SENSORY NEURON DEGENERATION IN VITAMIN DEFICIENCY

Degeneration of the Posterior Columns of the Spinal Cord, Peripheral Nerves, and Dorsal Root Ganglion Cells in Young Pigs Fed a Diet Containing Thiamin (B_1) and Riboflavin but Otherwise Deficient in Vitamin B Complex*

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Plates 6 to 9

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Since Eijkman demonstrated, in 1897, that atrophic degeneration of the medullary sheaths of nerves occurs in hens fed a diet of polished rice, attempts have been made to produce lesions of the nervous system experimentally by deficient diets. The earliest studies were concerned largely with deficiency of the antineuritic vitamin, B_1, but in recent years vitamin A and the B_2 complex have received attention. These investigations were in the main handicapped by inadequate information regarding the vitamins and by the error of attempting to produce absolute deficiency of the factor studied rather than a partial one. Such animals often died before clear cut lesions developed. Furthermore, drawings and retouched photographs were offered as evidence of nerve and spinal cord lesions, and it was later admitted that at least some of the supposed degenerations were artefacts. So unconvincing was this earlier work, that Grinker and Kandel (1) in 1933, after negative experiments of their own, decided that long standing severe vitamin A, B_1, and B complex deficiency causes no well defined histologic changes in the central nervous system.

In spite of this criticism, the subject has been pursued by several workers, particularly by Zimmerman and his associates (2–5). They have offered further evidence that changes in the medullary sheaths of peripheral nerves and of scat-

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tered fibre tracts in the spinal cord, follow chronic deficiency of vitamin A (2, 3) and that similar changes, together with degeneration of axis cylinders, follow deficiency of the heat-stable vitamin, B_2 (4). In a recent report (5) which appeared while the present work was in progress, Zimmerman and his coworkers described changes in the peripheral nerves, the posterior columns of the spinal cord, and the posterior nerve roots of adult dogs. Their experiments were well controlled and the animals carefully studied. The changes in the spinal cord, however, were not very marked. No description is given of the dorsal root ganglia.

The following observations were made in the course of an attempt to produce in young animals a condition similar to pernicious anemia. Such animals were chosen because it had been found that the blood of the mammalian fetus as well as the bone marrow, is similar to that of patients with this disease and during development undergoes changes similar to those caused by liver extract in pernicious anemia (6). The pig was used, as it is from the liver and the stomach of this animal that substances for the treatment of pernicious anemia are derived. Furthermore, it had been found that the content of anti-anemic principle in the liver of pig fetuses is very low as compared with that of adult animals (7). Consequently it was assumed that a condition resembling pernicious anemia might be produced more readily in the young pig than in other animals.

Methods

The basal diet (Table I), the ratio of protein to carbohydrate and fat, the amount of food and the salt mixture given, were determined after many trials in young animals, details of which are given elsewhere (8). Until the animals were 4½ months of age, acid-washed casein was used but after this time the crude casein was given, as it was found that the crude casein failed to support growth in rats when given without yeast. Butter was the chief source of fat at first but, at the times indicated in the charts, it was later replaced by lard. The amount of food shown in Table I represents a kilo unit, this amount, or a multiple of it, being given daily per kilo of body weight. The food was mixed with a small quantity of water and offered as a gruel.

Vitamins A and D were furnished in fresh cod liver oil (Mead Johnson, 1800 units A, 175 units D per gram) and for a short time, as above indicated, A was available in butter as well. Vitamin C was given in the quantities shown in the charts by administering orally a freshly made solution of cevitamic acid (Squibb)[1] by means of a medicine dropper three times a week. Vitamin B was furnished

[1] Furnished through the courtesy of Dr. George A. Harrop.

by giving whole dehydrated yeast (Northwestern) in the amounts shown in the charts.

All the animals which are the subject of this report were Duroc Jersey pigs of the same litter.[2] One (A 4-60) was received at 10 days of age, whereas the remainder were 23 days old on arrival in Baltimore.

That the diet was adequate as long as sufficient yeast was given, is indicated by comparison with the growth curve of the control animal (A 4-67) which received the same number of calories in cow's milk and sugar, supplemented with cod liver oil and cevitamic acid. The growth curves for these animals during the period when sufficient yeast was furnished, also compare well with the growth of pigs on optimal diets in an agricultural experiment station (9).

As shown in the charts, once growth and development had been well established, the quantity of yeast was gradually decreased. From this time, thiamin (vitamin B_1, betaxin)[3] and, in one animal, riboflavin (lactoflavin)[3] were adminis-

TABLE I

Basal Diet. One Kilo Unit

	gm.
Casein (new process, Sheffield) (protein 84.4 per cent)	9.5
Sucrose	21.0
Butter or lard (washed)	4.0
Cod liver oil	0.5
Salt mixture	2.2
Total	37.2

Caloric value 156.5.

tered by intramuscular injection twice a week in the amounts shown in the charts. These amounts were thought to be adequate or in excess of the requirement for these substances (10, 11).

Studies of the blood were made each week or oftener, and the ascorbic acid content of the blood[4] was determined at frequent intervals. Gastric analyses were carried out from time to time. These will be discussed elsewhere (8). The animals were observed daily and, when neurologic signs developed, photographs and cinematographic records as well as neurologic examinations were made.

Pathological Studies.—Complete autopsies were performed. The tissues of the nervous system were fixed immediately after removal in 10 per cent neutral formol-saline. Whole blocks were taken of the brain through the level of the

[2] Furnished by the Bureau of Animal Industry, United States Department of Agriculture, Beltsville, Maryland.

[3] Furnished by the Winthrop Chemical Company.

