Acta Neurophatologica 1, 313—334 (1962)

From the Armed Forces Institute of Pathology, Washington D. C.,
the Central Anatomic Laboratory, Department of Mental Hygiene, Baltimore, Md.,
and the National Institutes of Health, Bethesda, Md.

Systemic Axonal Dystrophy in Vitamin E Deficient Adult Rats

With Implication in Human Neuropathology * **

By
ANGEL PENTSCHEW and KLAUS SCHWARZ

With 17 Figures in the Text (2 coloured)

(Received March 18, 1961)

Interest in vitamin E deficiency was greatly stimulated when GOETTSCH and PAPPENHEIMER reported the development of "nutritional muscular atrophy" in rabbits and guinea pigs deprived of vitamin E. Because of some similarities in the pathological changes in muscles in these animals and in progressive muscular dystrophy in man, numerous patients with progressive muscular dystrophy were treated with vitamin E, but with little success. The subject was again given impetus when RINGSTED demonstrated that a spectacular neurological syndrome could be produced by depriving adult rats of vitamin E — a syndrome characterized by ataxia and paresis and evolving slowly over a period of 6 to 15 months. The development of this syndrome in rats has been subdivided into the following 4 stages (EINARSON 1952; EINARSON and RINGSTED; EINARSON and TELFORD).

Stage 1. Initially, gait disturbance often slight, intermittent, accompanied by fine tremor. Then ataxic, slow, cautious and somewhat waddling, and frequently jumping or limping. In later stages hindlimbs widely separated, gait shuffling and somewhat straddling. Slight muscular atrophy in hindlimbs often visible early, at a time when muscle power still strong.

Stage 2. Pronounced ataxia with straddling of the legs in walking, the feet and toes being kept in plantar flexion. Distinct atrophy and flabbiness of the musculature of the hindquarters. In moving, animal's hindquarters close to the ground, the forelimbs normally active.

Stage 3. Hindlimbs persistently abducted. Hindquarters drag along the ground and sway with the tail, from side to side, at every step. Grotesque attitudes in walking. Hypaesthesia and hypalgesia in some animals. Decreased mental alertness. No decrease in food consumption.

Stage 4 (Fig. 1). Inability to walk. Constant lying on side, with legs and tail in bizarre postures. On attempting to stand, falling and rolling on the side. Slight to moderate involvement of forelegs in some animals. Marked sensory loss. Ataxia and dysmetria progressive. In defensive reactions, animals unable to locate a stimulated spot. Hindlegs and toes crooked. Muscular atrophy sometimes extreme. Slow and dull mentally. Animals sleep quietly when left alone.

On microscopic examination of the nervous system of such rats the most pronounced lesions have been observed in the dorsal funiculi of the spinal cord, especially the fasciculus gracilis (EINARSON 1952; EINARSON and RINGSTED; EINARSON and TELFORD; LUTTRELL and MASON; MALAMUD, NELSON and EVANS). The primary change in this region was considered to consist in demyelination with subsequent fragmentation of axis cylinders and reduction in their number.

* Presented at the 36th Annual Meeting of the American Association of Neuropathologists, in Boston, Mass., June 11—12, 1960.

** This investigation was supported by Research Grant B-2275, from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland.

According to EINARSON and TELFORD, the glial reaction was somewhat variable. As a rule, however, demyelinated areas contained a rather marked increase in glial fibers and scattered hypertrophic astrocytes. The numerous scavenger cells present contained abundant lipoid breakdown products which took on a brilliant red with scarlet R. Observed also were amorphous pigment deposits, insoluble in fat solvents, which stained red-orange with scarlet R; they were acid-fast in sections stained by ZIEHL-NEELSEN's carbol fuchsin, and positive in PAS-stained material, displaying a bright yellow primary fluorescence when exposed to ultra-violet light. Within regions of most marked degeneration these pigment deposits were found in scavenger cells, in or around astrocytes and oligodendrocytes, and in the walls of small vessels. EINARSON (1952) mentioned merely that degeneration of the dorsal roots was also observed. MALAMUD, NELSON and EVANS found that dorsal roots were slightly demyelinated and showed a slight increase in endoneurial cells; no changes were detected in spinal ganglia, ventral roots, or peripheral nerves. EINARSON (1952) and EINARSON and TELFORD postulated that the presence of irreversible "lipodystrophic" changes in the nerve cells of the anterior horn "leave their imprint on the picture of the muscular atrophy; in other words features of a late spinal atrophy are gradually superimposed in the early muscular dystrophy". On the other hand, MALAMUD, NELSON and EVANS noted that some anterior horn nerve cells were hyperchromatic, sclerotic, vacuolated and showed a slight increase in fat content, but that such changes were inconstant and varied in intensity in the vitamin E deficient animals and in different levels of the spinal cord in any given animal. They could find no correlation between the changes in the anterior horn nerve cells and the pathological changes elsewhere or with the severity of the clinical signs. Moreover, they not infrequently found hyperchromatic or sclerotic cells in otherwise normal areas of the nervous system not only in the vitamin E deficient animals but also in the normal control animals.

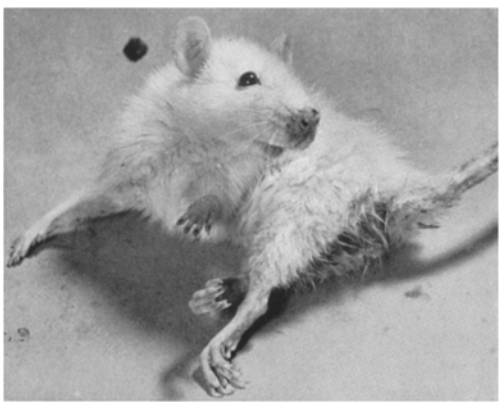

Fig. 1. Vitamin E deficient rat in the last stage, lying on side with legs and tail in bizarre postures. On attempting to stand, falling and rolling on the side. Slight involvement of forelegs. Hind legs and toes crooked

Material and Methods

The rats used in our study, from the inbred Fischer 344 strain, were selected at random from groups of 10 to 40 rats being used in an investigation of combined vitamin E-Factor 3*

* Factor 3 is a dietary agent effective in the prevention of dietary necrotic liver degeneration in the rat, multiple dietary necrosis in the mouse, exudative diathesis in chicks and a variety of other syndromes in different species. It was identified in 1957 as an organic comdound of the element selenium. It can be replaced by inorganic selenium in the form of sodium selenite (SCHWARZ and FOLTZ 1957, 1958). The minute amounts of selenium required physiologically for the prevention of these diseases are found widely distributed in dietary agents and foods, and also in normal tissue. They amount to only $^1/_{50}$ to $^1/_{100}$ of the minimum chronic toxic dose.

deficiency diseases, as indicated elsewhere (SCHWARZ 1960). The rats were given vitamin E deficient diets at weaning (ca. 21 days after birth) and kept in individual cages under standardized conditions. The composition of the basal ration is shown in Table 1. The ration was, in general, that of a semipurified dietary regimen in which casein was replaced by *Torula* as a source of protein, supplying 13—14% digestable protein to the animals (SCHWARZ 1951). The basal diet contained a supplement of 10 μg of selenium (in the form of sodium selenite) as a source of Factor 3 activity. In conventional vitamin E deficient diets, Factor 3 selenium

Table 1. *Composition of Basal Diet in Percentage Values*

Torula yeast[1]	30
Sucrose	59
Lard, vitamin-E-free[2]	5
Salts[3]	5
Vitamin powder[4,5]	1

[1] Dried yeast, *Torula utilis*, manufactured by Lake-States Yeast Corp., Rhinelander, Wisconsin.

[2] Vitamin E-free animal fat, stripped by molecular distillation (Distillation Products Div., Rochester, N. Y.).

[3] $CaCO_3$, 543 g; $MgCO_3$, 25 g; $MgSO_4$, 16 g; NaCl, 69 g; KCl, 112 g; KH_2PO_4, 212 g; $FePO_4$, 4 H_2O, 20.5 g; KI, 0.08 g; $MnSO_4$, 0.35 g; NaF, 1 g; $Al_2(SO_4)_3 \cdot K_2SO_4$, 0.17 g; $CuSO_4$, 0.9 g.

[4] Lactose, 88.68 g; thiamine HCl, 40 mg; riboflavin, 25 mg; pyridoxine HCl, 20 mg; D-calcium pantothenate, 200 mg; choline chloride, 10 g; niacin, 1 g; menadione (2-methyl-1, 4 naphtoquinone), 10 mg; folic acid, 20 mg; biotin, 10 mg; Vitamin A acetate (1 mg-%) and vitamin D (1 mg-%), dissolved in a small amount of ethanol (0.5 cc per 100 g of diet), were added to the diets.

[5] Factor 3 was added in the form of 10 μg of selenium, as sodium selenite, per 100 g of diet, except for 2 animals (AFIP Accs. 954293 and 954294) which received only 4 μg-% of the element throughout the experiment.

is normally supplied in the casein, which contains ample Factor 3 activity (SCHWARZ 1952). In the absence of Factor 3, liver necrosis inevitably develops and is fatal in approximately 21 days, while with selenium the animals live out most of their normal life span but manifest serious vitamin E deficiency. Used as controls were animals kept on the *Torula* regime supplemented with 50 mg of D,L-alpha tocopheryl acetate per 100 g of diet. Fischer 344 strain rats on *Torula* diets grow steadily at a rate of approximately 2 g per day until they reach a weight of ca. 175—200 g. Supplementation of tocopherol does stimulate growth but only very slightly. Dietary intake is not affected, except in the most advanced stage of the disease. With exception of a few rats studied at this stage, there were no great differences in weights between controls and deficient animals used. Since the basal diet was somewhat insufficient in its content of sulfur amino acids, the diet of a group of 20 animals was supplemented with 0.64% of D,L-methionine. In this group the incidence and severity of the neurological damage were much the same as in groups without methionine. Vitamin E was given therapeutically in some of the animals. Of group of 10 rats, of which 4 had died with severe paralysis and muscular wasting, 4 were given the vitamin E supplemented diet starting in the 13th month of the experiment. They were sacrificed after $2^1/_2$ months of treatment. The remainder served as untreated controls.

