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Differentiation between brain lesions in experimental thiamine deficiency

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Summary. Dietary deprivation of thiamine combined with pyrithiamine administration in rats was used for pathophysiological and morphological investigations. The animals passed through three different symptomatic stages, ranging from slight neurological abnormalities to generalized seizures from day 8 up to day 11. Hypothermia was a consistent finding during the second week. Histological examination revealed two types of neuropathological lesions in the rats. Those in the colliculi inferiores and the vestibular nuclei were characterized by a bullous spongiform appearance of the neuropil with severely damaged, pale and oedematous nerve cells. Alterations in the thalamus and inferior olives, however, showed eosinophilic nerve cell necrosis of the ischaemic type which resembles the thalamic pathology found in human cases of Wernicke's encepalopathy.

Key words: Metabolic Brain Diseases – Thiamine deficiency – Wernicke's Encephalopathy – Hypothermia

Introduction

Neuropathologic changes in Wernicke's encephalopathy involve the mammillary bodies, the thalamus and structures in the brainstem (Victor et al. 1971). Other investigations have demonstrated that lesions in the thalamus and inferior olives are structurally different from those in the mammillary bodies and in the floor of the fourth ventricle (Colmant 1965; Torvik 1985). In animal experiments, thiamine deficiency has been shown to induce brain lesions which are quite similar, but not identical to those observed in human beings (Watanabe and Kanabe 1978; Troncoso et al. 1981; Hakim and Pappius 1983). We further investigated the possible development of two different patterns of neuropathology in rats which were fed a thiamine-deficient diet. Indeed, we found two types of pathologic changes and these in two different topological distributions in experimental thiamine deficiency.

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Materials and methods

Six male albino rats (Chbb:THOM, Dr. Karl Thomae, Biberach-Riss, FRG), 4 weeks old, weighing 115–125 g at the start of the experiment, were used. They were kept in individual cages, were fed a thiamine-deficient diet (Altromin C 1021 Vitamin B1-arme Diät, 4937 Lage/Lippe, FRG) and received daily subcutaneous injections of pyrithiamine hydrochloride (Sigma Chemicals, St. Louis, USA) in a dosage of 50 microgram/100 g body weight. Six control animals received a normal thiamine containing diet ad libitum. The rats were weighed and examined daily. Temperature was measured beginning from the 5th day on. After development of severe neurological manifestations and hypothermia, which occurred after 10 or 11 days, the animals were sacrificed by intracardiac perfusion in ether narcosis with a solution containing 4% formaldehyde under a pressure of 100 cm H_2O. Perfusion was finished after 15 min and the bodies were kept in toto in formaldehyde for several days. The brains were removed and sectioned in the coronal plane. Additionally, the sciatic nerves were removed. The sections were dehydrated and embedded routinely and stained with hematoxylin-eosin, cresyl violet, elastica van Gieson and partly with Klüver-Barrera.

Results

Clinical manifestations

During the first week the animals gained weight. Subsequently there was steady loss of body weight of the animals on thiamine-deficient diet (Fig. 1), whereas the control animals gained weight normally. We observed 3 clinical stages. Stage 1 was

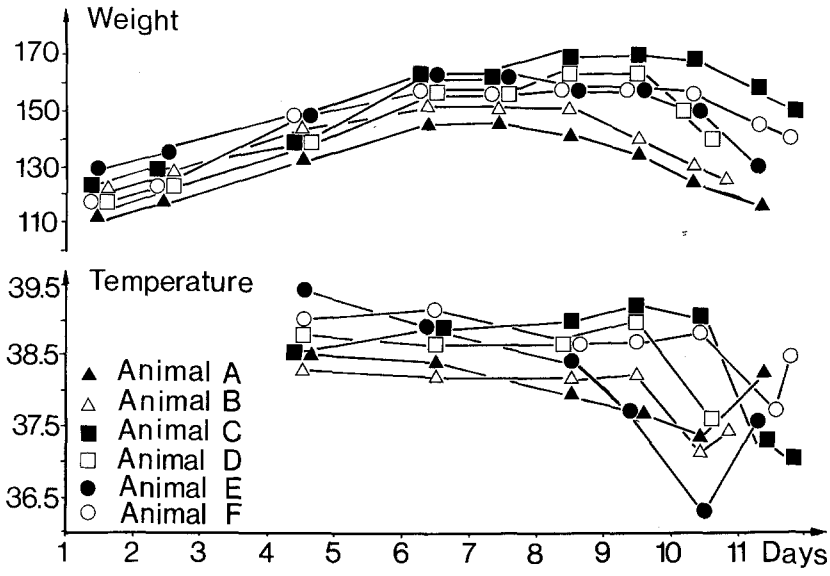

Fig. 1. Course of body weight and body temperature of the six thiamine deficient rats