[4] Carried out by Dr. Laslo Kajdi, Department of Pediatrics.

mamillary bodies and including the basal ganglia, the pons, upper and lower medulla including the cerebellum, and at various representative levels of the spinal cord. The spinal roots and dorsal root ganglia were also removed from various levels and pieces of the brachial, sciatic, peroneal, and tibial nerves were obtained. All blocks were embedded in paraffin and stained with hematoxylin and eosin, thionin (for cells), and the Mahon modification (12) of the Weigert stain (for myelin). The Mahon technique permits uniform treatment of tissue from experimental animals and controls, and eliminates differentiation of individual sections. Glia were stained by Mallory's phosphotungstic and hematoxylin method and axis cylinders by the Bielschowsky technique. Frozen sections of the spinal cords and peripheral nerves were stained for fat with Sudan III.

RESULTS

Protocols, growth curves, and blood findings in four animals are shown in Charts 1 to 4. Except when otherwise indicated on the charts, the animals ate well and consumed all their food in a short time. Only in the last month was their appetite seriously affected, but even then the day's food was usually entirely consumed in the course of 24 hours. In pig A 4-60 (Chart 1), at 70 days of age the hind legs became weak and by the 73rd day the animal was unable to stand; on this day thiamin, 25 mg., was injected. The next day the animal was able to walk and within a week had completely recovered. Following this, the history of the three animals receiving the artificial diet was the same. As the quantity of yeast given was gradually reduced, weight gain diminished, ceased, or loss occurred. Their hair became dirty and matted. The administration of thiamin (vitamin B_1) was followed by a resumption of growth. This effect was temporary, however, even in spite of the excessive amounts which A 4-60 received during its last month. Pig A 4-64 (Chart 2) received riboflavin continuously during the latter half of its life period. This too failed to delay the onset of the signs to be described, or to prevent death.

First in pig A 4-60, then in A 4-64 and A 4-65, at the time indicated on the charts (N.S.), a picture developed which was similar in each (Figs. 1, 2, 3). Noticed casually as an awkwardness in the hind legs while walking, on more careful observation it was seen that each pig had developed a peculiar slapping gait, in which the hind legs were placed widely apart rather than close together as in the normal animal. The ataxia progressed and the animals stumbled frequently. They

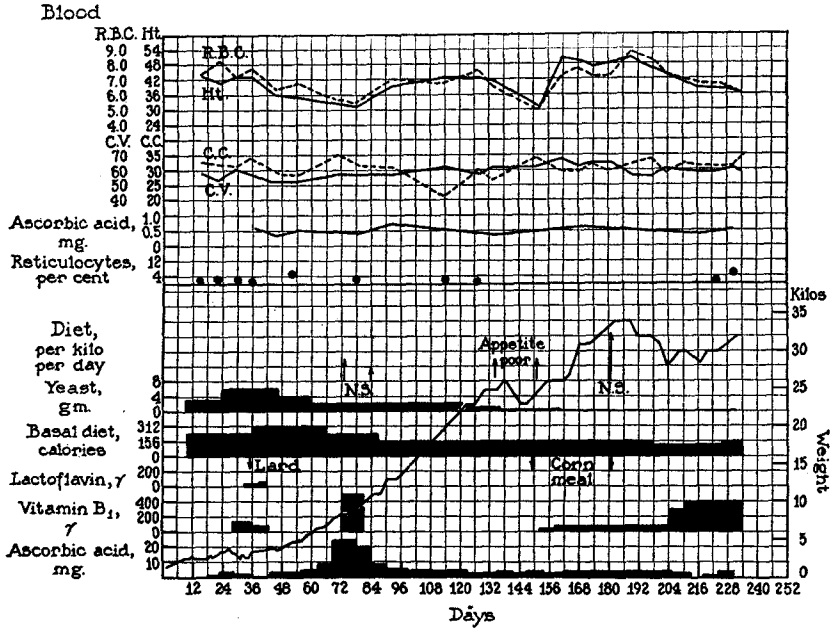

CHART 1. Diet, growth curve, and blood findings in pig A 4-60, male.

Legends.—All dietary data are shown as the amount per kilo body weight daily. At the point indicated, lard replaced butter in the basal diet. The quantities of thiamin (B_1) and riboflavin (lactoflavin) given are shown as micrograms per kilo per day, although actually they were injected intramuscularly twice each week.

The red cell counts (R.B.C.) are represented in millions per c.mm.; the volume of packed red cells (Ht.) in cc. per 100 cc. blood; the mean corpuscular volume (C.V.) in cubic microns; and the mean corpuscular hemoglobin concentration (C.C.) in per cent.

Protocol.—The quantity of yeast given was successively reduced from 6 gm. per kilo daily, to 4 gm., 2 gm., 0.5 gm., 0.2 gm., and finally 0.1 gm. A total of 373 gm. were given per kilo, an average of 1.6 gm. per day. The total amount of thiamin given was 540 mg., of which 360 mg. was given during the last month. Only 22.5 mg. of riboflavin were given. During the period between arrows marked corn meal, this animal received 15 gm. yellow corn meal instead of the same weight of sucrose in its basal diet.

A brief and temporary early period of weakness (see text) is represented between two arrows. The onset of the marked ataxia described in the text is indicated by a long arrow near the right (N.S.)