Observations

The clinical disturbances in our series of 7 vitamin E deficient adult rats (Table 2) were, on the whole, highly similar to those described by EINARSON et al. Incipient neurological signs and wasting of musculature developed consistently in all animals on the vitamin E deficient diet 6 to 9 months from the beginning of the experiment. These symptoms increased greatly in severity with

time*. Animals given the vitamin E supplement maintained a healthy condition throughout the experimental period. The period of observation before sacrificing the animals ranged from $14^1/_2$ to 23 months.

Table 2. *Animals Investigated*

Diet	Animal No.	AFIP No.	Duration of Experiment (months)	Clinical Features
I. Basal Vitamin E-deficient	1	954295	23	Severe, typical neurological signs
	2	954293	23	Severe, typical neurological signs
	3	954294	23	Severe, typical neurological signs
	4*	954304	$14^1/_2$	Severe, typical neurological signs
	5	954305	$14^1/_2$	Severe, typical neurological signs
II. Basal plus vitamin E	6	954298	23	No abnormalities
	7	958183	23	No abnormalities
	8	958180	23	No abnormalities
	9	958181	23	No abnormalities
	10+	958175	—	No abnormalities
III. Therapeutic trial: basal vitamin E deficient for 12 months, then vitamin E supplemented for $2^1/_2$ months	11	954302	$14^1/_2$	Severe, typical neurological signs
	12	954303	$14^1/_2$	Several, typical neurological signs

* Untreated, vitamin E deficient controls for Group III (therapeutic trial).

+ Random control from stock of AFIP, kept approximately 18 months on "Purina" diet (Laboratory Chow), manufactured by Ralston Purina Company, St. Louis 2, Missouri.

At autopsy no gross changes were seen in the brain, spinal cord, meninges or blood vessels in any of the animals. No hemorrhage or evidence of edema was noted. Microscopically, the changes observed in the 7 rats of the two groups were similar and thus will be presented as a whole.

Axonal Changes. In the affected grisea and fiber tracts of the spinal cord and medulla oblongata, in which, in Mallory preparations, the myelin sheaths were red and the axis cylinders blue, the essential pathological feature consisted in dystrophy of the axoplasm, manifested by swelling of axis cylinders up to 30 times their normal size. In cross section the swollen axis cylinders usually appeared as round or ellipsoid structures. Swollen axis cylinders could occasionally be identified by the still persisting red myelin sheath around them (Fig. 2). Sometimes only small remnants of myelin were seen about the swollen axons. In sections cut in various planes the axis cylinder swelling was found discontinuous, involving only short stretches. The forms in which the swelling appeared were diverse, e. g., moniliform (Fig. 3), fusiform, or torpedo-like. Now and then the axonal swelling involved longer stretches of an axis cylinder in an irregular manner. When the affected part of the axis cylinder reached a certain dimension

* The muscle lesions were investigated by Dr. KARL E. MASON of the University of Rochester, N. Y. They were not different from those ordinarily seen in vitamin E deficient animals.

its connections to the latter were severed and it led for quite a long period of time an "independent" existence. We apply to these formations the therm "axonal bodies" ("Schollen"). Most of the huge axonal bodies appeared homogenous, but PAS staining, which brought out the bodies most effectively, revealed in some a series of concentric rings and, in others, PAS-positive dots. Huge bodies often contained large vacuoles (Fig. 4). A further type of bodies encountered was granular with a halo. In Bodian preparations the granular core was argentophilic (Fig. 5), and in Mallory preparations most of the granules were stained red. The bodies lost their argentophilia on reaching certain dimensions, but many of them retained an outer argentophilic ring. The bizarrely shaped argentophilic bodies illustrated in Fig. 5 were exceptions to this rule. Some of the bodies contained round, spiral or otherwise shaped fibrils or crumbly material (Fig. 6). In Kluver-Barrera and cresyl echt violet preparations the bodies appeared as round or oval spaces (Figs. 7 and 8) which sometimes contained material which had a pale blue tint. When stained by hematoxylin-eosin the bodies were light pink.

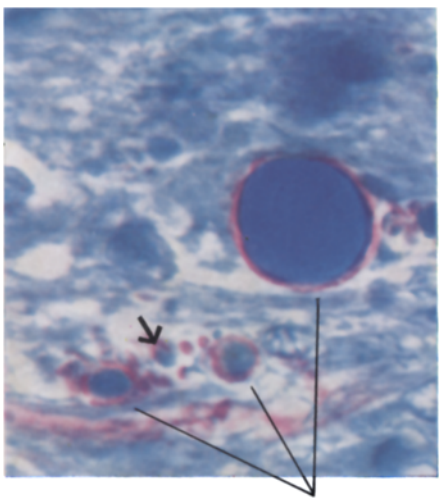

Fig. 2. Vitamin E deficient rat (AFIP Acc. 954294, Neg. 602443). Kodachrome. Nucleus gracilis. Cross section of 3 swollen axons (*sw. a.*), one of them gigantic. The swollen axons are identifiable as such by the still persisting red colored myelin sheaths. Normal sized axon (→). Mallory. ×900

Peculiar spiked ball-like structures ("Stachelkugeln") were also observed. They were relatively scanty and were seen in the nuclei cuneatus medialis and lateralis, in the sensory nuclei of the Vth nerve, and in the nucleus of the tractus solitarius (Figs. 9—11). They were most conspicuous in sections stained by the Mallory and PAS methods, but were also detected in H-E preparations. They had a blue (Mallory), light purple (PAS) or dark purple (H-E) compact nucleus from which many spikes radiated. The spiked balls were found to originate within huge axonal bodies (Fig. 12). Occasionally they had an outer shell of residue of the decomposed body (Fig. 11).

Changes in Grisea. Axonal dystrophy was noted in various grisea: the nuclei of the dorsal spinal horns, the cell group corresponding in position to the nucleus dorsalis (CLARKE's column in man*), the medioposterior cell column in the dorsal grey commisure, the nuclei gracilis, cuneatus medialis and lateralis, the sensory nuclei of the Vth nerve, and the nucleus of the tractus solitarius. In the upper cervical segments in one animal (Rat 5), the nucleus of Stilling was identified in a position corresponding to that of the nucleus dorsalis; it was severely affected.

* According to CRAIGIE, the nucleus dorsalis in the rat is not differentiated as a distinct group of cells. On the other hand, EINARSON and RINGSTED found, as we did, a very distinct and well defined cellular aggregate in this region, especially between the VIth thoracic and the upper lumbar segments.

In 2 animals (Rats 2 and 11) a few axonal bodies were seen in the close vicinity of large nerve cells in the ventral and lateral spinal horns.

Both the medial and the lateral nuclei of the fasciculus cuneatus exhibited the *florid stage* of the dystrophic process (Fig. 13). Because of the great number of

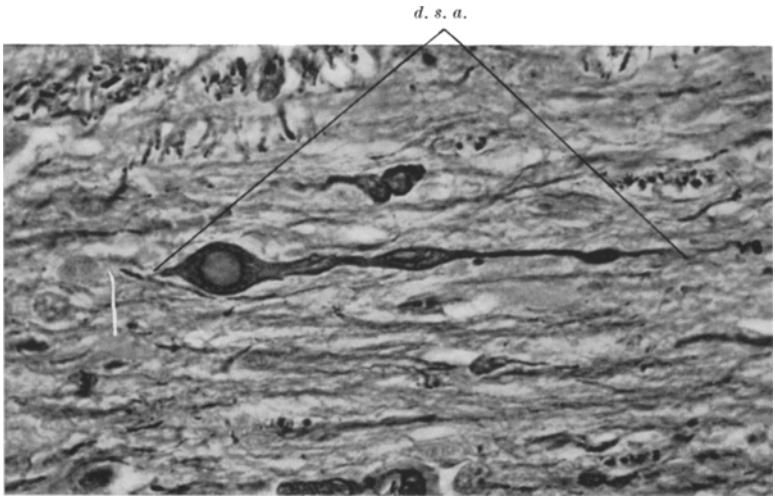

Fig. 3. Vitamin E deficient rat (AFIP Acc. 954302, Neg. 602454). Fasciculus gracilis. Longitudinal section. Discontinuous moniliform swelling of one axon (*d. s. a.*). The largest specimen has a round light core. Above and below, segments of swollen, irregularly shaped axons. The majority of the axons have disappeared, indicating an advanced state of axonal dystrophy. Bodian. ×575

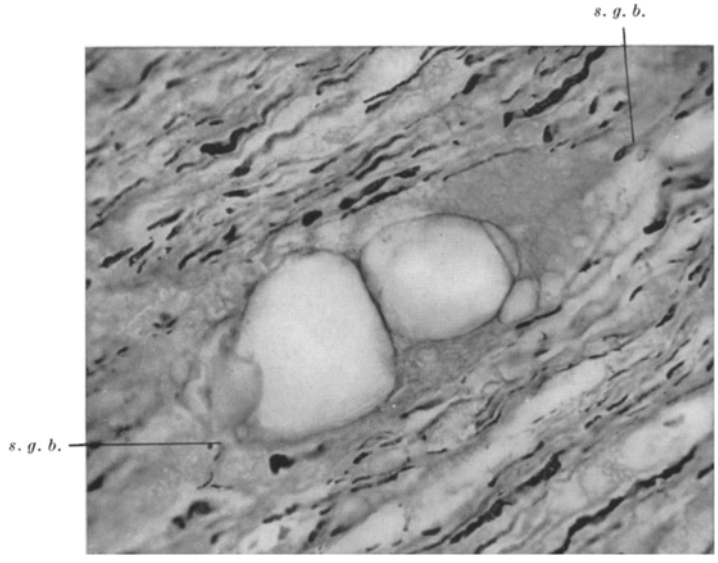

Fig. 4. Vitamin E deficient rat (AFIP Acc. 954305, Neg. 602838). Fasciculus gracilis. Single giant body (*s. g. b.*) with several larger and smaller vacuoles surrounded by well preserved axis cylinders. Bodian. ×550

giant axonal bodies, many of them conspicuously vacuolated, the pathological changes were evident even at low magnification. Except for the presence of PAS-positive deposits in their cytoplasm, the nerve cells were intact and their number was not appreciably decreased.

