observed in the neuron would also be of great interest. No examination was made of the *boutons terminaux* on the ganglion cells of the spinal cord. This is another phase of the problem which requires pointed study.

Attention must be called to the changes in the spinal ganglion cells in a few of the animals deficient in pantothenic acid. Such lesions were not found in pyridoxine-deficient swine. These changes may further indicate that pantothenic acid plays a more important rôle than pyridoxine in the metabolism of the perikaryon. In view of the predominance of changes in the sensory rather than the motor neuraxis, studies on the metabolism of dorsal root ganglion cells in relation to cells from other portions of the nervous system would be of great interest and might throw some light on the predominance of the sensory neuron lesions found in our experimental animals. Himwich *et al.* (12) have reported differences in the respiration of various portions of the brain of rats. Pearce and Gerard (13) have found differences in the oxygen consumptions (Q_{O_2}) of various structural regions of the frog's brain; the highest values were found in the cerebellum. No studies of dorsal root ganglion cells were made by these two groups of investigators, however. The responses of various cell regions to anoxia, drugs, and chemical poisons also point to varying

metabolic activities in different portions of the nervous system. Variations in acetylcholine content further indicate metabolic differences (14). Whether the metabolism of sensory and motor nerves of similar diameter is the same or not is a question that has yet to be settled. The experiments described in this report would indicate this. Certain physiological responses would indicate differences since sensory fibers are more easily engaged by interaction and have a lower stimulation threshold than motor nerves (15). Employing ultraviolet microscopic technique, Gersh and Bodian (8) have shown that large dorsal ganglion cells contain larger amounts of nucleotides than anterior horn cells. This finding may be of significance in explaining the pathogenesis of the neurological changes in swine deficient in pantothenic acid.

As noted above the gait of the two groups of animals differed. Whereas pyridoxine-deficient animals showed a swaying and twisting of the legs as an early sign, those deficient in pantothenic acid rarely showed this symptom, but would lift one leg suddenly off the ground as though it were painful. The latter animals developed a jerky almost military goose step whereas those lacking pyridoxine flung their legs about in a more irregular fashion. The onset of ataxia and the development of pathological lesions were earlier in the animals deficient in pantothenic acid. There was little difference, however, between the late manifestations in the two groups of animals.

The pigs studied in these experiments did not show the fatty degeneration of ganglion cells which Truex and Zwemer (16) have found in human material from the aged and which these investigators suggested might be due to dietary deficiency.

No adequate microscopic examinations of the tissues of animals deficient in pyridoxine or pantothenic acid have been reported by other writers. Using the Marchi technique, which frequently gives questionable results, Lippincott (17) noted myelin degeneration of the sciatic nerve and spinal cord of mice placed on a pantothenic acid-deficient diet. Street *et al.* (18), in dogs deficient in pyridoxine, noted degenerative changes in the myelin sheaths in the spinal cord and peripheral nerves. In neither of the above reports was there any mention of examination of the dorsal root ganglion cells.

In view of the common acceptance of thiamin as "the antineuritic vitamin" it would seem worthwhile to mention that we have failed to produce lesions of the nerves in swine deficient in this vitamin (19, 20).

SUMMARY

1. When pigs were fed diets deficient in pyridoxine or pantothenic acid, ataxia developed and lesions were found in the sensory neuron.

2. The morphological pattern of the lesions differed in the early stages of the two deficient states. Degeneration of the peripheral process of the sensory neuron was the initial and most prominent feature in pyridoxine deficiency.

Chromatolysis was the first evidence of damage to the afferent neuron in pantothenic acid-deficient animals.

3. The possible significance of this evidence of damage to different portions of the sensory neuron is discussed.

Vitamins used in these experiments were furnished by Merck and Company, Incorporated, and yeast and cod liver oil by Mead Johnson and Company.