Autopsy.—324th day. Animal killed by pentobarbital. Body moderately well nourished, hair a little dry but only slightly matted. Nervous system as described in the text (Figs. 5, 7, 8, 10, 16, 17, 18). Thoracic and abdominal organs normal, except for numerous pericardial adhesions, presumably caused by repeated cardiac punctures.

were particularly awkward in turning. One of the most striking features was a clonic scratch-like movement of one or other hind leg which often persisted for several seconds while the pig stood on its other feet. As time went on the animals became more and more reluctant to move about, and sat with their hind legs spread widely

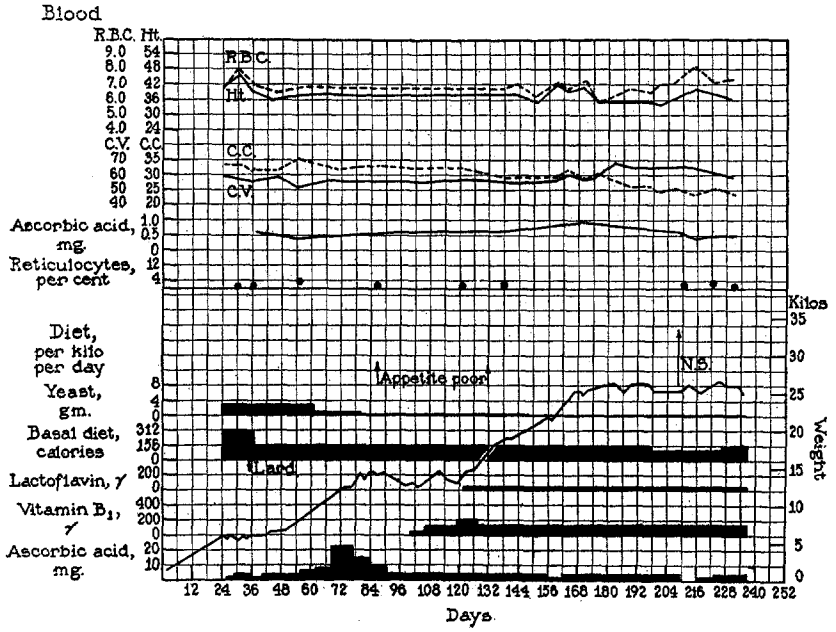

CHART 2. Diet, growth curve, and blood findings in pig A 4-64, female.

Legends.—Same as in Chart 1. N.S. denotes the onset of marked ataxia.

Protocol.—The quantity of yeast was successively reduced from 3 gm. per kilo daily, to 1 gm., 0.5 gm., 0.2 gm., and finally 0.1 gm. A total of 169 gm. were given per kilo, an average of 0.7 gm. per day. The total amount of thiamin given was 415 mg. and the total quantity of riboflavin was 103.8 mg.

Autopsy.—238th day. Animal found dead. Nervous system described in the text (Figs. 3, 11, 14). The thoracic and abdominal organs showed no changes. No cause of death was found.

apart or even sprawled forward beyond their fore legs. The effect of blindfolding on the gait and balance was tested, in order to see if the loss of visual control caused any greater defect. At the time this was done, however, the animals were already so ataxic that little difference was noticed.

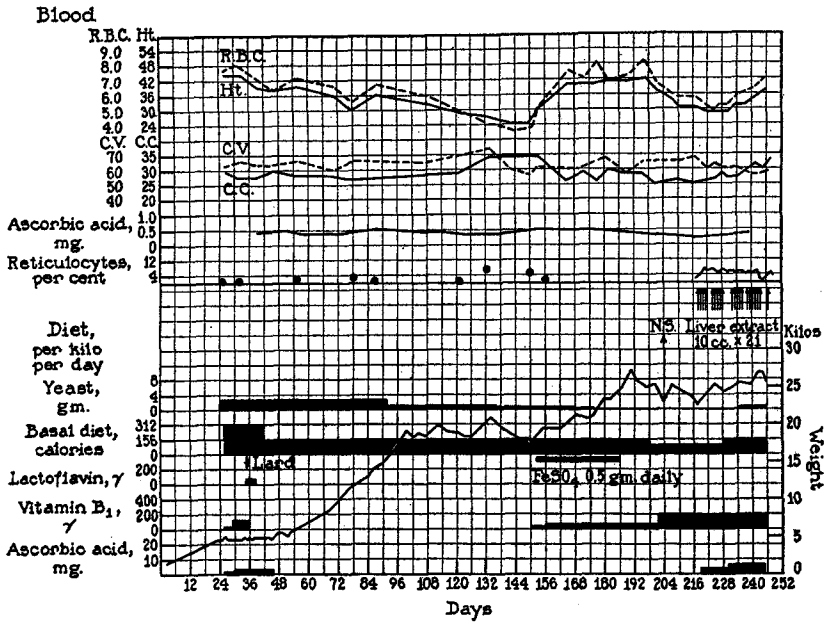

CHART 3. Diet, growth curve, and blood findings in pig A 4-65, female.

Legends.—Same as in Chart 1.

Protocol.—The quantity of yeast was successively reduced from 3 gm. per kilo daily, to 0.5 gm., 0.3 gm., 0.2 gm., and finally 0.1 gm. During the last 12 days, after 13 injections of liver extract had been given, the quantity of yeast was increased to 0.3 gm. A total of 247 gm. were given per kilo, an average of 1 gm. per day. The total amount of thiamin given was 312 mg., and the total quantity of riboflavin was 3 mg.

Autopsy.—247th day. Animal found dead. Ulcers between hind hoofs. Round firm fluctuant mass over the left buttock containing thick yellow pus. Tissues of the nervous system described in the text (Figs. 12 and 15). Muscles edematous and in places hemorrhagic in appearance. Abdomen distended by gas in the stomach and bowel; no gas or fluid found in the peritoneal cavity. Liver small and pale; spleen normal. Cut surface of the kidney showed mottling in the cortex. Right pleural cavity largely obliterated, but loculated areas contained pus; left cavity normal. Both lungs rather nodular, the right much more so than the left. General pulmonary congestion, no consolidation. Considerable enlargement of the root glands on both sides. Heart normal except for pericardial adhesions.

Histological studies showed abscesses in the lungs and kidneys. None found in the liver or spleen, nor in any of the nervous tissues examined. The small vessels in the affected organs contained enormous numbers of a large Gram-positive anthrax-like bacillus. Unfortunately no material was taken for cultural studies so that identification of the organism was not possible. The septicemia was thought to be entirely a terminal event and unassociated with the nervous condition. It is noteworthy that this animal was given very little ascorbic acid.