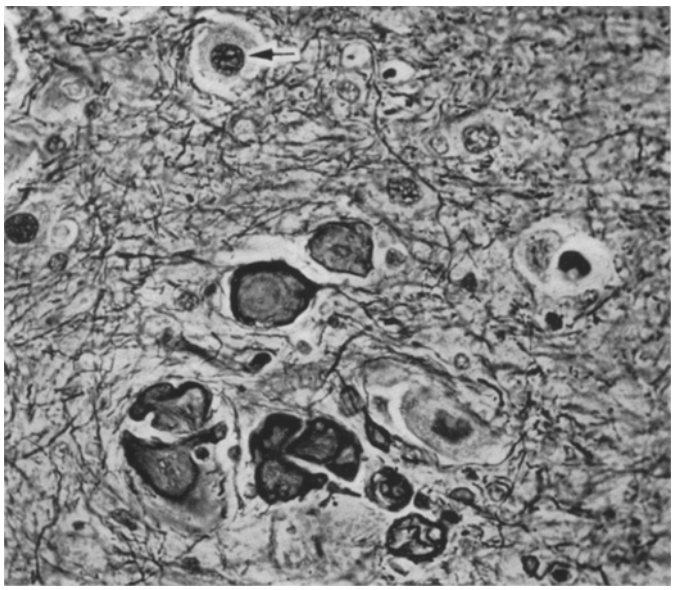

Fig. 5. Vitamin E deficient rat (AFIP Acc. 954294, Neg. 602441). Medio-posterior nerve cell column of dorsal grey commissure of spinal cord. Clusters of bizarre shaped highly argentophilic bodies. Above, a body with argentophilic core with halo (*arrow*). Bodian. ×400

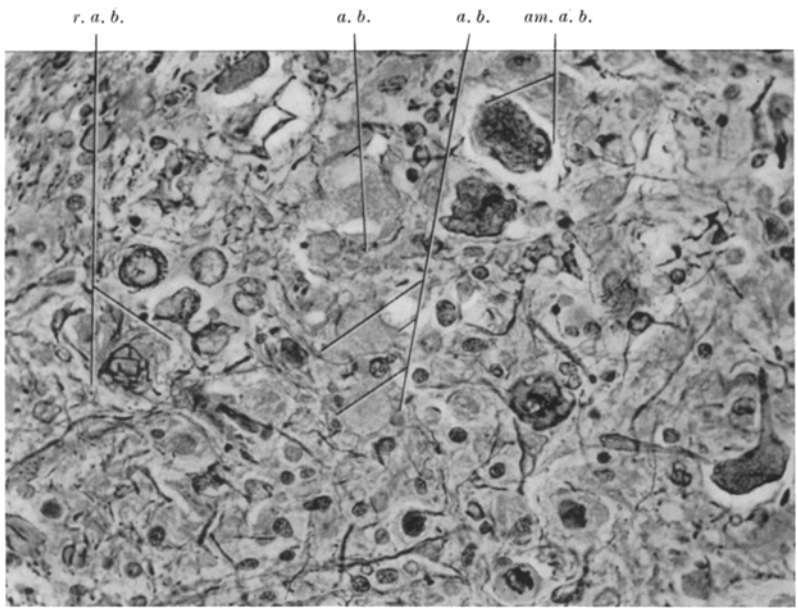

Fig. 6. Vitamin E deficient rat (AFIP Acc. 954293, Neg. 602336). Lateral cuneate nucleus. Bodies showing reticulate structure (*r. a. b.*), others containing amorphous argentophilic material. (*am. a. b.*) Many of the larger bodies (*a. b.*) are inconspicuous because of no argentophilia. Bodian. ×350

As to the principal sensory nucleus and the nucleus of the spinal tract of the Vth nerve, which in the rat form a unit which occupies most of the lateral part of the medulla oblongata (CRAIGIE), the alterations were, in general, much more

conspicuous in longitudinal than in cross section. In the dorsal spinal horns the damage was in all respects highly similar to that in the medial and lateral cuneate nuclei. Substantia gelatinosa and nucleus proprius were the sites of predilection of axonal involvement and the lower lumbar, sacral and coccygeal segments were often the most heavily damaged. Nucleus dorsalis (Fig. 14) and the nerve cell group in the dorsal grey commissure, corresponding to the medioposterior cell column in man, showed equally severe and extensive axonal changes as the

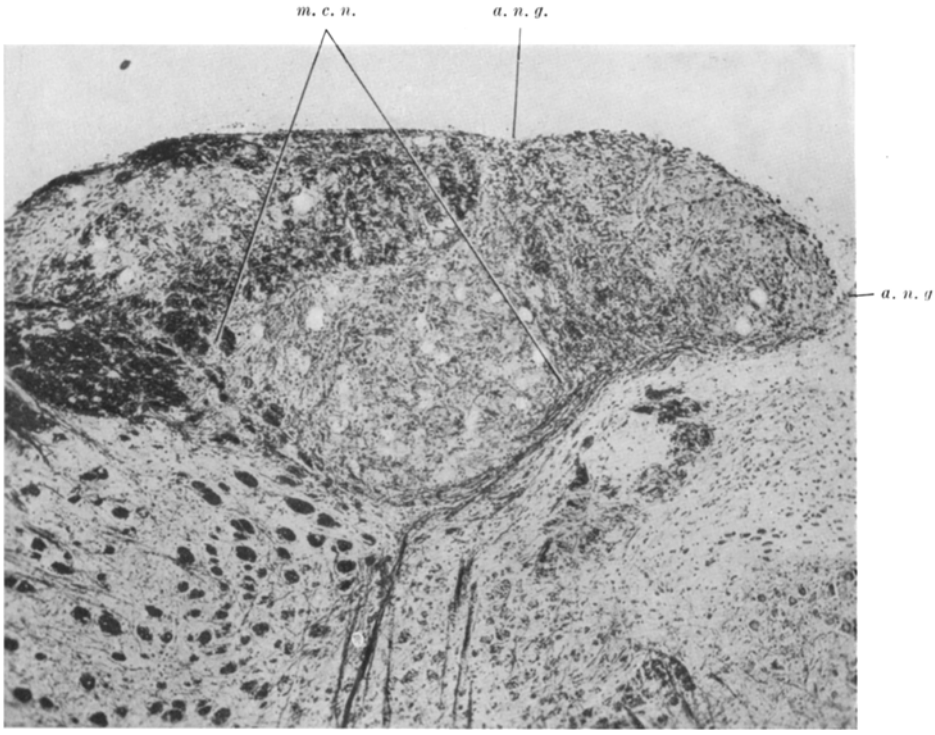

Fig. 7. Vitamin E deficient rat (AFIP Acc. 954295, Neg. 601940). Axonal dystrophy in the medial cuneate nucleus (*m. c. n.*) is evident from the large vacuoles within axonal bodies. In contrast, the atrophic nucleus gracilis (*a. n. g.*) is inconspicuous except for the increased cellularity. Kluver-Barrera. × 65

medial and lateral cuneate nuclei. The alterations in the nucleus tractus solitarius were less conspicuous but nonetheless evident, spiked balls also being present.

Abundant, large or giant (Fig. 15) bizarrely shaped "naked" astrocytes sometimes clustered in honeycomb fashion, and numerous small PAS-positive astrocytes with eccentric nucleus completed the spectacular histological picture presented by all these nuclei. Patchy tissue disintegration of involved grisea was another feature of the pathological picture; it was most pronounced in the sensory nuclei of the Vth nerve.

Due to absence or paucity of axonal bodies the alterations in the nucleus gracilis were so inconspicuous (Fig. 7) that they could easily have been overlooked. However, the gross atrophy of the nucleus with resulting compactness of its nerve cells, the increased number of small astrocytes and the many PAS-positive phagocytes strongly suggested a *terminal stage* of the same pathological process

present in the other affected nuclei. No fibrous astrocytes were present in HOLZER preparations.

In some of the dorsal roots, isolated medium-sized axonal bodies were observed. They were situated in the glial division of the roots adjacent to the cord. Except for the presence of PAS-positive pigment in the nerve-cell bodies, the spinal ganglia appeared normal.

