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Originalarbeiten · Original Investigations · Travaux originaux

Neurofibrillary Degeneration of Nerve Cells after Intracerebral Injection of Aluminium Cream***

HENRYK WIŚNIEWSKI*, WITOLD KARCZEWSKI**, KRYSTYNA WIŚNIEWSKA*

The Institute of Neuropathology and the Institute of Experimental Pathology, Laboratory of Electrophysiology, Polish Academy of Sciences, Warsaw

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In earlier publications (WIŚNIEWSKI et al., 1965; KLATZO et al., 1965), it was found that alum phosphate produces, besides epilepsy, neurofibrillary degeneration of nerve cells. It occurred to us that it would be interesting to examine the morphological changes which might be produced by other aluminium compounds, in particular whether they also produced neurofibrillary changes. One such compound which has been extensively used experimentally by BARRERA et al. (1944), GASTAUT et al. (1959), is aluminium cream or aluminium hydroxide (KOPELOFF et al., 1942; MAYMAN et al., 1965; WESTRUM et al., 1964). Here though extensive morphological studies were made, neurofibrillary changes were not reported. We therefore decided to re-examine the effects of this compound varying the dosage and examining specifically for neurofibrillary tangles.

Material and Methods

Experiments were carried out with 15 rabbits of both sexes, weighing 2.5—3 kg each. Since four rabbits died within 48 hours after the procedure, the observations pertain to the remaining 11 rabbits. In 6 of the 11 rabbits (Table, No. 1—6), 0.5 ml of aluminium cream as a gelatinous form of aluminium hydroxide was injected into the basal region of the left temporal lobe; in 2 rabbits (No. 7 and 8), 0.5 ml of aluminium hydroxide was injected into the base of the pons, and in the remaining 3 rabbits (No. 9—11), 0.1 ml was injected into the base of the left temporal lobe. The aluminium cream prepared by the method described by KOPELOFF et al. (1944), was injected by means of a stereotaxic apparatus. Electroencephalograms were made with an Alvar Rega 8 apparatus with surface (NARĘBSKI, 1958) and inbuilt electrodes. In rabbits which died in status epilepticus during the night, the brain and spinal cord were removed and fixed in 10% formalin solution. The remaining rabbits were sacrificed by perfusion with 10% isotonic solution of formalin according to the technique of CAMMERMEYER (1961). For microscopic examination, numerous sections from both hemispheres, pons, medulla oblangata, cerebellum and spinal cord were embedded in paraffin and stained with hematoxylin-eosin and by the methods of KLÜVER and BIELSCHOWSKY.

Results

Clinical observations

On the day after the injection and during the next three weeks all the rabbits remained in good condition and exhibited nothing abnormal. After three weeks,

* Institute of Neuropathology, Polish Academy of Sciences, Warsaw (Director Prof. Dr. E. OSETOWSKA).

** Institute of Experimental Pathology, Laboratory of Electrophysiology, Polish Academy of Science, Warsaw.

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Table. *Summary of the results*

No. of the rabbit	Site of injection of the paste		Dose injected ml	Survival time of the animal	Clinical symptoms		Pathological EEG	Histological changes	
	base of temp. lobe	base of the pons			epi. seiz	paresis of extrem. ataxia.		neurofibrillary degeneration	nonspecific local reaction
1*	+	0	0.5	6 w	+	+	+	+	+
2*	+	0	0.5	7 m	+	0	+	0	+
3*	+	0	0.5	6 m	+	0	+	0	+
4*	+	0	0.5	6 m	+	0	+	0	+
5**	+	0	0.5	12 m	0	0	+	0	+
6**	+	0	0.5	12 m	0	0	+	0	+
7*	0	+	0.5	3 w	+	+	+	+	+
8*	0	+	0.5	4 w	+	+	+	+	+
9*	+	0	0.1	8 m	+	0	+	0	+
10*	+	0	0.1	7 m	+	0	+	0	+
11**	+	0	0.1	12 m	0	0	+	0	+

* = rabbits which died in status epilepticus.