In spite of their locomotor disabilities the motor power was not greatly diminished, as judged from the animals' movements when venepunctures or gastric analyses were performed. There was no sign of muscular wasting. The tendon reflexes were absent or only doubtfully obtained. From the ataxia, loss of reflexes, and intact motor power, it was evident that a sensory defect existed. Further-

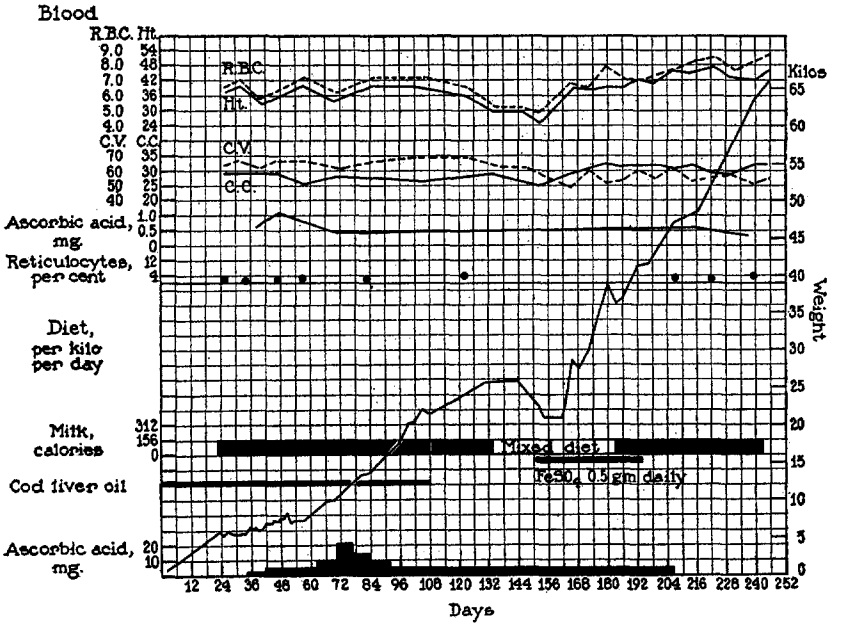

CHART 4. Diet, growth curve, and blood findings in pig A 4-67, female.

Legends.—Same as in Chart 1.

Protocol.—For 108 days this animal received cow's milk and sugar, 156 calories per kilo daily. After this time it received a mixed diet consisting of waste from the hospital kitchen. The cod liver oil was given in amounts of 0.5 gm. per kilo daily.

Autopsy.—247th day. Tissues of the nervous system as described in the text (Figs. 6, 9, 13, 19). The thoracic and abdominal organs were normal, both in the gross and microscopically.

more, ulcers developed on the hind limbs and above and between the hoofs, and these seemed to be insensitive to pin pricks. On pinching of the tendons, the animals showed no discomfort.

In marked contrast was the gait and general appearance of the control animal (A 4-67) (Fig. 4). Not only was this pig larger and heavier

but the body and hair were clean, and no ulceration occurred. Tendon reflexes were readily elicited. The animal moved about quickly and naturally with the hind feet close together. Blindfolding had no effect on the animal's movements and it could swing about with ease.

In none of the animals were cranial nerve changes observed: vision, eye movements, swallowing, and voice were normal. The jaws were strong and their snouts were sensitive to pin pricks.

Pigs A 4-60 and A 4-67 were killed by the intravenous injection of pentobarbital but A 4-64 was found dead. It was decided to attempt treatment of A 4-65 by the intramuscular injection of a crude liver extract (campolon, Winthrop Chemical Company) (Chart 3). Slight improvement followed. The animal finally died, however, of septicemia.

Pathological Studies.—The findings of main interest were in the nervous system.

1. Peripheral Nerves.—In general the changes found were the same in the three affected animals (pigs A 4-60, A 4-64, A 4-65), although they differed in severity. Demyelination was extreme in the sciatic nerves and their branches (Figs. 14 and 15). In the brachial nerves this was also present but was less extensive (Fig. 17). In pig A 4-60, which developed neurological signs before the others, the demyelination was most advanced. It was observed in the transverse sections that certain bundles showed almost complete loss of myelin whereas others were little changed (Fig. 17). The significance of this will be discussed later. With the loss of myelin a considerable increase in the number of neurilemma cells was seen and they were frequently found enlarged and swollen. This was most marked in A 4-65, the animal which died of terminal septicemia. Everywhere the loss of myelin was more widespread than the damage to the axis cylinders. The latter were swollen and in many places fragmented (Fig. 18); the changes again were more marked in A 4-60 than in A 4-64 or A 4-65.

Pig A 4-67 (control) showed an entirely different picture (Figs. 13 and 19). Myelin stained consistently in all the nerves examined, appearing in the transverse sections as regular rings (Fig. 13). The neurofibrils appeared as continuous, heavily impregnated fibres and the neurilemma cells were seen as very thin fusiform nuclei lying parallel to and along the myelin sheaths (Fig. 19).