Table 1. Duration of the different clinical stages (hours); the sum gives the duration of the neurological illness

Stage:	1	2	3	Sum
Animal A	48	20	shortly	68
Animal E	38	5	17	60
Animal F	43	0	12	55
Animal B	28	15	9	52
Animal C	40	8	shortly	48
Animal D	0	15	7	22

Stage 1: Slight neurologic abnormalities
Stage 2: Staggering gait, frequent falling to one side, lethargy and lying on one side
Stage 3: Generalized, spontaneous or easily elicited seizures, opisthotonus and extensive rolling to one side

characterized by minor neurologic abnormalities, such as wide-based posture, which were reversible after irritation of the animal. Stage 2 consisted of staggering gait, frequent falling to one side, lethargy and lying on one side. Stage 3 was characterized by generalized, spontaneous or easily elicited seizures, opisthotonus and extensive rolling to one side. The duration of the clinical stages is shown in Table 1. Two animals (A, C) were perfused a short time after the onset of generalized seizures; the other animals were perfused 7–17 h after the first observation of convulsions. In all animals there was a decrease in body temperature which developed gradually over 1 to 2 days during the course of the neurological illness. Temperature minimums were at least 1.0 degree Kelvin below starting level (Fig. 1). At the final stage of the disease, however, immediately before the perfusion was performed, the temperature rose again in four animals. In general, the longer the period of neurological abnormality, the more severe the neuropathological lesions were.

Histopathological findings

Under the light microscope there were no pathologic alterations in the peripheral nerves and no brain lesions in the control animals. All thiamine-deficient animals showed well demarcated areas in certain regions of the brainstem, characterized by a bullous, spongiform change of the brain tissue. Some neurons were pale and highly oedematous. Several cells showed vacuoles and reduced chromatin. Sometimes chromatin was condensed around the nucleolus. Haemorrhages were also found in these areas, but intact vessels were noted to have distended walls and patent lumina. Astroglial and oligodendroglial cells appeared normal and no microglial reaction was visible.

This type of lesion was always found in the colliculi inferiores (Fig. 2). Animals A, E and F showed these lesions inside the vestibular nuclei, ignoring the borders between them (Fig. 3). Animals B, C and D showed massive haemorrhages in the vestibular complex. Less severe lesions of this type were found in the superior olivary complex, with relatively large vacuoles in the neuropil appearing isolated or forming small clusters. Cellular elements were almost completely spared; only the centre of the lesion showed some involvement of neurons (Fig. 4). Similar lesions were also found in the griseum centrale and in the nuclei oculomotorius and interstitialis.

Examination of the thalamus revealed a different type of lesion. Circumscribed pale areas were