Changes in White Matter. The axonal changes were restricted to the spinal cord and medulla oblongata, involving selectively the fasciculi gracilis and cuneatus,

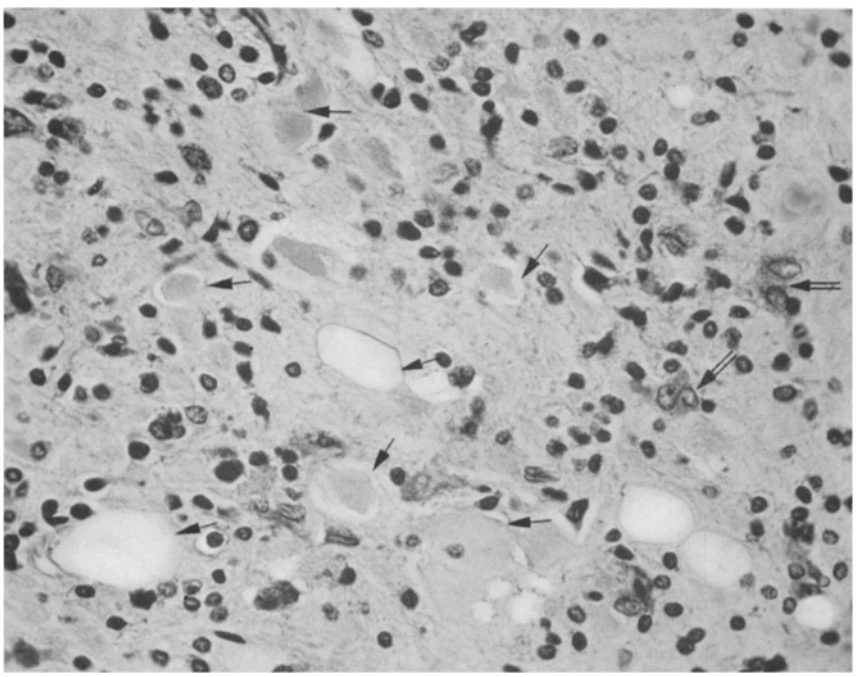

Fig. 8. Vitamin E deficient rat (AFIP Acc. 654295, Neg. 601937). Lateral cuneate nucleus. Some of the bodies lightly stained (←). Vacuoles within giant bodies. Increased number of glia cells. The nerve cells (⇐) are well preserved. Cresyl echt violet. ×350

LISSAUER's zone, spinal tract of Vth nerve, and the tractus solitarius (Table 3). At lumbo-sacral levels almost the entire area of the dorsal funiculi was affected, while at progressively higher thoracic and cervical levels the lesions were more and more restricted to the fasciculus gracilis. The lesions in the dorsal fasciculi were not continuous; in some cross sections they were missing. No changes were seen in the pyramidal tracts (which in the rat occupy a small ventral area in the dorsal funiculi) or in the lateral and ventral funiculi.

In the *incipient stage* the dystrophy was in the fasciculus cuneatus, spinal tract of the Vth nerve, LISSAUER's zone and the tractus solitarius, while in the *advanced stage* they were present only in the fasciculus gracilis. The incipient lesions were characterized by the presence of a few mediumsized bodies without glial reaction. In the advanced stage, as seen in the fasciculus gracilis, pronounced demyelination was present, axis cylinders were sparse, the majority of the axonal

Table 3. *Sites and Stages of Axonal Dystrophy in Vitamin E Deficient and Control Adult Rats*

Animal Nos.	GRISEA							WHITE MATTER				
	Dorsal Spinal Horn	"Column of Clarke"	Medio-Post. Cell Column	Nucleus Gracilis	Nuclei Cuneatus Med. & Lat.	Sensory Vth Nuclei	Nucleus Tractus Solitarius	Fasciculus Gracilis	Fasciculus Cuneatus	LISSAUER's Zone	Spinal Tract of Vth Nerve	Tractus Solitarius
	I. Basal Vitamin E Deficient Diet											
1	2+	2+	2+	3+	2+	2+	2+	2+	1+	1+	1+	1+
2	2+	2+	2+	3+	2+	2+	2+	2+	1+	1+	1+	1+
3	2+	2+	2+	3+	2+	2+	—	2+	1+	1+	1+	—
4	2+	2+	2+	3+	2+	2+	—	2+	1+	1+	1+	—
5	2+	2+	2+	—	—	2+	—	2+	1+	1+	1+	—
	II. Basal plus Vitamin E Diet (Control Animals)											
6	0	0	0	2+	0	0	0	0	0	0	0	0
7	0	0	0	1+	0	0	—	0	0	0	0	—
8	0	0	0	2+	0	0	0	0	0	0	0	0
9	0	0	0	2+	0	0	0	0	0	0	0	0
10	0	0	0	2+	0	0	0	0	0	0	0	0
	III. Basal Vitamin E Deficient Diet, then Therapeutic Vitamin E Trial											
11	2+	2+	2+	3+	2+	2+	2+	2+	1+	1+	1+	1+
12	2+	—	2+	—	2+	2+	2+	2+	1+	1+	1+	0

1+, incipient stage; 2+, florid stage; 3+, end stage; —, information not available; 0, no changes.

bodies had undergone dissolution, and intense glial proliferation had occurred. The few bodies that remained were usually large.

Abundant glial reaction was evident in the fasciculus gracilis. The glial cells were somewhat difficult to identify. Most of them were large and spherical and they had a hyperchromatic nucleus and distinct cytoplasm. Second most abundant were glial cells of a type never seen in the control animals. Their nuclei were huge, pale, usually elongated, lobulated or sausage-shaped, and "naked"; some of them were gigantic. Scattered glia of this kind, apparently astrocytes, were divided into two more or less equal parts connected by a small bridge, suggesting abortive amitotic division. In 3 animals typical plump astrocytes were seen; they were most abundant in the 2 animals given vitamin E after neurological manifestations had fully developed (Rats 11 and 12). In these 2 rats the dimensions of the plump astrocytes exceeded by 2 or 3 times the size of the plump astrocytes present in an untreated animal (Rat 3). Fibrous astrocytes were not seen in HOLZER preparations. Large phagocytes (macrophages) were found only in the transitional zone between the nucleus and fasciculus gracilis. They contained relatively large PAS-positive particles.

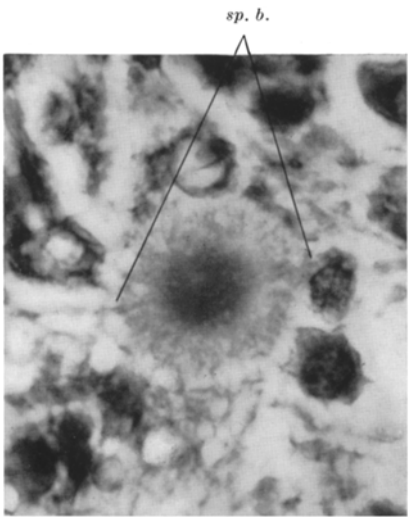

Fig. 9. Vitamin E deficient rat (AFIP Acc. 954304, Neg. 606959). Lateral cuneate nucleus. Characteristic spiked ball (*sp. b.*). Mallory. ×1300

Changes Unrelated to Axonal Dystrophy. The PAS method disclosed widespread numerous small microglial cells with pyknotic, eccentric nuclei and homogenous PAS-positive crimson cytoplasm. The principal areas in which such cells were found were the grey matter of the spinal cord, most of the nuclei of medulla oblongata, pons, midbrain and thalamus, the granular layer of the cerebellar cortex, and the intrafolial cerebellar white matter. A few PAS-positive cells were observed in the perivascular space and adjacent to the subarachnoidal space, suggesting that these cells were mobile and were engaged in the removal of PAS-positive material. PAS-positive particles were sometimes seen outside of cell bodies. Except for the ependymal cells of the central canal of spinal cord, fixed gial cells did not contain PAS-positive pigment.

Another characteristic finding which was not seen in the controls was the presence of PAS-positive pigment in most of the nerve cells in the spinal cord, brain stem, cerebellum and, in less intensity, in the thalamus and cerebral cortex. It was most conspicuous in the motor nerve cells of spinal cord and brain stem. In most of the nerve cells the PAS-positive material had the same location as lipofuscin, i. e., it was accumulated next to the nucleus or in the periphery of the cytoplasm. In other nerve cells it was diffusely scattered in the cytoplasm. In Bodian preparations the PAS-positive deposits in the nerve cells proved to be argentophilic; they had a granular or reticular structure. Otherwise, the nerve cells appeared normal, with no nuclear displacement. Many nerve cells of the spinal ganglia contained copious deposits of PAS-positive material but otherwise appeared normal. They contained no PAS-positive phagocytes.

Vacuolar Nerve Cell Changes. In most of the animals varying numbers of nerve cells contained vacuoles of varying dimensions. In motor nerve cells of the spinal cord and medulla oblongata they were, as a rule, single and some were almost as large as the nerve-cell body.

In some smaller nerve cells they were multiple and small, giving the cellular periphery a fenestrated appearance as if gnawed at. Characteristic of this nerve-cell change was the integrity of the nucleus and of the cytoplasm. Only a small number of the nerve cells showed pyknotic nuclei and hyperomatic cytoplasm.