2. Spinal Roots and Posterior Root Ganglia.—Here again the normal appearance of cells and fibres in pig A 4-67 contrasted with the varying degrees of demyelination and chromatolysis in the three animals A 4-60, A 4-64, and A 4-65. As before, the changes were most marked in pig A 4-60. The extreme loss of myelin in the posterior root fibres contrasted strikingly with the normal anterior roots

(Figs. 7 and 8). This was seen in all the spinal roots examined including those from the cervical region as well as those in the sacral, although it was less marked in the former. This finding perhaps explains the fact referred to above, namely that in the peripheral nerves some bundles appeared well myelinated while others showed almost complete degeneration. In other words the demyelination appears to have been confined to the afferent fibres. The ganglion cells in the three animals which were given a deficient diet showed changes which varied in degree from some loss of Nissl substance, to severe chromatolysis. The cells of the control animal showed a large central nucleus, with a deeply stained nucleolus, and rather heavy Nissl granules (Fig. 6). The ganglion cells in the affected animals appeared to be diminished in number and many showed little or no Nissl substance except around the nucleus (Fig. 5). In some cells, the nuclei were eccentric and no Nissl substance whatever was observed. In pig A 4-60 alone, however, was chromatolysis frequent and well developed.

3. Spinal Cord.—The uncut cord appeared normal to the naked eye. On looking at the cut transverse surface, the posterior columns stood out clearly by their white color which contrasted with the more yellow appearance of the rest of the cord. The sections of the spinal cords in pigs A 4-60, A 4-64, and A 4-65, in the myelin stain showed a definite pallor of the posterior columns (Figs. 10, 11, 12). Throughout the posterior columns large round, punched-out areas were seen and in these spaces no axis cylinders were apparent. There was considerable variation in the size and shape of the myelin rings. This was in marked contrast to the regularity of the myelin sheaths and the central position of the axis cylinders in the lateral and anterior columns of these three animals, and in all parts of the cord in A 4-67 (Fig. 9). Stained by Sudan III, some globules of free fat were seen in the posterior columns. Although with the Mallory stain some glial growth was observed in the posterior columns, nowhere was it conspicuous and there was no dense scarring. Examination of the cells in the anterior horns revealed no abnormalities. At most there was some vacuolation of these cells in the affected animals which was not present in the control pig. The cells of the dorsal nuclei and substantia gelatinosa Rolandi showed no changes. The membranes and blood vessels of the cord were normal except for the injection of the latter in the animal which died of septicemia (A 4-65).

4. Brain.—With the exception of the changes previously described in the posterior columns, which could be followed as high as the cuneate and gracile nuclei in the lower medulla, the brain stem and brain were quite normal.

In summary, the process seemed to consist of a quite selective degeneration of the peripheral sensory neuron involving its cell body in the posterior root ganglion, the peripheral axon, and the central axon included in the posterior roots and in the posterior columns. No clear cut abnormalities were seen in the anterior roots or anterior horn cells and many bundles of well myelinated fibres, probably motor, were evident in sections of the peripheral nerves.

The tissues other than those of the nervous system were either normal, or showed the results of secondary infection. Details are given in the descriptions of Charts 1 to 4.

DISCUSSION

The neurological signs in these animals were so well marked, the changes in the nervous system so well defined, and there was such a close correlation between the signs and the histologic findings that there can be no doubt regarding the pathologic lesion. Selective degeneration of the sensory neuron occurred.

It is thought that the changes in the nervous system were dependent upon a dietary deficiency of one or more components of the vitamin B complex, other than thiamin (B_1) or riboflavin. The animals received a diet which was adequate in proteins, carbohydrates, fats, unsaturated fatty acids, minerals, and vitamins A, C, and D, and which was supplemented at first by adequate amounts of yeast. As the quantity of yeast was gradually reduced, growth ceased. The appetite of the animals became poor, although they still consumed their daily ration during the 24 hour interval between feedings. No neurological signs were observed, however. Shortly after administration of thiamin was started, appetite and growth were restored. It was not until several months had elapsed that the described neurological signs appeared. In one of these animals thiamin was given even in excessive amounts. We believe, therefore, that the disorder could hardly have been the result of insufficient vitamin B_1.

The neurological changes did not seem to be caused by a lack of riboflavin, for A 4-64 developed well marked neurological signs similar to those observed in pigs A 4-60 and A 4-65. It may be mentioned, however, that in this animal the neurological signs and the changes found in the tissues of the nervous system, were less marked than in the animals which received very little riboflavin, in spite of the fact that it had received less yeast than A 4-65. In experiments which are now in progress, the significance of riboflavin, nicotinic acid, vitamin B_6 and other components of the B_2 complex, is being studied, and the possibility of preventing the development of the described neurologic changes by the administration of large amounts of yeast, is being investigated.

The relation if any, of the neurological changes observed to those

occurring in nutritional disorders in man, such as pernicious anemia, and pellagra, is at present a matter only for speculation. It is of interest, however, that the diet which these animals received seems to be low in its content of "extrinsic factor" (8). Comparing the clinical picture in the affected animals to the common neurologic disorders of man, the similarity with that of tabes is striking. In view of the ataxia, the animals immediately impressed one as having predominant posterior column pathology rather than a peripheral neuritis or subacute combined degeneration. There was no evidence of spasticity.

Pathologically the picture resembles none of the diseases mentioned above. The degeneration of the central axon and cell body is not a part of the usually recognized pathology of sensory neuritis in man. In tabes dorsalis, degeneration of the posterior root fibres and posterior columns, as well as replacement by a dense glial scar is seen, but lesions have not been reported in the peripheral axon or posterior root ganglion. In the animals here described, the whole neuron was involved and glial reaction was minimal. The peripheral neuritis and posterior column changes without glial reaction are quite similar to those seen in subacute combined degeneration of the cord, but in no instance were any changes observed in the lateral columns. In human beings, the lateral columns as well as areas in the anterior columns, and even in the brain stem and brain, may be involved. These animals also failed to show the diffuse degeneration of the cord, the pigmentary changes in the ganglion cells, or the π granules of Wilson in the peripheral nerves, which have been described in pellagra.

It must be kept in mind, when comparison is made between changes produced in animals by experiment, and those lesions discovered in man, that in human beings nutritional deficiencies are usually multiple in character. Dietary defects exactly comparable to that produced in the animals here described, may not occur.