** = rabbits which showed no abnormalities to the end of the experiment and were anesthetized.

the first neurological symptoms appeared in the rabbits in which 0.5 ml of aluminium cream had been injected into the base of the pons (Table, No. 7 and 8). These symptoms consisted of epileptic convulsions, ataxia and marked paresis of all four extremities.

After three days of illness, status convulsivus developed in both rabbits and the animals, being in agonal condition, were perfused with 10% isotonic formalin solution. Of the 6 rabbits in which 0.5 ml of aluminium cream had been injected into the base of the temporal lobe, one rabbit (No. 1) died after 6 weeks exhibiting symptoms of ataxia, paresis of the extremities and status epilepticus. In three rabbits (No. 2, 3 and 4) isolated attacks of epileptic convulsions were observed from the eighth week after the injection.

Two of the animals (No. 3 and 4) died in status epilepticus after 6 months observation, and one rabbit (No. 2) after 7 months. Apart from the epileptic fits, no other abnormalities were observed. The remaining two rabbits (No. 5 and 6) were sacrificed under anesthesia after 12 months observation, having exhibited no neurological symptoms apart from the pathological EEG pattern. Of the three rabbits (No. 9, 10 and 11) in which 0.1 ml aluminium paste had been injected into the base of the temporal lobe, in two of them (No. 9 and 10) a few generalized epileptic seizures were observed starting the third month after the procedure. Rabbit (No. 10) died in status epilepticus after 7 month observation, and rabbit No. 9 after 8 months. The third rabbit of this group (No. 11) was anesthetized and sacrificed after having survived for one year and having shown no symptoms until the end except a pathological electroencephalographic pattern.

In summary, of 11 rabbits, three (No. 1, 7 and 8) died in status epilepticus with symptoms of ataxia and paresis of the extremities; five rabbits (No. 2, 3, 4, 9 and 10) died in status epilepticus without neurological symptoms and the remaining three rabbits (No. 5, 6, 11) were sacrificed after one year exhibiting no symptoms apart from abnormal EEG and no epileptic seizures.

EEG studies

After injecting the aluminium paste, EEG records were made in the rabbits at frequent intervals beginning from the third week. At the first examination all the animals exhibited pathological EEG patterns regardless of the amount of aluminium gel injected. These patterns were characterized by random spikes and series of spikes. On the whole, the tracings exhibited the character of desynchronization with amplitudes of 40 μV and frequency of 46/sec. The character of the EEG tracings remained unchanged troughout the period of observation of the animals (Figs. 1 and 2). The EEG curves in the rabbits which had no epileptic seizures to

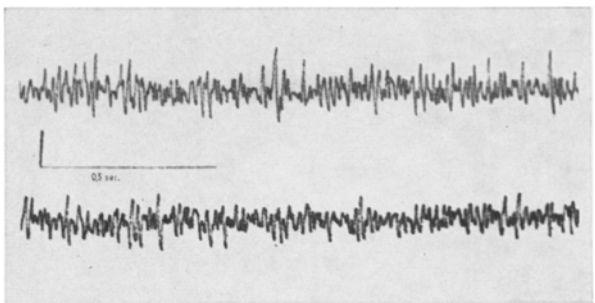


Fig. 1

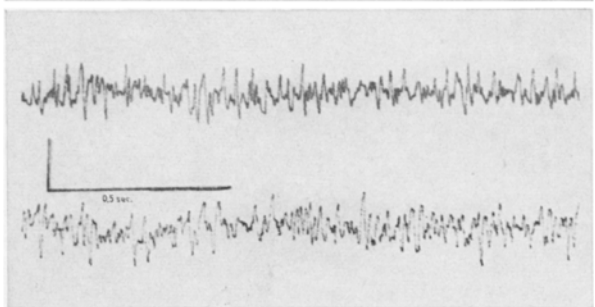


Fig. 2

Fig. 1. Rabbit No. 2. the table. I. Six weeks after injection of 0.5 ml aluminium paste on the base of the left temporal lobe. Note isolated spike and series of spikes

Fig. 2. Same rabbit as in Fig. 1. A portion of the EEG recorded 6 month after operation. Note practically the same curve as in Fig. 1

the end of the experiment (No. 5, 6 and 11) did not differ from the recordings of those animals which displayed epileptic convulsions and died in status epilepticus.