Control Animals. In the 4 control animals given the vitamin E supplemented diet (Rats 6—10 in Table 3) grisea and fiber tracts were normal. However, the nucleus gracilis showed axonal swelling, similar to that described above for the medial and lateral cuneate nuclei of the vitamin E deficient rats. The changes,

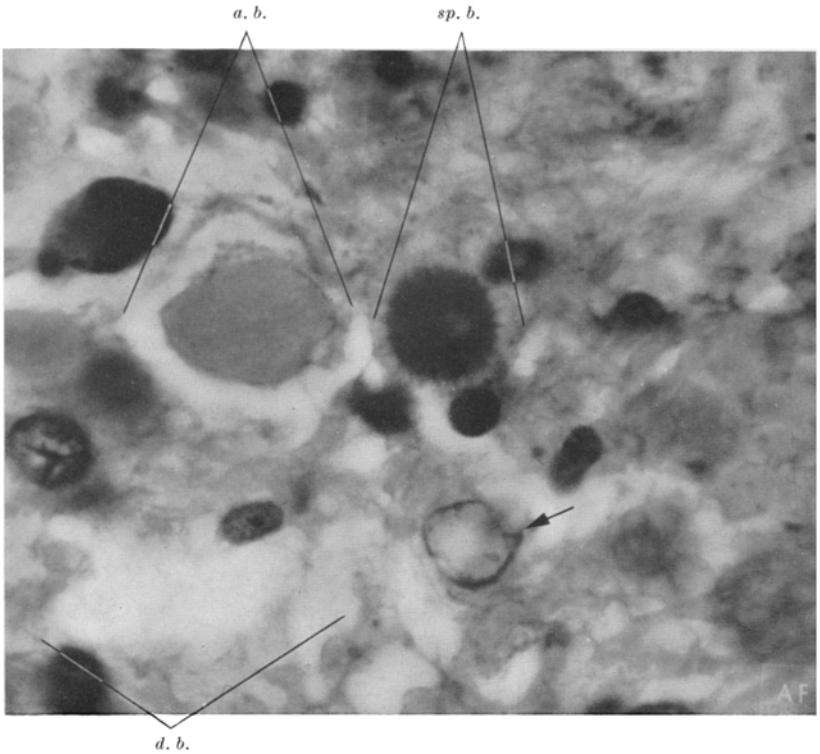

Fig. 10. Vitamin E deficient rat (AFIP Acc. 954304, Neg. 606958). Lateral cuneate nucleus. Spiked ball with huge core (*sp. b.*), axonal body with halo (*a. b.*); almost completely disintegrated body (*d. b.*), not to be confused with vacuole; huge astrocytic nucleus (*arrow*). PAS. ×1300

seen only in the nucleus gracilis, were not identical to those in the vitamin E deficient groups. They were much less severe, and neither "naked" astrocytic nuclei nor PAS-positive small phagocytes could be observed. A control rat taken at random from the A.F.I.P. stock showed the same changes of nucleus gracilis. This adult rat had been raised and maintained on Purina laboratory chow.

Axonal Dystrophy in Man

Changes similar to those seen in vitamin E deficient rats were found in 4 human cases. The similarity was most striking in Case 1.

Case 1 (C. A. L. 581/60). The patient, a 51-year-old Negro, was first admitted to a mental hospital when he was 42. Previously he had been locked up at least six or seven times for drunkenness, confusion, and wild behavior. He was mentally defective and had been epileptic

for many years. One year prior to death in 1959 he was admitted for the third time to a hospital for chronic alcoholism and tuberculosis. A serological test for syphilis was positive, but the cerebrospinal fluid was normal. The EEG was abnormal. It was thought that he had WERNICKE's encephalopathy.

Grossly, there was slight atrophy of the medulla oblongata and of the cerebellar cortex in the border zones of supply of the posterior inferior cerebellar arteries, and old contusion foci in left gyrus rectus and both temporal poles. Microscopic examination revealed axonal dystrophy in the nuclei gracilis, cuneatus medialis (Fig. 16) and lateralis, and of the nucleus of Stilling with numerous typical axonal bodies and scanty spiked balls. (Only cervical segments

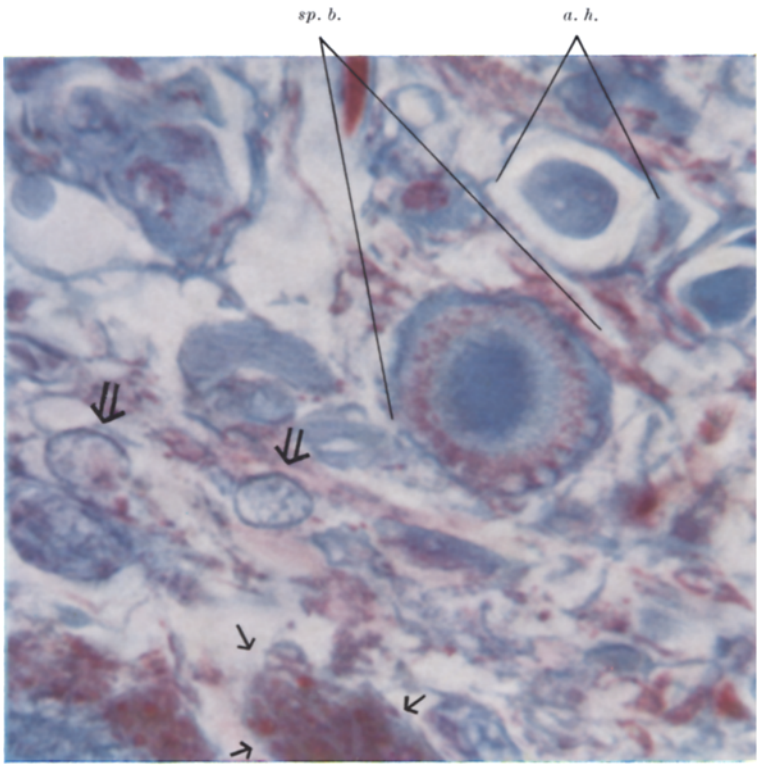

Fig. 11. Vitamin E deficient rat (AFIP Acc. 954304, Neg. 606961). Kodachrome. Lateral cuneate nucleus. Peculiar spiked ball with outer shell containing red granules, Peculiar presumably residues from the axon in which the spiked ball was "born" (*sp. b.*) (see also Fig. 12). Axonal body with halo (*a. h.*). Axonal body with red spots (←); huge astrocytic nuclei (⇐). Mallory. × 1300

were available so that the nucleus spinalis, of which STILLING's nucleus is a homologue, could not be examined.) The cerebellar changes were those of the familiar lobular sclerosis. No alterations suggesting WERNICKE's disease were found.

Case 2 (C. A. L. 897/60). This case, too, was one of chronic alcoholism. The patient was a 50-year-old Caucasian male. The changes in the nuclei gracilis and cuneatus medialis were similar although less conspicuous than in case 1. A small number of spiked balls were observed. Lateral cuneate nucleus and spinal cord were not available.

Case 3 (AFIP Acc. 991260). In this case of chronic alcoholism associated with epileptic seizures, the patient was a 73-year-old Caucasian man. The changes in the nuclei gracilis and cuneatus were similar to those in the previous two cases and were more pronounced than in case 2. Characteristic for this case were the perivascular infiltrates of lymphocytes located in the dorsal part of the lower medulla oblongata. Only a small piece of the lower medulla was available.

Case 4 (AFIP Acc. 808153). This case concerned a 66-year-old Caucasian man. The only clinical history available indicated chronic psychosis and sporadic grand mal of undetermined character and etiology. The nuclei gracilis and cuneatus medialis were involved. (Lateral cuneate nucleus and spinal cord as well as cerebrum and cerebellum were not available.) The tremendous swelling of the axons, leading to the formation of many giant axonal bodies, produced a striking pathological picture (Fig. 17).

Discussion

The nutritional aspects of the Torula yeast ration used in this study have been discussed in detail elsewhere (SCHWARZ et al.). The diet is limited in sulfur amino acids, but this aspect does not significantly enter into the etiology of the CNS

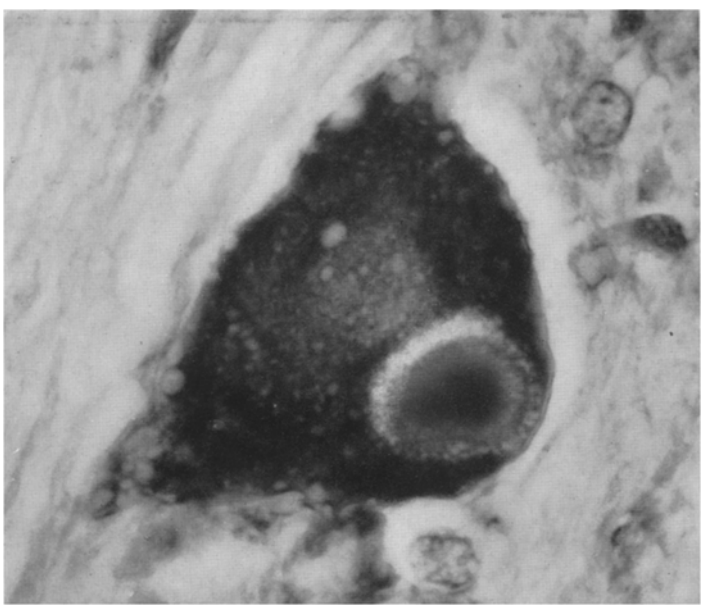

Fig. 12. Vitamin E deficient rat (AFIP Acc. 954304, Neg. 606960). Longitudinal section. Fasciculus cuneatus not far from its nucleus. Spiked ball in the process of formation within a giant axonal body. Mallory. ×1300

changes which are the subject of this paper, as shown by supplementation of methionine. The lack of Factor 3-selenium, also characteristic of Torula yeast diets, has been eliminated by supplementation of the selenite-selenium, added in very small, physiological dose levels. All other known dietary trace factors have been supplied at optimal levels (with the exception of the glucose tolerance factor, recently identified with trivalent chromium, K. SCHWARZ and W. MERTZ). Thus the described changes, preventable by tocopherol, are related to vitamin E deficiency, with the exception of minor axonal swellings restricted to the nucleus gracilis in the controls. The latter lesion is of unknown etiology. It was also seen in a stock animal kept on normal breeding ration. Its origin is under further investigation.