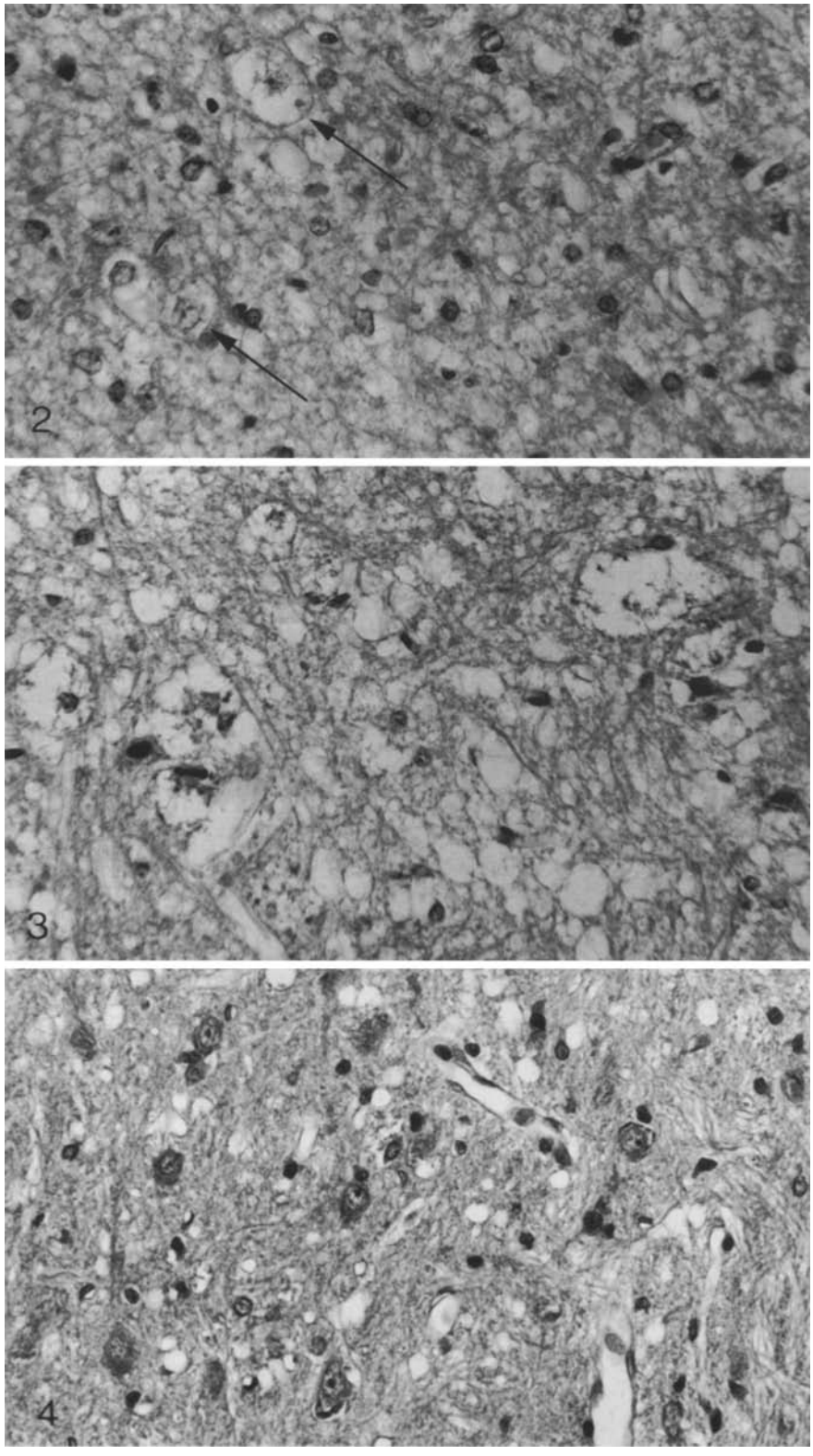

Fig. 2. Inferior colliculi (animal F): Spongiform tissue destruction. Two nerve cells are marked by an *arrow*. Haematoxylin and eosin stain, ×100

Fig. 3. Vestibular complex (animal E): Spongiform tissue destruction. Haematoxylin and eosin stain, ×100

Fig. 4. Superior olivary complex (animal D): Spongy alteration of the neuropil with relative sparing of cellular elements. Haematoxylin and eosin stain, ×100