SUMMARY

Young pigs were given an artificial diet presumably adequate in all respects. As they developed, the quantity of yeast was gradually reduced while thiamin (vitamin B_1) and riboflavin were given in its place. The rate of growth decreased, the general condition of the

animals became impaired, and marked ataxia without motor weakness developed. Histologically, severe degeneration of the posterior columns of the spinal cord, the dorsal root ganglion cells, and the peripheral nerves was found.

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EXPLANATION OF PLATES

PLATE 6

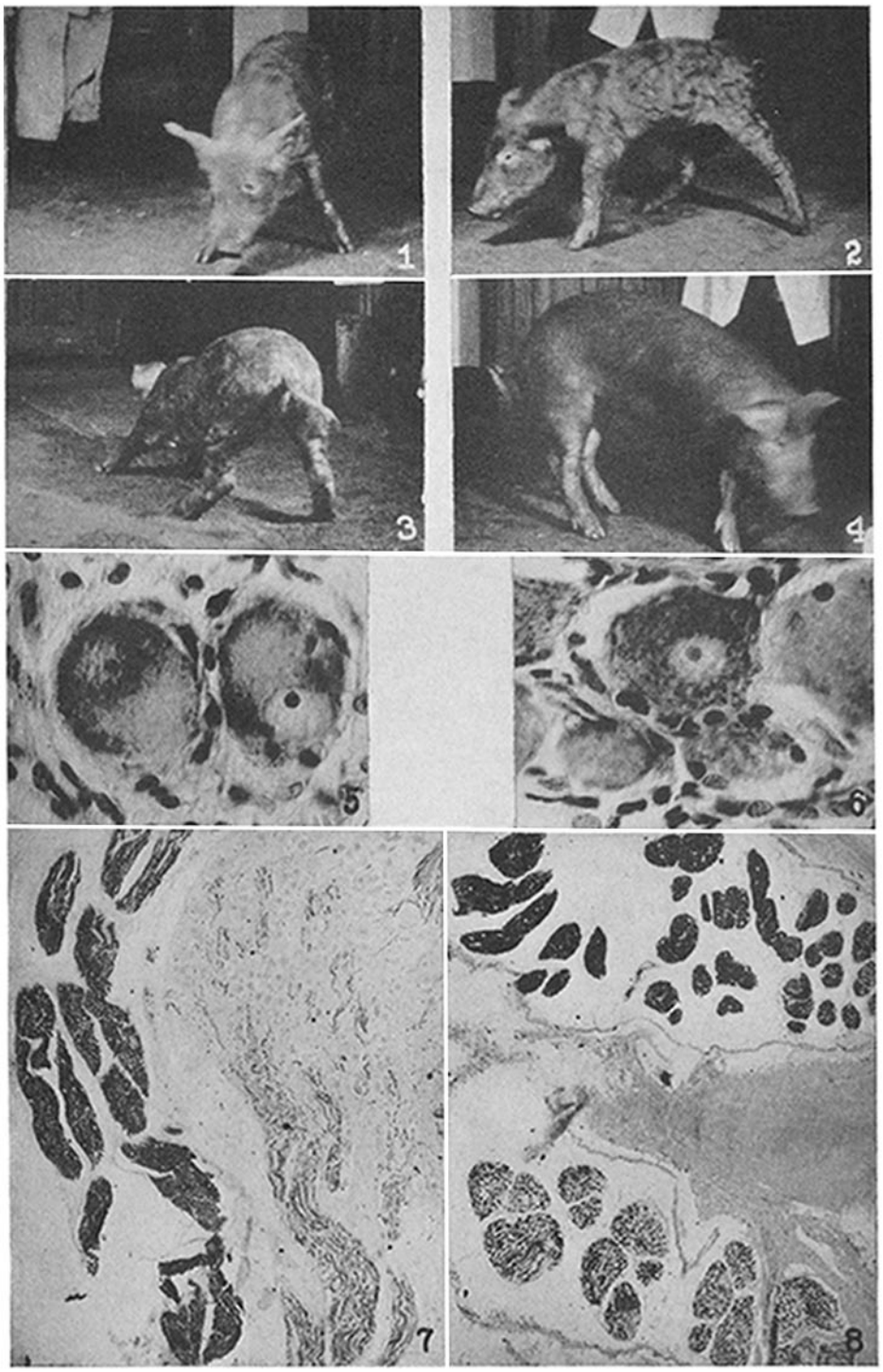
FIGS. 1 to 4. Reproduced from cinematograph film; showing wide base of support used by affected animals (A 4-65, Figs. 1 and 2, A 4-64 Fig. 3); the relative size and stance of the control animal (A 4-67) are shown in Fig. 4.

FIG. 5. Pig A 4-60. Posterior root ganglion cells showing eccentricity of nucleus and loss of granules except around the nucleus. Nissl stain, thionin. × 300.

FIG. 6. Pig A 4-67 (control). Posterior root ganglion cells as in Fig. 5, showing central nucleus and normal Nissl substance.

FIG. 7. Pig A 4-60. Anterior and posterior roots, posterior root ganglion, longitudinal section. Myelin normal in anterior roots; largely lost in posterior. Mahon stain. × 100.

FIG. 8. Pig A 4-60. Anterior and posterior roots, transverse section. Anterior root shows normal myelin; the posterior root is somewhat demyelinized.