Our vitamin E deficient rats developed the same advanced neurological syndrome produced by RINGSTED. In our series, although only animals in advanced stages of vitamin E deficiency were available, the evolution of the dystrophic process of the axis cylinders was traceable. The *initial stage*, visible in the

fasciculus cuneatus, spinal tract of the Vth nerve, LISSAUER's zone and the tractus solitarius, was characterized by the presence of only a few small or medium sized axonal bodies without glial response. Myelin sheaths about the bodies were usually preserved. In the *florid*, or second stage, observed in all involved grisea except the nucleus gracilis, the main features consisted in the presence of great numbers of huge axonal bodies in different phases of disintegration, characterized mainly by vacuolization, and in the presence of many huge "naked" astrocytic nuclei. Myelin around the bodies had virtually disappeared, apparently as a consequence of the axonal swelling. It was evident that the demyelination was of

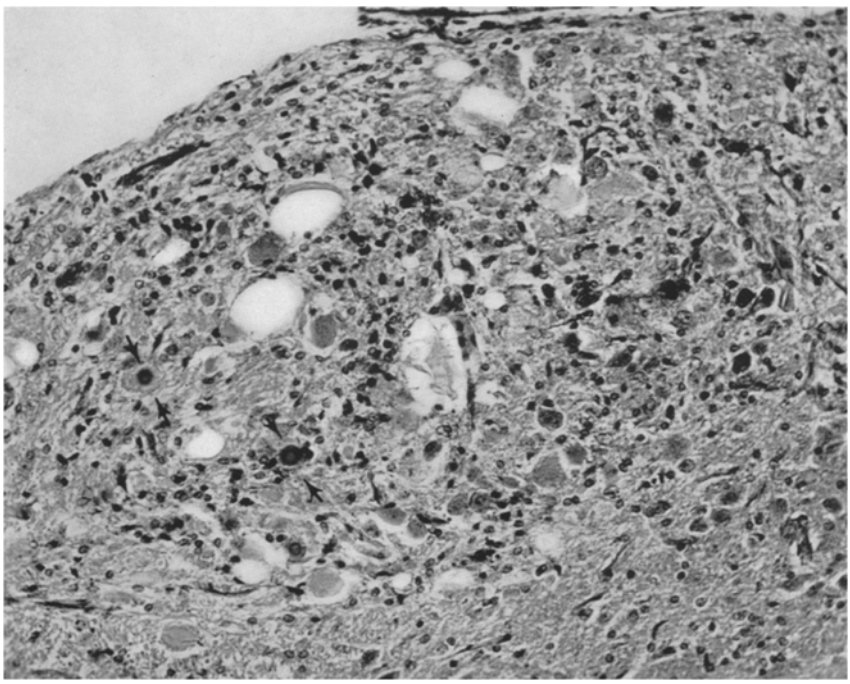

Fig. 13. Vitamin E deficient rat (AFIP Acc. 954293, Neg. 602338). Lateral cuneate nucleus. Florid stage of axonal dystrophy with huge axonal bodies containing large vacuoles; glial proliferation. Spiked balls within axonal bodies (arrows). PAS. ×165

secondary nature. In some grisea the tissue showed patchy disintegration. The *terminal*, or third stage, present only in the nucleus gracilis, was characterized by absence or paucity of axonal bodies, gross atrophy of the nucleus with preservation of the nerve cells, increased number of small astrocytes, and by the presence of large phagocytes containing PAS-positive material. The fewness of the axonal bodies was attributed to their dissolution. Likewise, fasciculus gracilis was the most extensively demyelinated and it showed many huge "naked" astrocytic nuclei and a relatively small number of axonal bodies, most of them gigantic. In 2 of the 3 animals in which vitamin E was given therapeutically (Rats 11 and 12), plump astrocytes of huge dimensions were present in the fasciculus gracilis. Neither in the nucleus gracilis nor in fasciculus gracilis was there HOLZER-positive gliosis. The absence of astrogliosis was interpreted as due to

the inability of the astrocytes to form HOLZER positive fibers. On the other hand, MALAMUD, NELSON and EVANS as well as EINARSON and TELFORD found HOLZER astrogliosis in the demyelinated areas of the dorsal funiculi.

From the pathological changes it seemed that the dystrophic process commenced within the nucleus gracilis and extended subsequently in a discontinuous manner and presumably at a slow pace to the fasciculus gracilis. It would appear

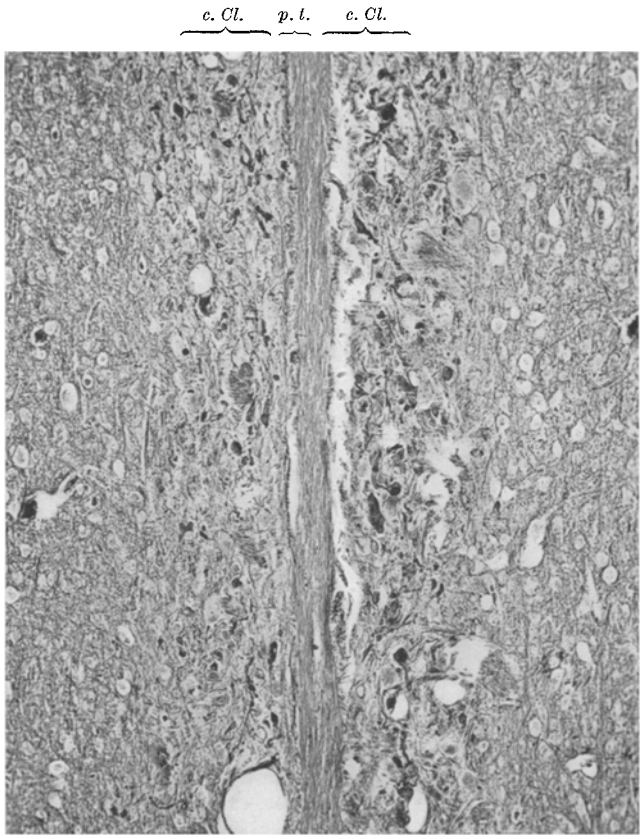

Fig. 14. Vitamin E deficient rat (AFIP Acc. 954302, Neg. 602447). Longitudinal section of the thoracal spinal cord at the level of the pyramidal tracts (*p. t.*), which, in the rat, occupy the most ventral field of the dorsal column. Severe axonal dystrophy with disintegration of the tissue restricted to two small paramedial fields on both sides of the pyramidal tracts, corresponding to the columns of Clarke (*c. Cl.*). Bodian. ×80

that the dystrophic process then began in the other grisea, the last affected being the fasciculus cuneatus, the tracts of the sensory nuclei of the Vth nerve and of the LISSAUER's zone, and the tractus nucleus solitarius.

Of interest were the spiked ball-like structures found in all affected grisea except for the nucleus gracilis. The originated within axonal bodies and were more resistant to disintegration than the axonal bodies. The spiked balls were only one type of various bizarre structures resulting from decomposition of the dystrophic axons. They were clearly not senile plaques. The spiked balls were reminiscent of the "Stachelkugeln", or "Morgensterne", found together with "torpedos" in the molecular and granular layers of the cerebellar cortex in systemic degene-

ration of the cerebellar cortex (ULE), and in cerebellar cortical degeneration following dimethyl mercury poisoning (HUNTER and RUSSELL). The spiked balls were probably identical with the bodies found by KLATZO et al. in the granular layer and occasionally in the molecular layer of the cerebellar cortex and in the basal ganglia and cerebral cortex in 6 of 12 cases of Kuru. In the 2 of the 3 cases of chronic alcoholism (cases 1 and 2) in which axonal dystrophy was evident in the nuclei gracilis and cuneatus spiked balls were also found. Some of them were lying within axonal bodies.

An interesting point was the integrity of the nerve-cell bodies in all affected grisea. From this observation it may be concluded that transneuronal degeneration did not take place. Another conspicuous nerve cell change was the cytoplasmic vacuolation in the nerve cells. EINARSON and TELFORD also described this change

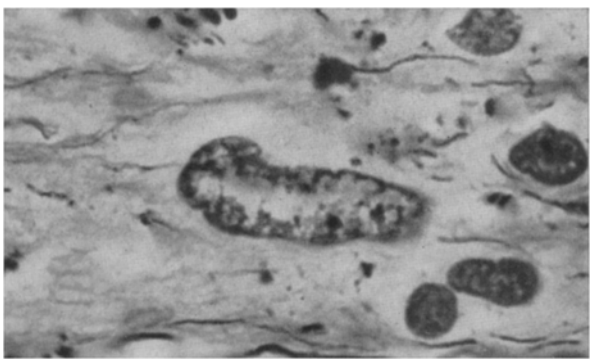

Fig. 15. Vitamin E deficient rat (AFIP Acc. 954294, Neg. 606711). Fasciculus gracilis. Gigantic naked astrocytic nucleus among normal glia cells. Residues of axons. Bodian. ×1300

in vitamin E deficient mice. It closely resembled the nerve cell changes observed in cats exposed to chronic tellurium poisoning (PENTSCHEW).

Another characteristic finding was the presence of huge, "naked" astrocytic nuclei, which were elongated, sausage-like shaped and sometimes spherical. They were most numerous in the grisea showing the greatest axonal dystrophy and were present also in the fasciculus gracilis, where the axonal damage was very advanced. Possibly the patchy tissue disintegration observed in the dorsal horns, the column of CLARKE and in the sensory nuclei of the Vth nerve was attributable to dysfunction of these astrocytes.