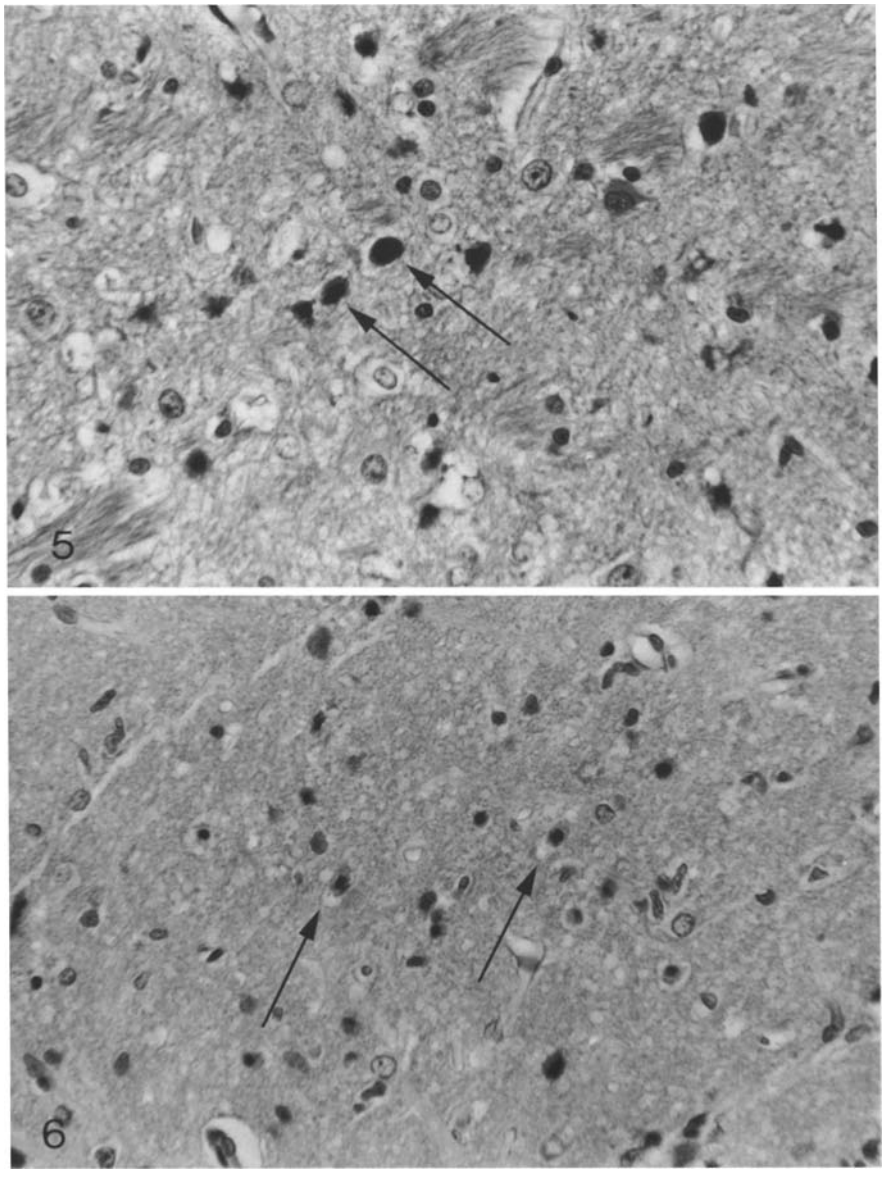

Fig. 5. Thalamus (animal B): Eosinophilic nerve cells. Two of them are marked by an *arrow*. Haematoxylin and eosin stain, ×100

Fig. 6. Corpus geniculatum mediale (animal C): Eosinophilic nerve cells. Haematoxylin and eosin stain, ×100

seen with a slight spongiform state in the neuropil. Most nerve cells were shrunken with pericellular vacuoles and pyknotic nuclei. Some of them were condensed to intensely, that the nucleus was hard to distinguish from the cytoplasm. The vessels appeared normal, though occasional haemorrhages were visible. Astrocytic nuclei were slightly swollen, the oligodendrocytes were normal, and there was no microglial reaction. The ventral and lateral part of the nucleus ventralis thalami (Fig. 5) and the corpus geniculatum mediale (Fig. 6) were always involved. Only in the severe cases the whole nucleus ventralis thalami was altered, together with the surrounding structures. In animal E the entire thalamus, including the raphe, was involved. Figure 7 shows the differential extent of thalamic lesions in the six animals.

Similar lesions were present in the inferior olivary complex. Neurons demonstrated eosinophilia of the cytoplasm with condensation of the nuclear chromatin (Fig. 8, 9). The oral part of the nucleus showed this type of nerve cell damage always occurring in the dorsal loop of the principal nucleus, whereas the nucleus accessorius dorsalis was spared. Following the inferior olivary complex in a caudal direction (Fig. 10), the medial part of the nucleus accessorius dorsalis was also involved. In caudal sections, the nucleus olivaris accessorius

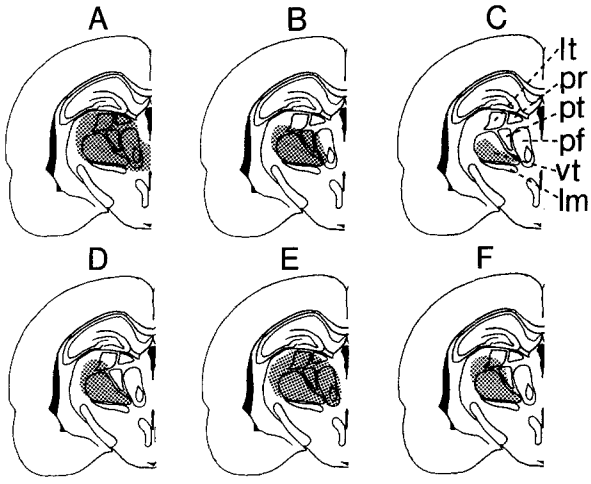

Fig. 7. Damaged areas in the thalamus of the six thiamine deficient rats; *pt* = Nucleus posterior thalami, *lt* = pars posterior nuclei lateralis thalami, *vt* = nucleus ventralis thalami, *lm* = lemniscus medialis, *pf* = nucleus parafascicularis, *pr* = nucleus praetectalis