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EXPLANATION OF PLATES

PLATE 28

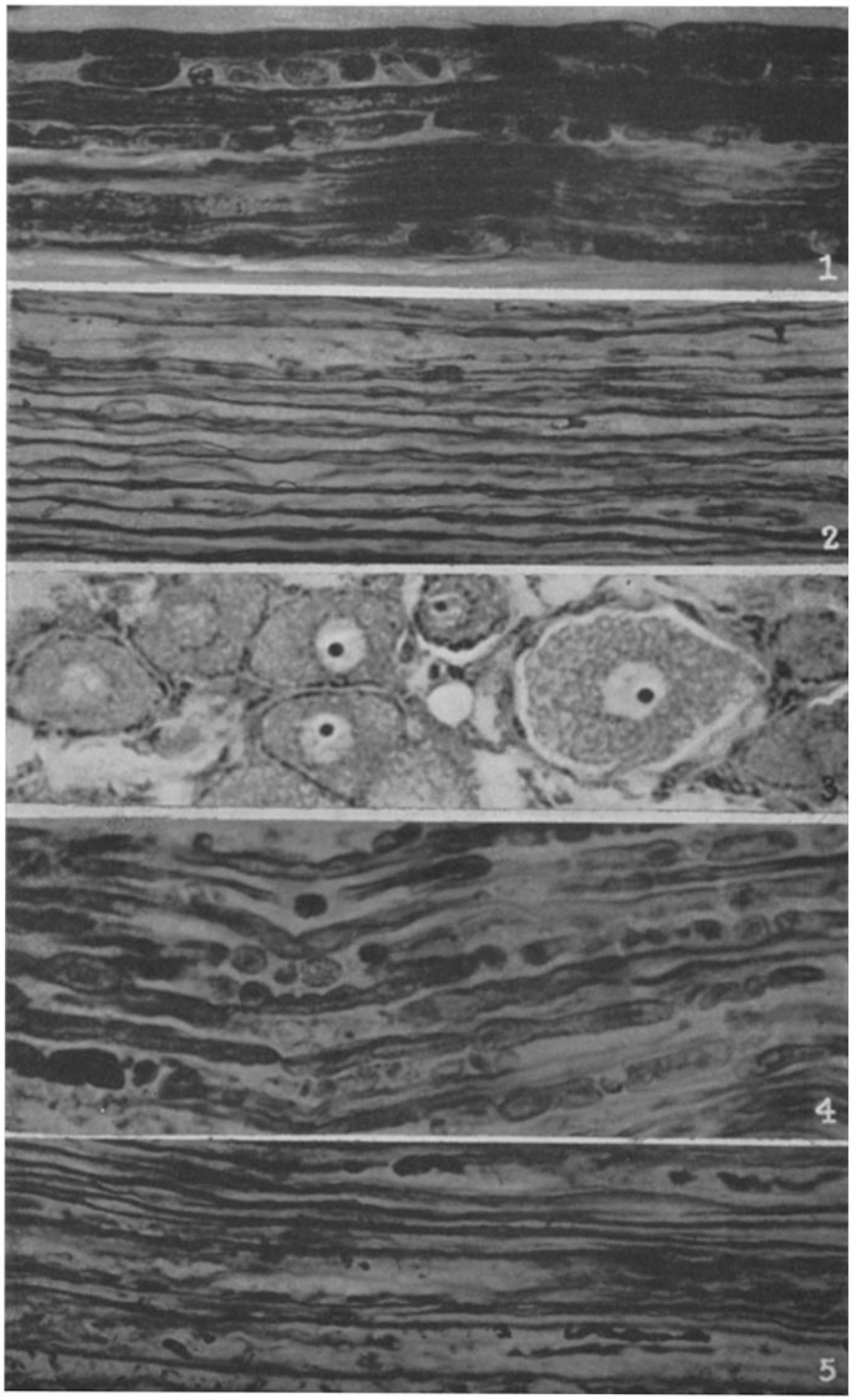
FIG. 1. Pig 7-49. This animal died after being on the pyridoxine-deficient diet for 90 days. Suspicious ataxia developed after 40 days which became definite on the 72nd day. Sciatic nerve. Several nerve fibers show characteristic demyelinization. Modified Weigert stain. ×470.

FIG. 2. Pig 7-49. Sciatic nerve. Axis cylinder in upper portion of field has disappeared. Bodian silver stain. ×470.

FIG. 3. Pig 7-49. Dorsal root ganglion, lumbar region. Note normal size and appearance of cells. Methylene blue stain. ×470.

FIG. 4. Pig 7-47. This animal died after being on the pyridoxine-deficient diet 101 days. Suspicious ataxia developed after 41 days and was definite after the 72nd day. Sciatic nerve. Fibers show much more extensive degeneration than those in Fig. 1. Modified Weigert stain. × 470.

FIG. 5. Pig 7-47. Sciatic nerve. Degenerated and fragmented axis cylinders are present. This was wide spread. Bodian silver stain. ×470.