Conspicuous also in the pathological process were the numerous small PAS-positive phagocytes scattered throughout practically all the grisea, mainly those in the spinal cord and medulla oblongata. Quite distinct from them were large phagocytes which contained relatively coarse PAS-positive particles; they were infrequent and were found only in the nucleus gracilis and its tract. The small phagocytes were apparently related partly to the disturbance of lipofuscin metabolism characteristic of vitamin E deficiency (BECKMANN) and partly to the breakdown of myelin and perhaps of axoplasm.

Dystrophic axonal changes have been found under numerous disease conditions. They have been extensively studied in the proximal stump after severance of the neurite by CAJAL and by SPATZ, the latter concluding that the axonal

changes were the outcome of "acute retrograde reaction". The resemblance (SPIELMEYER; SEITELBERGER 1957) to the findings in the vitamin E deficient rat is impressive despite the fact that the axonal changes in the latter start in the most distal part of the nerve fiber. Axonal swelling is seen also in the region of ischemic areas, e.g., in the spinal cord in decompression sickness (HAYMAKER). It also has been observed in various degenerative diseases, such as amyotrophic lateral sclerosis, olivo pontocerebellar dystrophy, and familiar diffuse leucoencephalopathy (VAN BOGAERT and SCHOLZ). Under such conditions the axonal swelling has, however, been found more or less incidental.

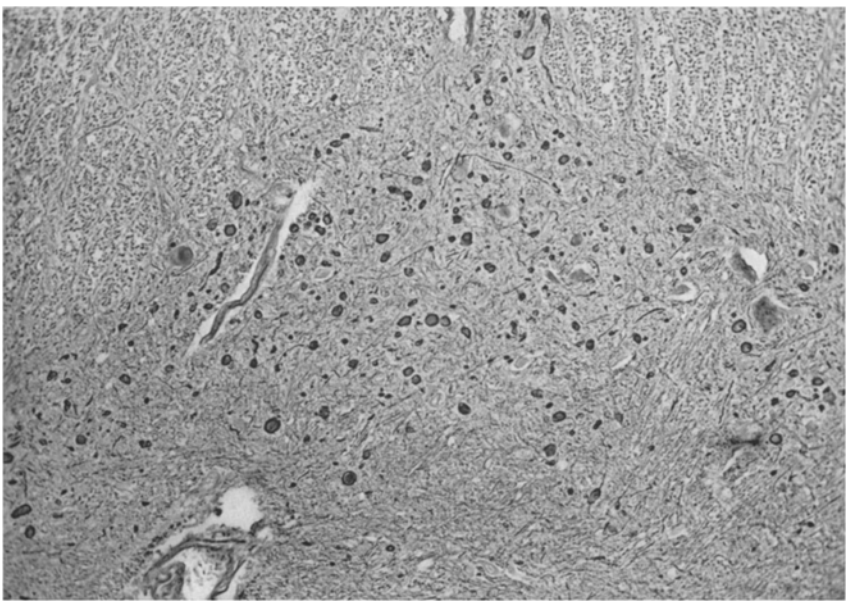

Fig. 16. 51-year-old man (CAL 581/60, AFIP Neg. 607093). Chronic alcoholism. Nucleus cuneatus medialis. The nucleus sharply delineated by the presence of a great number of argentophilic axonal bodies. Bodian. ×115

SEITELBERGER and GROSS were the first to draw attention to Hallervorden-Spatz disease, including an infantile form (SEITELBERGER 1953), as the prototype of an encephalo-myelopathy in which a systemic axonal dystrophy of endogenous nature is the main feature. (See also SEITELBERGER and GROSS and GROSS et al.) In their appearance and staining properties the axonal lesions described by SEITELBERGER et al. were highly similar to those observed in vitamin E deficient rats. The distribution of the changes differed, however, in most points. Besides globus pallidus and red zone of substantia nigra, where a great number of huge axonal bodies were regularly seen, they were often found in the red nucleus, the ventral nucleus of thalamus, the tegmentum of brain stem, and the dorsal horns and dorsal fasciculi of spinal cord. Axonal bodies were found also in the ansa lenticularis and the white matter of the cerebellum. The nuclei gracilis and cuneatus medialis were affected in a similar manner (SEITELBERGER 1960). No information was available on the lateral cuneate nucleus.

Our 3 cases of chronic alcoholism may readily be included in the group of the systemic axonal dystrophic encephalomyelopathies. This is true especially of

case 1 in which the appearance and staining properties of the axonal lesions as well as their distribution were strikingly similar to those in vitamin E deficiency disease of the rat. In cases 2 and 3 of chronic alcoholism, the topography of the axonal swelling was not as clear because the nucleus cuneatus lateralis and the spinal cord were not available for study. In 2 of the 3 cases spiked balls were present. We gained the impression that in the 3 cases the axonal lesions were a manifestation of some impairment other than thiamine deficiency associated with chronic alcoholism. The perivascular lymphocytic infiltrates in case 3 are secondary changes of an inflammatory character. They have been occasionally

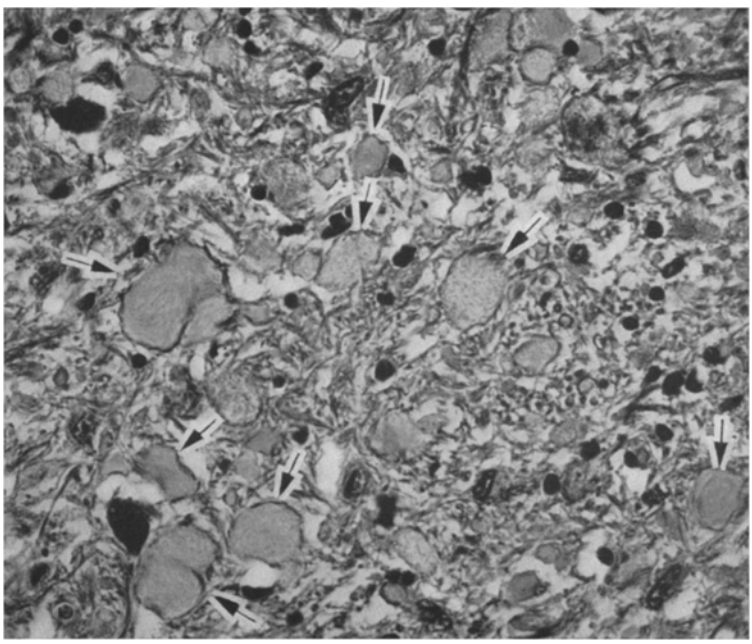

Fig. 17. 66-year-old man with chronic psychotic condition of undetermined etiology (AFIP Acc. 808153, Neg. 602830). Nucleus gracilis. Great number of large and small axonal bodies. (←) H-E. ×440

seen in cases of WERNICKE's encephalopathy (KANT; LÜTHY and WALTHARD; NEUBÜRGER) and have been found to be related to extracerebral infectious processes.

Case 4 was difficult to evaluate because of the paucity of medulla oblongata available and because of the very limited clinical history. However, the location of the characteristic axonal lesions and their extensiveness left little doubt that axonal dystrophy of endogenous origin was concerned. Further studies are required before it can be determined whether Hallervorden-Spatz disease and our 3 cases of chronic alcoholism were related to vitamin E deficiency or whether they were basically different metabolic disorders leading to morphologically similar systemic axonal lesions.

Summary and Conclusions

1. In rats maintained on a synthetic vitamin E deficient diet for $14^1/_2$ to 23 months, with *Torula* yeast as the source of protein, severe neurological disabilities developed. The neurological syndrome was practically the same as described by

RINGSTED. Hitherto undescribed pathological changes were found. The most impressive alteration consisted in axonal dystrophy of terminal twigs of nerve fibers within the grisea of the spinal cord and medulla oblongata belonging to the afferent system. These were nerve fibers originating in the spinal ganglia and in the ganglia of the V, VII, IX, and X cranial nerves. The demyelination that occurred was secondary to the axonal damage. The evidence suggested that the pathological process started in axis cylinders in the grisea and then spread proximally into their respective fiber tracts. Nerve cells in the affected grisea were not materially altered. The pattern of distribution of the alterations suggested a disorder of a systemic nature.

2. The dystrophy of the axis cylinders resulted in axonal swelling and subsequently in formation of large axonal bodies. Of special interest was the formation of spiked ball-like structures within the axonal bodies. They corresponded to the "Stachelkugeln" in human neuropathology.

3. Huge "naked" astrocytes were found in the affected grisea and fiber tracts. They did not show fibers in HOLZER preparations, however Cajal's method allowed to identify them as hypertrophic fibrous astrocytes. Small PAS-positive phagocytes of microglial origin were ubiquitous, but were most prominent in the affected grisea.

4. In the 4 control animals given the vitamin E supplemented diet, grisea and fiber tracts were normal. However, axis cylinders in the nucleus gracilis showed swellings similar, but not identical, to those seen in the medial and lateral cuneate nuclei of the vitamin E deficient rats. The same alterations were found in a stock animal kept on Purina laboratory chow. Their etiology is not clear.

5. The axonal changes found in the vitamin E deficient rats were practically the same as in certain human cases of chronic alcoholism and in Hallervorden-Spatz disease. The distribution of the lesions in the rats closely simulated that in the cases of chronic alcoholism but was quite different than in Hallervorden-Spatz disease. Whether the axonal dystrophy in these disorders was related to some specific deficiency is not known.