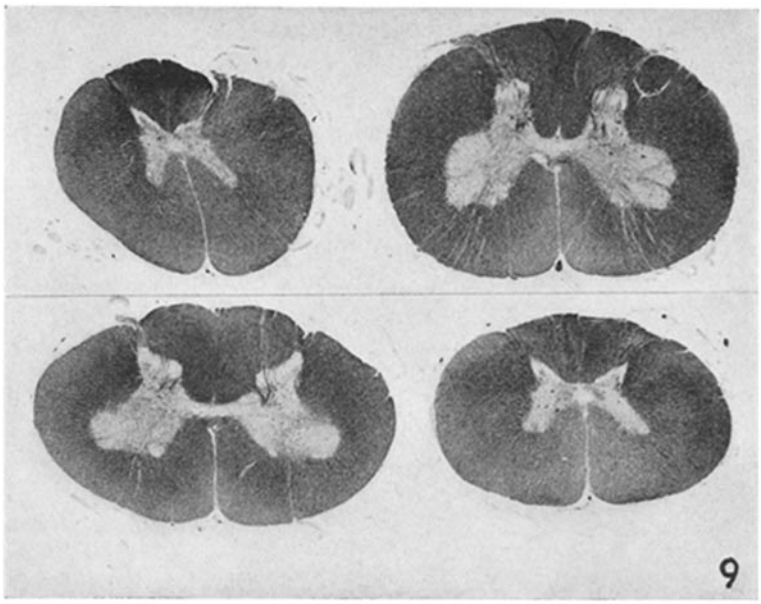


(Wintrobe *et al.*: Neuron degeneration in vitamin deficiency)

PLATE 7

FIG. 9. Pig A 4-67 (control). From left to right, transverse sections of spinal cord of the upper thoracic, lower cervical, sacral, and lumbosacral regions. Mahon stain. × 5.

FIG. 10. Pig A 4-60. Spinal cord (same order as in Fig. 9): pallor of posterior columns at all levels, indicating loss of myelin. Mahon stain. × 5.



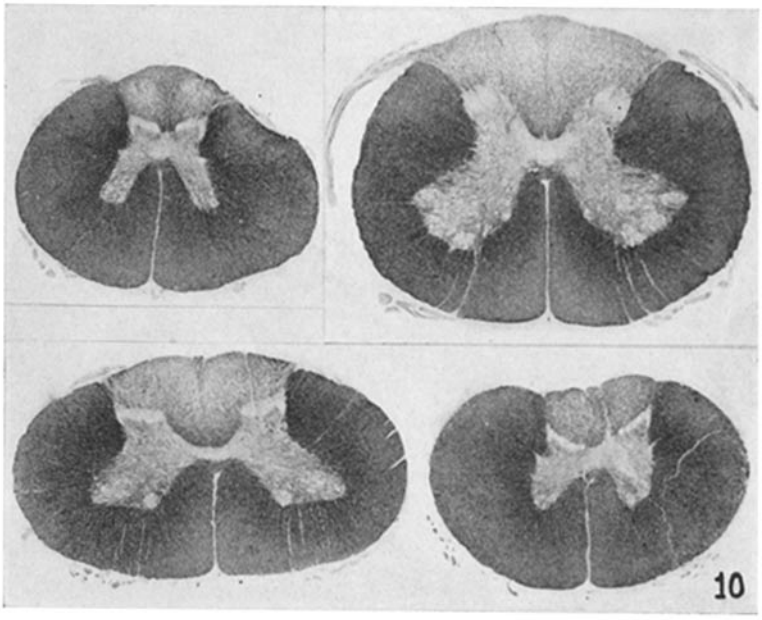


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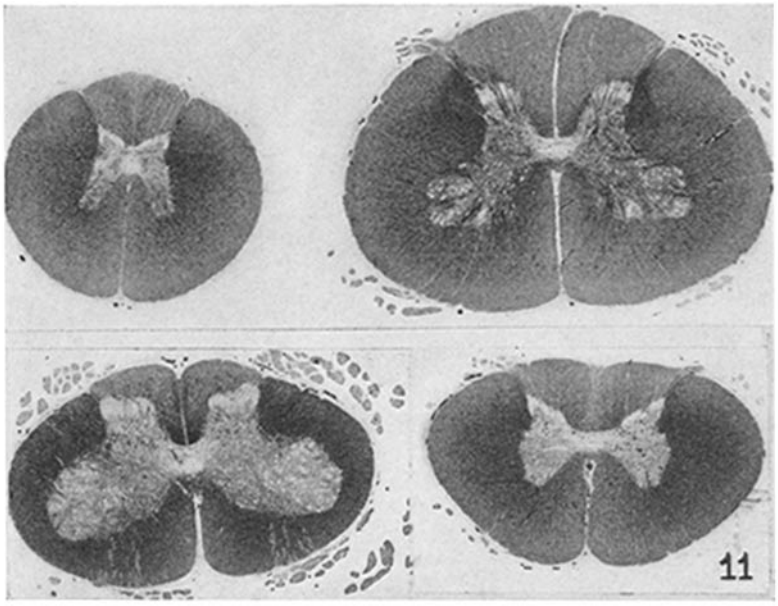
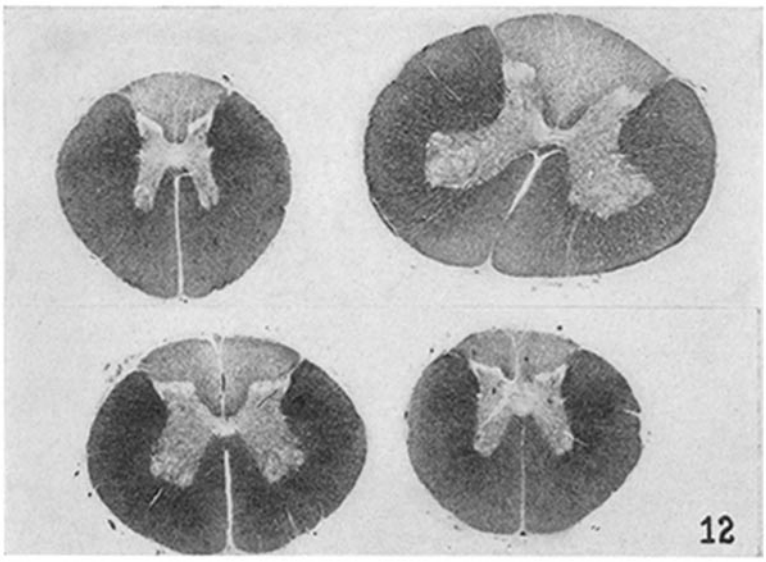
PLATE 8

FIG. 11. Pig A 4-64. Spinal cord (same order as in Fig. 9): pallor of posterior columns. Mahon stain. × 5.