Morphological studies

In rabbits in which 0.5 ml of aluminium gel was injected into the base of the temporal lobe, extensive tissue defects were observed at the site of injection the floor of which was formed by the temporal horn of the lateral ventricle. In animals in which 0.1 ml of aluminium cream was injected, the tissue defects were shallow with dimensions not exceeding 0.3×0.5 cm. In rabbit No. 1, which died in the sixth week after injection of aluminium gel, a considerable amount of the cream was found in the floor of the defect. In the remaining animals only small remnants of aluminium gel were found at the sites of injection. In rabbits in which aluminium gel was injected into the base of the pons, the lower surface of the cerebral

peduncles, pons and medulla oblongata were covered with the paste, and the leptomeninges were fibrotic.

In rabbits in which aluminium cream was injected into the base of the temporal lobe, microscopic observation revealed numerous macrophages and deposits of homogeneous strongly eosinophilic material in the margins of the tissue defects. The zone surrounding the tissue defect exhibited a spongy appearance and the glial reaction was moderately pronounced. In rabbits in which aluminium paste was given into the base of the pons, the subarachnoid space was filled with macrophages and deposits of strongly eosinophilic material. The pontine tissues (apart

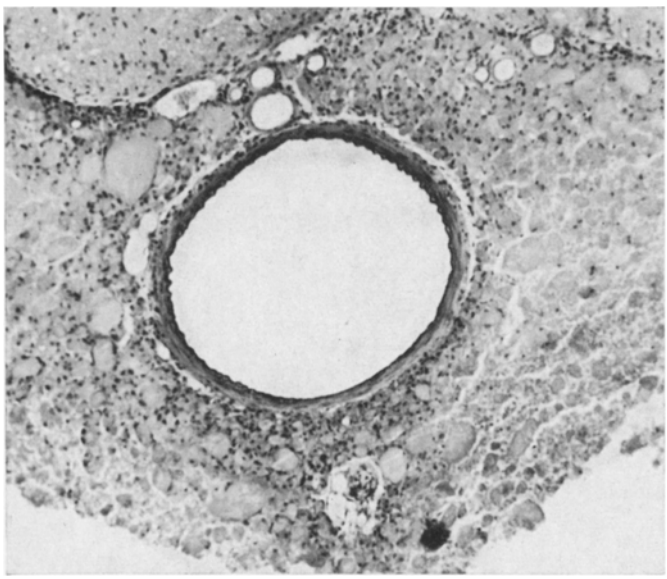


Fig. 3. Arteria basilaris surrounded by macrophages and amorphous material without signs of inflammation. H & E, ×60

from the tract of the injection) and the medulla were undamaged. No other reactive cells apart from macrophages were observed in most of the studied sections.

The blood vessels of the subarachnoid space lying among the macrophages and amorphous material were patent and their walls showed no signs of inflammation (Fig. 3). Small groups of macrophages were present also in the injection tract. A few inflammatory cells were observed under the ependyma and in the choroid plexus. Nerve cells, exhibiting focal clearings in the cytoplasm filled by neurofibrillary tangles were seen in only three rabbits (No. 1, 8, 7) in which 0.5 ml of aluminium gel had been injected (Figs. 4—6). In the remaining rabbits even the neurons directly adjacent to the paste were unaltered.

The topographical distribution of the neurofibrillary changes in rabbit No. 1, in which the aluminium gel had been injected into the basal region of the pole of the temporal lobe, was as follows: In the spinal cord neurons of all cell groups throughout the length of the spinal cord were affected. In the medulla oblongata and pons nearly all the neurons of the nuclei of the olivary system and reticular formation exhibited neurofibrillary degeneration. In the cranial nerves, the most pro-