(Follis and Wintrobe: Pyridoxine and pantothenic acid deficiencies)

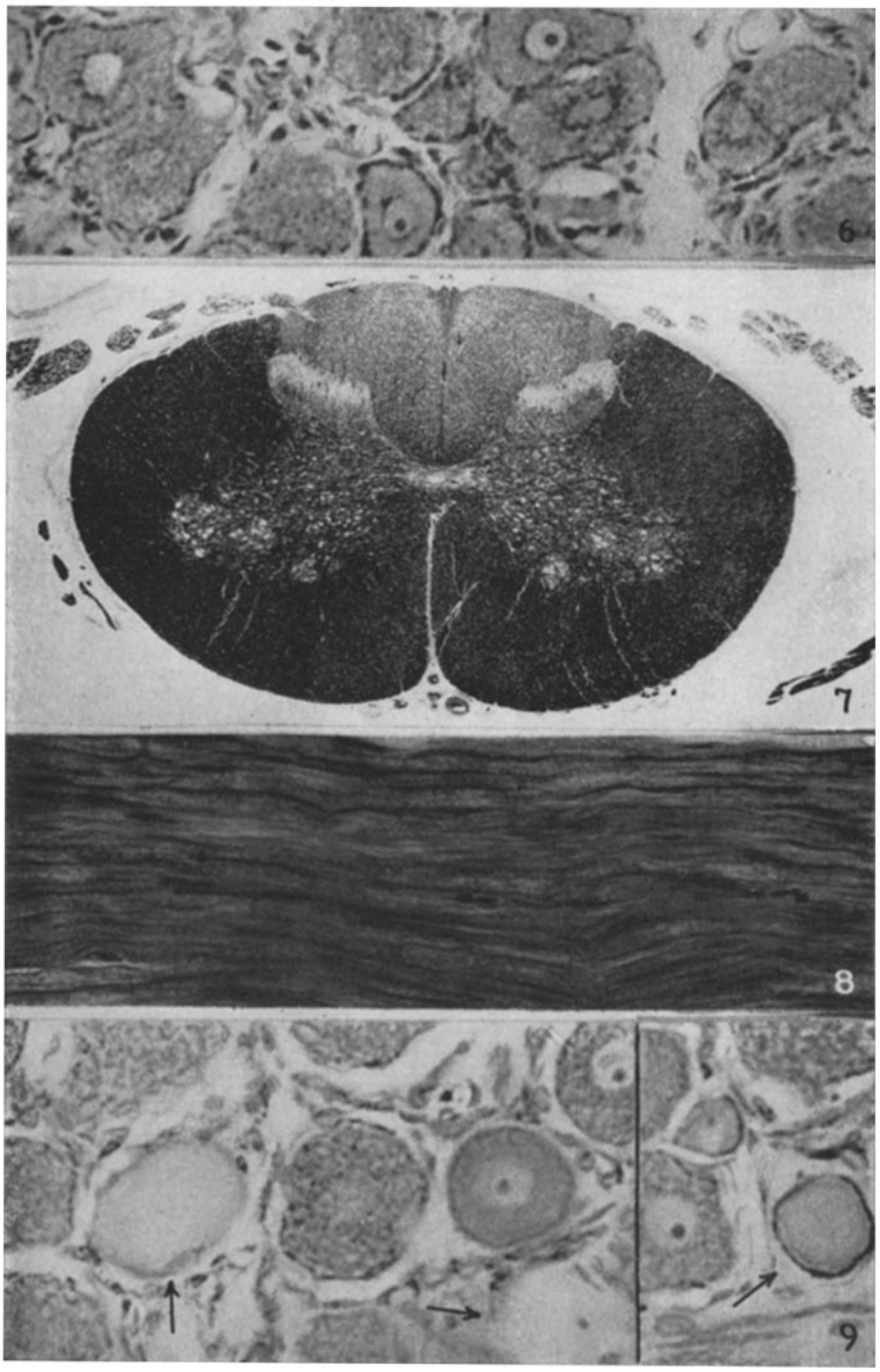
PLATE 29

FIG. 6. Pig 7-47. Dorsal root ganglion, lumbar region. Note absence of chromatolysis. Cells are atrophic when compared with those in Fig. 3. Methylene blue stain. ×475.

FIG. 7. Pig 7-47. Spinal cord and roots, lumbar region. Note diffuse demyelinization of dorsal columns. Contrast dorsal and ventral roots. The former are much lighter indicating demyelinization. Modified Weigert stain. ×29.

FIG. 8. Pig 7-52. This animal was killed after being on the pantothenic acid-deficient diet for 32 days. Ataxia was never definite but had been suspected since the 25th day. Sciatic nerve. Normal. Modified Weigert stain. ×475.

FIG. 9. Pig 7-52. Dorsal root ganglion, lumbar region. Cells are not much decreased in size; note chromatolytic cells (arrows). Methylene blue stain. ×475.