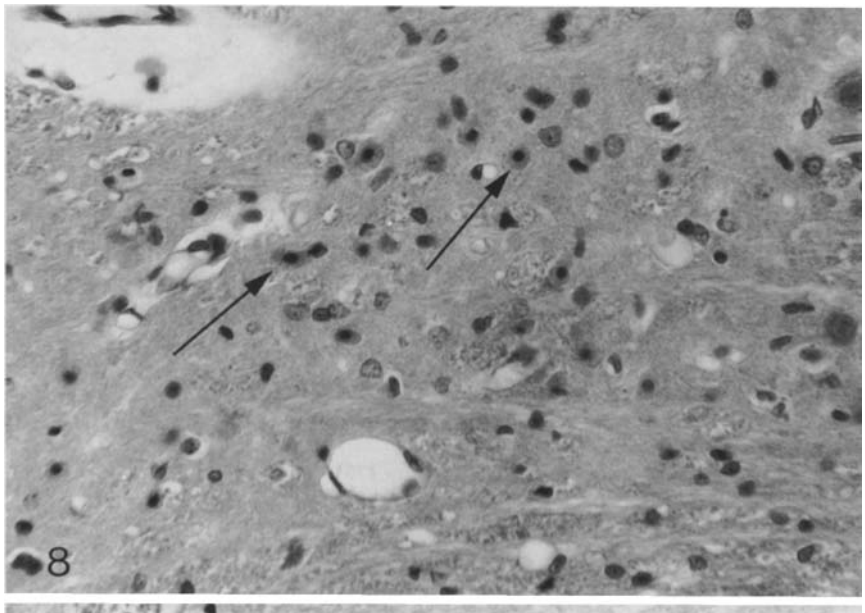

Fig. 8. Inferior olivary complex (animal C): Eosinophilic nerve cells, condensation of the nuclear chromatin. Haematoxylin and eosin stain, × 100

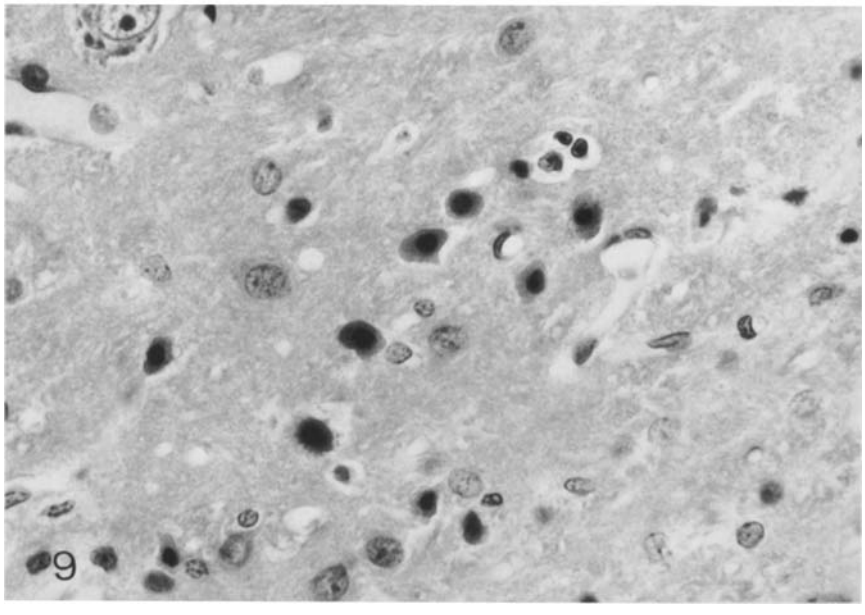

Fig. 9. Inferior olivary complex (animal F): Eosinophilic nerve cells, condensation of the nuclear chromatin. Haematoxylin and eosin stain, × 100