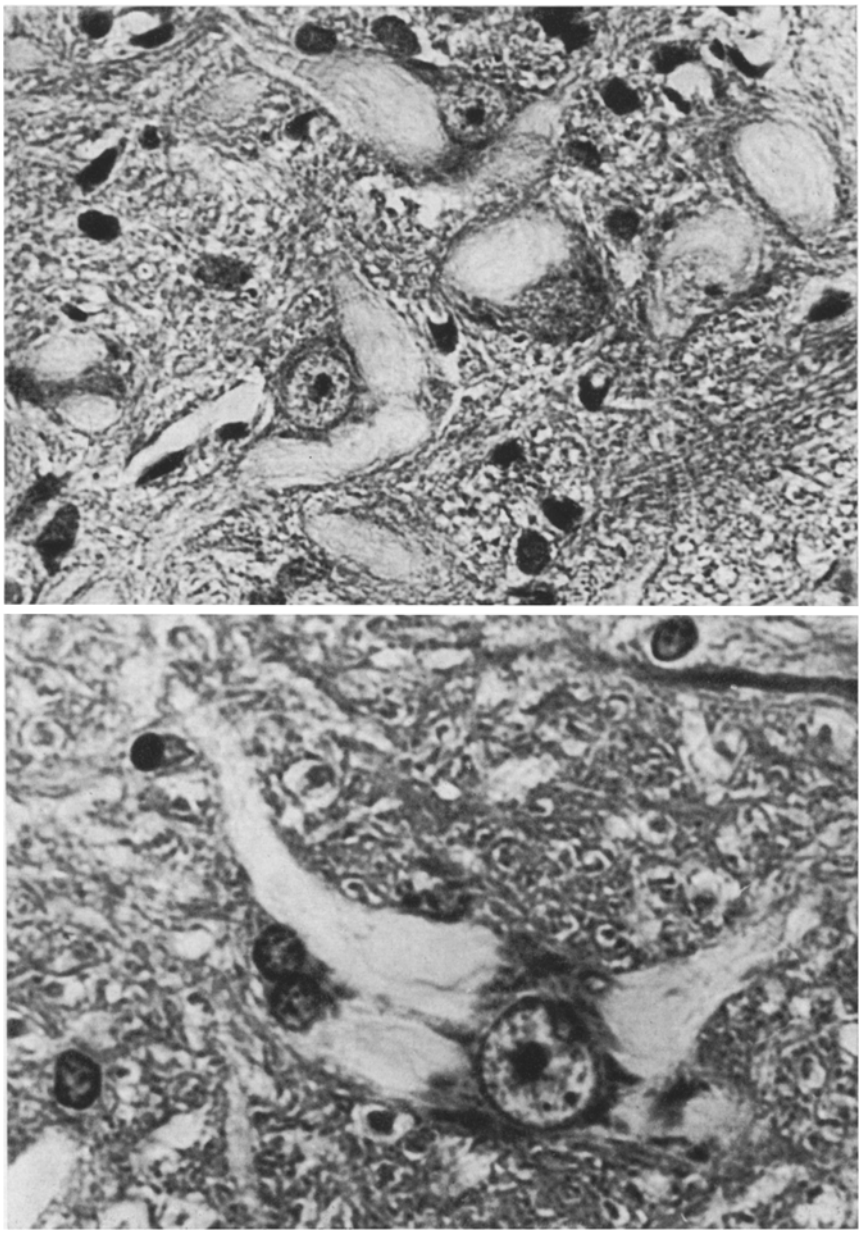


Fig. 4 a and b, Pons. Focal clearings in the nerve cell cytoplasm filled by neurofibrillary tangles. Rabbits No. 7 and 8 the table. a ×300; b ×600, H & E.

nounced changes were present in the cochlear nerve, spinal trigeminal nucleus and dorsal motor nucleus of the vagus nerve.