Zusammenfassung

1. Ratten, welche $14^1/_2$—23 Monate eine Vitamin E freie Diät (Torula-Hefe als Proteinquelle) erhalten hatten, entwickelten das von RINGSTED gezeigte, neurologische Syndrom. Es wurden bisher nicht beschriebene, neuropathologische Befunde erhoben. Die eindrucksvollste Veränderung bestand in einer axonalen Dystrophie der Endzweige von Nervenfasern innerhalb dem afferenten System zugehöriger Grisea des Rückenmarkes und der Medulla oblongata. Es handelte sich um Nervenfasern, welche ihren Ursprung in den Spinalganglien und den Ganglien von Nn. V, VII, IX und X haben. Die Entmarkung war sekundär, eine Folge der Axonschädigung. Es wurde angenommen, daß der pathologische Vorgang seinen Anfang in Achsencylindern innerhalb der Grisea nahm und sich dann proximal auf die zugehörigen Faserbündel ausbreitete. Die Nervenzellen in den betroffenen Grisea waren intakt. Das Verteilungsmuster der Läsionen legte das Vorliegen einer systematischen Erkrankung nahe.

2. Die dystrophischen Vorgänge in den Achsencylindern manifestierten sich in Axonschwellung und späterhin Bildung von großen, schollenartigen Gebilden.

Von Interesse war die Entstehung eigenartiger kugeliger Gebilde innerhalb der Schollen, welche den „Stachelkugeln" der menschlichen Neuropathologie entsprechen. Sie waren der auffälligste Typ bizarrer Strukturen, welche mit dem Abbau der dystrophischen Achsencylinder zusammenhingen.

3. Große, „nackte" Astrocyten wurden in den betroffenen Grisea und in schwer geschädigten Faserbündeln gefunden. Sie zeigten in Holzer-Präparaten keine Gliafasern, konnten jedoch mit der Cajalschen Goldsublimatmethode als hypertrophische Faserbildner identifiziert werden. Kleine PAS-positive Phagocyten mikrogliöser Abstammung lagen überall verstreut, waren aber am ausgesprochensten in den betroffenen Grisea.

4. In den vier Kontrolltieren, die eine mit Vitamin E versehene Diät erhalten hatten, zeigte der Nucleus gracilis Axonveränderungen ähnlich, aber nicht identisch mit denen in den Nuclei cuneatus medialis und lateralis der Vitamin E-Mangel-Ratten. Diese Veränderungen wurden auch in einem normalen Kontrolltier vorgefunden, das mit einer gewöhnlichen Zuchtdiät aufgezogen war.

5. Die Achsencylinderveränderungen in den Vitamin E-Mangel-Ratten waren außerordentlich ähnlich solchen, die bei bestimmten Fällen von chronischem Alkoholismus und bei der Hallervorden-Spatz-Krankheit beobachtet wurden. Die Ausbreitung der Läsionen war außerordentlich ähnlich in den Fällen von chronischem Alkoholismus und ziemlich verschieden in letzterer Krankheit. Es erhebt sich die Frage, ob die Achsencylinderveränderungen in diesen Erkrankungen in Beziehung zu bestimmten Mangelzuständen stehen.

References

Beckmann, R.: Vitamin E (Physiologie, pathologische Physiologie, klinische Bedeutung). Z. Vitamin-, Hormon- u. Fermentforsch. Sonderdruck Bd. VII 1—166; p. 65 (1955).

Bogaert, L. van, and W. Scholz: Klinischer, genealogischer und pathologisch-anatomischer Beitrag zur Kenntnis der familiären diffusen Sklerose. Z. ges. Neurol. Psychiat. **141**, 510—561 (1932).

Cajal, R. y.: Les métamorphoses précoces des neurofibrilles dans la régénération et la dégénération des nerfs. Trav. Labor. Rech. Biol. Univ. Madrid **5**, 47—104 (1907).

Craigie, E. H.: An introduction to the finer anatomy of the central nervous system, based upon that of the albino rat. Philadelphia: Blackston, 1925; pp. 25, 27, 31.

Einarson, L.: Critisizing review of the concepts of the neuro-muscular lesions in experimental vitamin E deficiency, preferably in adult rats. Acta psychiat. scand. Suppl. **78**, 9—76 (1952).

—, Deposits of fluorescent acid-fast products in the nervous system and skeletal muscles of adult rats with chronic vitamin E deficiency. J. Neurol. Neurosurg. Psychiat. **16**, 98—109 (1953).

—, and A. Ringsted: Effect of chronic vitamin E deficiency on the nervous system and the skeletal musculature in adult rats. A neurotropic factor in wheat germ oil. Copenhagen: Levine & Munksgaard-Ejnar Munksgaard, and London: Oxford Univ. Press 1938; pp. 23 to 27, 51—52.

—, and I. R. Telford: Effect of vitamin E deficiency on the central nervous system in various laboratory animals. Biol. Skr. Dan. Vid. Selsk. **11**, Nr 2, København, 1960; pp. 1 to 81.

Goettsch, M., and M. Pappenheimer: Nutritional muscular dystrophy in the guinea pig and rabbit. J. exp. Med. **54**, 145—165 (1931).

Gross, H., E. Kaltenbäck and B. Uiberrak: Über eine spätinfantile Form der Hallervorden-Spatzschen Krankheit. I. Klinisch-anatomische Befunde. Dtsch. Z. Nervenheilk. **176**, 77—103 (1957).

Haymaker, W.: Decompression sickness. In Henke-Lubarsch' Handbuch der speziellen pathologischen Anatomie und Histologie. Bd. XIII/1 B, p. 1636, Fig. 11 A. Berlin, Göttingen, Heidelberg: Springer 1957.

HUNTER, D., and D. S. RUSSELL: Focal cerebral and cerebellar atrophy in a human subject due to organic mercury compounds. J. Neurol. Neurosurg. Psychiat. **17**, 235—241 (1954).

KANT, F.: Die Pseudoencephalitis Wernicke der Alkoholiker (Polioencephalitis haemorrhagica superior acuta). Arch Psychiat. Nervenkr. **98**, 702—768 (1932).

KLATZO, I., D. C. GAJDUSEK and V. ZIGAS: Pathology of Kuru. Lab. Invest. **8**, 799—847 (1959).

LÜTHY, F., and K. M. WALTHARD: Über Polioencephalitis haemorrhagica superior (WERNICKE). Z. ges. Neurol. Psychiat. **116**, 404—422 (1928).

LUTTRELL, C. N., and K. E. MASON: Vitamin E deficiency, dietary fat, and spinal cord lesions in the rat. Ann. N. Y. Acad. Sci. **52**, 113—120 (1949).

MALAMUD, N., M. M. NELSON and H. M. EVANS: The effect of chronic vitamin E deficiency on the nervous system in the rat. Ann. N. Y. Acad. Sci. **52**, 135—138 (1949).

NEUBÜRGER, K.: Über Hirnveränderungen nach Alkoholmißbrauch (unter Berücksichtigung einiger Fälle von Wernickescher Krankheit mit anderer Ätiologie). Z. ges. Neurol. Psychiat. **135**, 159—209 (1931).

PENTSCHEW, A.: Die vitale Tellurfärbung und Speicherung, sowie ihre Bedeutung für die Lehre vom Stoffaustausch zwischen dem Zentralnervensystem und dem übrigen Körper. Arch. Psychiat. Nervenkr. **102**, 749—787 (1934).

RINGSTED, A.: A preliminary note on the appearance of paresis in adult rats suffering from chronic avitaminosis E. Biochem. J. **29**, 788—795 (1935).

SCHWARZ, K.: Production of dietary necrotic liver degeneration using American Torula yeast. Proc. Soc. exp. Biol. (N. Y.) **77**, 818—823 (1951).

—, Casein and Factor 3 in dietary necrotic liver degeneration. Concentration of Factor 3 from casein. Proc. Soc. exp. Biol. (N. Y.) **80**, 319—323 (1952).

—, Factor 3, Selenium and vitamin E (Editorial). Nutr. Rev. **18**, 193—197 (1960).

—, and C. M. FOLTZ: Selenium as an integral part of alpha-Factor 3 against necrotic liver degeneration. J. Amer. chem. Soc. **79**, 3292—3293 (1957).

—, and C. M. FOLTZ: Factor 3 activity of selenium compounds. J. biol. Chem. **233**, 245—251 (1958).

—, and W. MERTZ: Chromium(III) and the Glucose Tolerance Factor. Arch. Biochem. **85**, 292—295 (1959).

SEITELBERGER, F.: Zur Morphologie und Histochemie der degenerativen Axonveränderungen im Zentralnervensystem. IIIe Congrès internat. neuropath. Bruxelles 1957. Acta. med. belg., Bruxelles; pp. 127—147.

—, Personal communication to the authors, 1960.

—, and H. GROSS: Über eine spätinfantile Form der Hallervorden-Spatzschen Krankheit. II. Histochemische Befunde. Erörterung der Nosologie. Dtsch. Z. Nervenheilk. **176**, 104—125 (1957).

SPATZ, H.: Über die Vorgänge nach experimenteller Rückenmarksdurchtrennung mit besonderer Berücksichtigung der Unterschiede der Reaktionsweise des reifen und des unreifen Gewebes. NISSL-ALZHEIMERS Histol. Arb. Großhirnrinde, Erg. Bd., (1921), pp. 49—367.

SPIELMEYER, W.: Lehrbuch der speziellen pathologischen Anatomie des Nervensystems, pp. 42—43, 259—261. Berlin: Springer 1922.

ULE, G.: Die systematischen Atrophien des Kleinhirns. In: HENKE-LUBARSCH, Handbuch der speziellen pathologischen Anatomie und Histologie. Bd. XIII/1 A. pp. 950—951. Berlin, Göttingen, Heidelberg: Springer 1957.

Prof. Dr. A. PENTSCHEW, Department of Mental Hygiene, Central Anatomic Laboratory, 700 Fleet Street, Baltimore 2, Maryland, U.S.A.