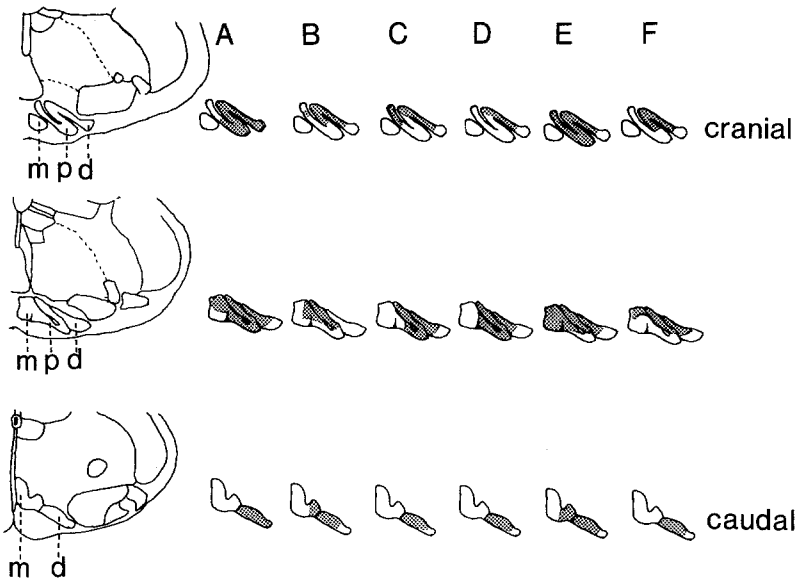

Fig. 10. Damaged areas in the inferior olivary complexes of the six thiamine deficient rats from cranial (*above*) to caudal direction; *m* = Nucleus olivaris accessorius medialis, *p* = Nucleus olivaris principalis, *d* = Nucleus olivaris accessorius dorsalis

dorsalis was mainly affected. The lesions of animals A, B, E and F extended to the area of the nucleus accessorius medialis.

Histologic studies disclosed haemorrhages which infiltrated the surrounding tissue in many regions of the brain stem. Animals A and E, which had the greatest extent of spongiform alterations, demonstrated the most extensive haemorrhages, both in number and in severity. All animals showed gross haemorrhages in the colliculi inferiores and the vestibular nuclei. With exception of these nuclei, haemorrhages in other regions of the brainstem did not follow a symmetrical distribution.

Discussion

Our pathophysiological and behavioural findings agree with previous investigations of experimental thiamine deficiency (Watanabe and Kanabe 1978; Troncoso et al. 1981; Hakim and Pappius 1983). Hypothermia was described only by Troncoso, and Watanabe and Kanabe. Our rats also developed neurological disease and hypothermia within 1 to 2 days. There are several reports describing the occurrence of hypothermia in patients with Wernicke's encephalopathy (Koeppen et al. 1969). It was suggested that the hypothermia is caused by lesions in the posterior hypothalamus. Thorough examination of our rat brains, however, revealed no alterations in this area. Therefore the occurrence of hypothermia in experimental thiamine deficiency still eludes explanation. This holds, too, for the small re-increase of temperature measured in 4 of 6 animals, immediately before the perfusion was performed.

The histopathological changes of Wernicke's encephalopathy have been described as involving the mammillary bodies and the structures along the third and fourth ventricles and the aqueduct, including degeneration of the neuropil, endothelial proliferation of the capillaries and only relatively slight degeneration of nerve cells (Victor et al. 1971). This syndrome has not yet been exactly reproduced by experimental thiamine deficiency (Watanabe and Kanabe 1978).

Colmant 1965 and Torvik 1985 described different findings in the thalamus and the inferior olives with Wernicke's encephalopathy which are characterized by shrunken neurons with eosinophilic cytoplasm. Thus, the manifestation of this type of neuronal lesion resembles that of ischaemic neuronal necrosis. Our observations demonstrate that this morphological syndrome together with its peculiar localization can be induced in experimental animals by thiamine deficiency. Especially, we found further topological similarities in the human and the experimentally induced lesions in that there was a particular involvement of the dorsal loop of the principal nucleus of the inferior olive (Colmant 1965).

Of course, this interpretation of the lesions in the thalamus and the inferior olives implies that ischemic pathomechanisms of different etiology may be involved, e.g. seizure induced hypoxia. However, since the regions predilective for seizure induced lesions, e.g. the hippocampus, were intact in all animals, the seizures are not likely to be in-

volved in the pathogenesis of the alterations described.

Our findings confirm the structural dissimilarities between the experimentally induced brain stem lesions and the characteristic lesions of Wernicke's encephalopathy in the human mammillary bodies. The reason for this discrepancy is not yet clarified.

Concerning the thalamic and inferior olivary lesions, however, we described topological and structural similarities which suggest that at least this type of lesion reflects a metabolically caused, selective vulnerability to thiamine deficiency which is species independent.

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