The mesencephalic nucleus of the fifth nerve, n. prepositus hypoglossi and subnucleus of Deiters were unaltered. In the remaining nuclei of the cranial nerves, only a few neurons showed typical changes. In the midbrain, the neurons of the

stratum griseum profundum of the corpus quadrigeminum anticum regularly showed pronounced changes. Also, a high percentage of neurons were involved in the corpus quadrigeminum posticum, red nucleus and reticular formation. In the basal ganglia pronounced neurofibrillary degeneration was seen in the ventral nucleus of the thalamus. Similarly involved was the lateral geniculate ganglion. Less fre-

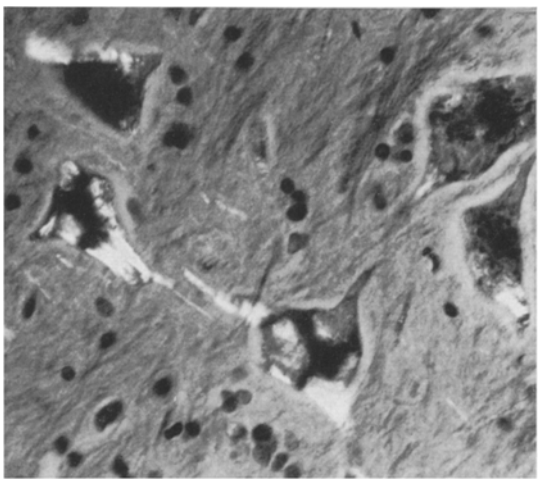
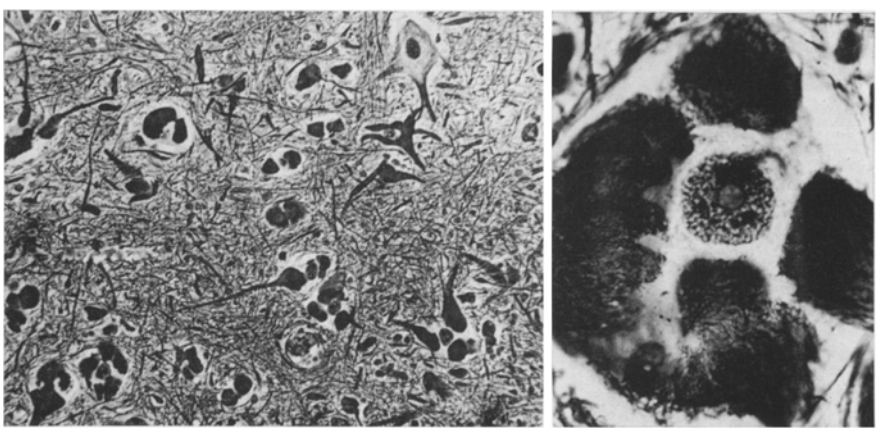


Fig. 5. Pons. Reticular formations. Neurons showing focal clearings in the cytoplasm. Cresyl violet; polarized light; ×600



a b

Fig. 6. a Medulla. Dense bundles of argentophilic material in the cytoplasm. Bodian stain; ×200. b Spinal cord. High magnification of the neuron filled by neurofibrillary tangles. Bodian stain; ×920

quently, degeneration was encountered in the neurons of the medial geniculate ganglion. In the mamillary body, the large cells of the lateral portion bordering medially on the descending column of the fornix frequently showed neurofibrillary changes. Only a few neurons were usually involved in the zona incerta and in the substantia nigra. Free of changes were the nucleus caudatus, globus pallidus, putamen, claustrum and the medial nucleus of the thalamus. In the cerebellum, the Purkinje cells and the neurons of the cerebellar nuclei were regularly affected.

In the cerebral cortex, the neurofibrillary changes were inconspicuous and were observed only occasionally in the large pyramidal neurons of the posterior part of the retrosplenial area, area limbica posterior, area praesubicularis and in Ammon's cortex. In the rhincephalon, almost all the large neurons of the tuberculum olfactorium Edinger were affected. The spinal ganglia were regularly free of changes. In rabbits No. 7 and 8, in which aluminium paste had been injected into the base of the pons, the topography of the neurofibrillary changes in spinal cord, medulla oblongata, pons and mesencephalon were similar to those in rabbit No. 1. Only random neurons in the tuberculum olfactorium Edinger and the large neurons in the retrosplenial area in the cerebral hemispheres were altered. The basal ganglia exhibited no changes.