(Follis and Wintrobe: Pyridoxine and pantothenic acid deficiencies)

PLATE 30

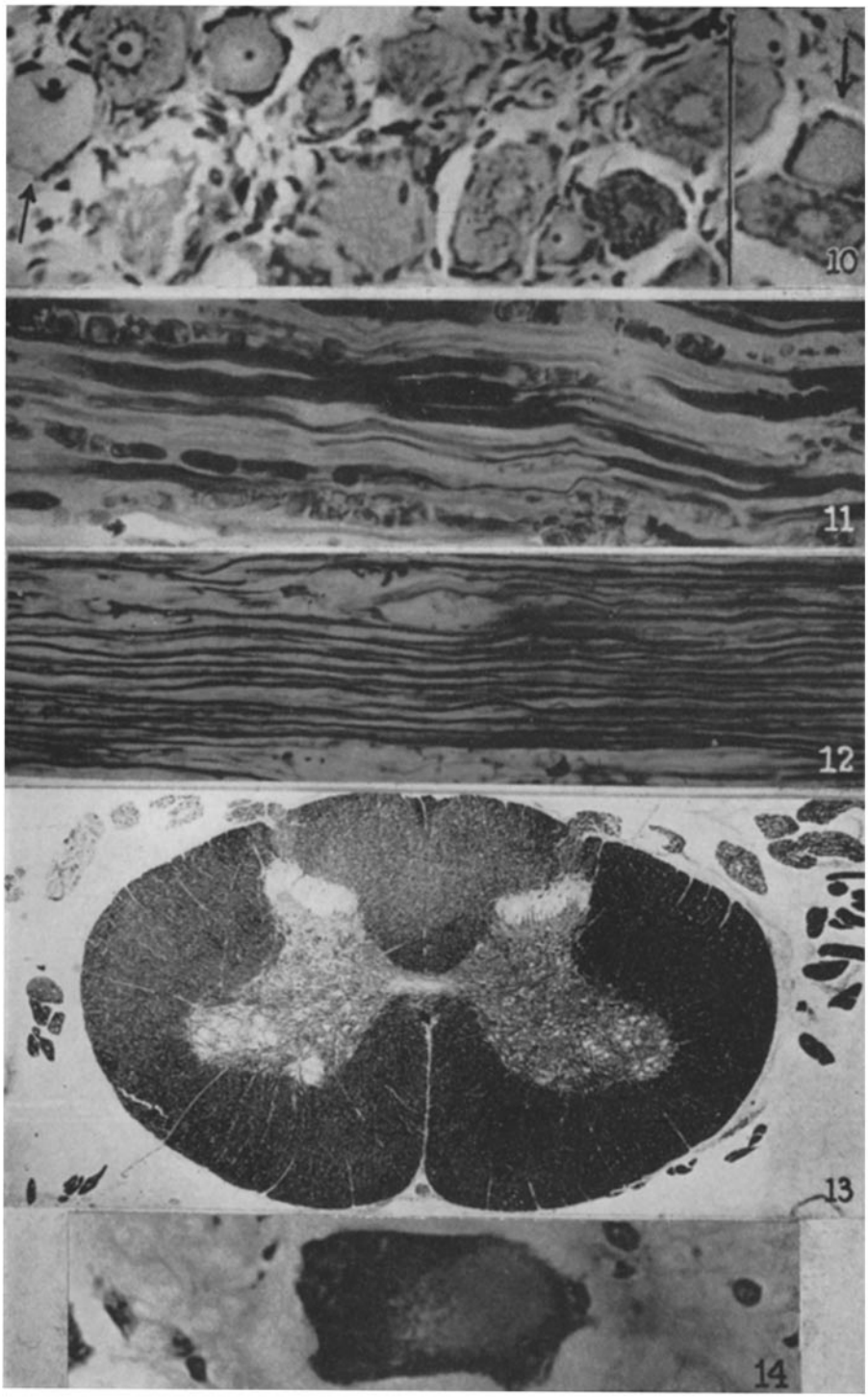
FIG. 10. Pig 7-62. This animal died after being on the pantothenic acid-deficient diet for 99 days. Small supplements of calcium pantothenate had been administered Compare size of cells with Fig. 9. Note chromatolytic cells (arrows). Methylene blue stain. ×470.

FIG. 11. Pig 7-62. Sciatic nerve. Fibers show demyelinization. Modified Weigert stain. ×470.

FIG. 12. Pig 7-62. Sciatic nerve. Axis cylinders show degeneration. Bodian silver stain. ×470.

FIG. 13. Pig 7-62. Spinal cord and roots, lumbar region. Note diffuse demyelinization of dorsal columns and compare with Fig. 7, where process is more extensive. Contrast dorsal and ventral roots. The former show demyelinization. Modified Weigert stain. ×28.

FIG. 14. Pig 7-53. This animal had been on the pantothenic acid-deficient diet for 44 days and had received no supplements of calcium pantothenate. Ganglion cell from intermediate gray matter of lumbar spinal cord. Cell shows chromatolysis. Methylene blue stain. ×760.



(Follis and Wintrobe: Pyridoxine and pantothenic acid deficiencies)