FIG. 12. Pig A 4-65. Spinal cord (same order as in Fig. 11): pallor of posterior columns. Mahon stain. × 5.

In all these spinal cord sections the slight peripheral pallor is considered an artefact.





(Wintrobe *et al.*: Neuron degeneration in vitamin deficiency)

PLATE 9

FIG. 13. Pig A 4-67 (control). Sciatic nerve: normal myelin in all nerve bundles. Mahon stain. × 100.

FIG. 14. Pig A 4-64. Sciatic nerve: almost no myelin in nerve bundles. Mahon stain. × 100.

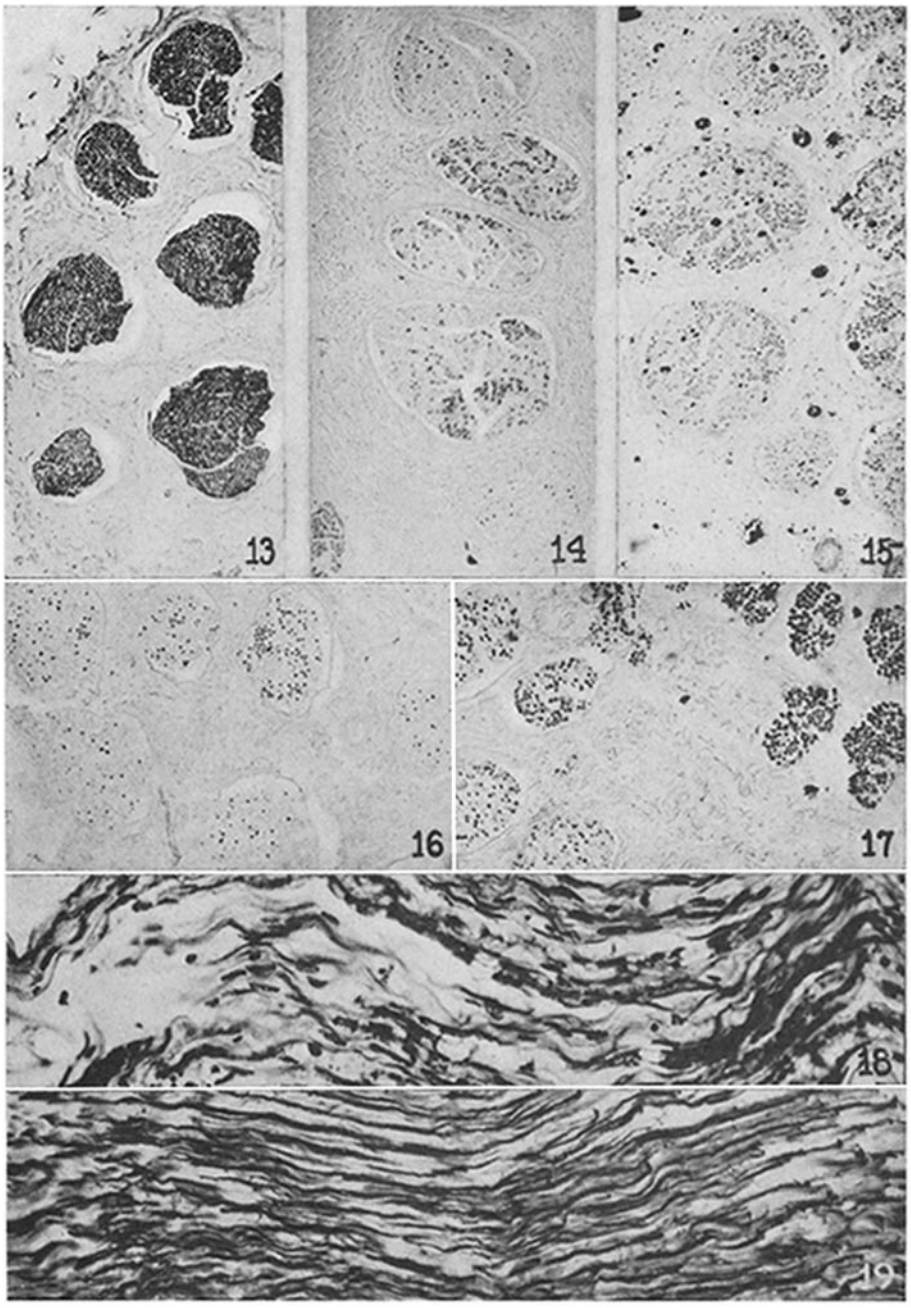
FIG. 15. Pig A 4-65. Sciatic nerve: again severe demyelinization (the black spots are dilated capillaries with red cells stained black). Mahon stain. × 100.

FIG. 16. Pig A 4-60. Peroneal nerve: extreme loss of myelin. Mahon stain. × 100.

FIG. 17. Pig A 4-60. Brachial nerve: compare with Fig. 16. Demyelinization is not so severe. Mahon stain. × 100.

FIG. 18. Pig A 4-60. Sciatic nerve: swelling and fragmentation of axis cylinders. Bielschowsky stain. × 300.

FIG. 19. Pig A 4-67 (control). Sciatic nerve: axis cylinders uniform and continuous. Bielschowsky stain. × 300.



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