Discussion

The epileptogenic properties of aluminium cream have been known for years (KOPELOFF et al., 1942), however, paresis of the extremities, ataxia and neurofibrillary degeneration are new observations not previously reported in animals exhibiting epilepsy after intracerebral injection of aluminium gel (GASTAUT et al., 1959; BARRERA et al., 1944).

In the rabbits in which 0.1 ml of aluminium cream was injected, pathological EEG patterns and epileptic seizures were observed, but there were no degenerative changes in the neurons. Paresis of the extremities, ataxia and neurofibrillary degeneration of neurons were observed only in rabbits in which 0.5 ml of aluminium cream had been injected. Apparently, the dose of 0.1 ml of aluminium gel is too small to produce neurofibrillary changes in rabbits.

The low percentage of animals with this alteration after injection of 0.5 ml of aluminium gel was probably due to the method of preparing the cream, and not to the individual sensitivity of the animals. Aluminium cream i.e. aluminium hydroxide, begins to precipitate at pH 4, and at pH 6.3—7.5 precipitation is complete: at pH 10 and 11 it redissolves (GREENFIELD and BUSWELL, 1922). Biologically, aluminium cream should be indifferent, but since it is not, it must contain readily soluble aluminium compounds or dissociate. Water-soluble ammonium-aluminium sulfate is the starting product af aluminium cream. Presumably, it is impossible to remove the ammonium-aluminium sulfate from the cream completely when the aluminium hydroxide is prepared by the method described by KOPELOFF et al. (1942). Because of the physical properties of the gel, small amounts of the sulfate are unevenly distributed in it. The dose of 0.5 ml of aluminium cream probably failed to produce neurofibrillary changes in the neurons of all the rabbits because not every portion contained the same amount of readily soluble aluminium. Stechiometrical calculation shows that in 0.1 ml of aluminium cream should be about 0.0105 g of aluminium. Used by KLATZO et al. (1965) Holt's adjuvant contained 0.00029 g of aluminium in 0.1 ml. However, despite the fact that aluminium cream contain in 0.1 ml more aluminium than Holt's adjuvant the last compound was more toxic. In our opinion higher toxicity of Holt's adjuvant was due to that this compound was more contaminated with soluble aluminium compounds than aluminium cream. Using insoluble aluminium compounds we never know even approximatively the dosis of aluminium entering the brain, therefore now for

production of neurofibrillary tangles or EEG changes we use only water soluble aluminium compounds.

In principle, the topography of the neurofibrillary changes was the same as that observed by KLATZO, WIŚNIEWSKI and STREICHER (1965) in rabbits after injection of aluminium phosphate. Absence of neurofibrillary changes in the basal ganglia of the rabitts in which aluminium paste was injected into the base of the pons may be attributed to the early death of the rabitts in status epilepticus soon after the appearance of ataxia and paresis of the extremities. Whether the neurofibrillary changes and epilepsy are related remains unclear for the present. The problem of correlating the EEG activity with morphologic alterations of the nerve cells will be the purpose of our further investigations.

Summary

Intracerebral administration of aluminium cream (aluminium hydroxide) in rabbits resulted in the development of pathological EEG, convulsive seizures, paresis of the extremities and neuronal changes consisted in a focal clearings in the cytoplasm filled by neurofibrillary tangles. In principle, the topography of the neurofibrillary changes was the same as that observed by KLATZO, WIŚNIEWSKI and STREICHER in rabbits after injection of aluminium phosphate.

Zusammenfassung

Intracerebrale Applikation von Aluminiumpaste (Aluminiumoxyd) bei Kaninchen führte zum Auftreten von pathologischen EEG-Veränderungen, Krampfanfällen, Extremitätenparesen sowie von Nervenzellveränderungen in Form fokaler Cytoplasmaaufhellungen, die von verplumpten Neurofibrillen erfüllt sind. Die Topographie der neurofibrillären Veränderungen entsprach grundsätzlich den Beobachtungen von KLATZO, WIŚNIEWSKI u. STREICHER an Kaninchen nach Injektion von Aluminiumphosphat.

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Dr. H. WIŚNIEWSKI
Institute of Neuropathology Polish Academy
of Sciences, Pasteura 3
Warsaw, Poland