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VIII NERVE IN EXPERIMENTAL LEAD POISONING

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Forty healthy guinea pigs were poisoned with repeated peritoneal injections of lead acetate. After 7 weeks the animals were sacrificed and the inner ear with VIII nerve were examined histologically. The lead level in the blood was estimated in all animals after poisoning and in 10 healthy guinea pigs (control group). The sensory cells of the inner ear, the spiral and vestibular ganglion cells appeared to be normal. The VIII nerve in the majority of poisoned animals showed segmental demyelination and axonal degeneration. Theories on etiology and pathogenesis of the neuropathological changes in lead poisoning are discussed.

Among the many symptoms of lead poisoning are found complaints of auditory defects and vertigo (Ciurlo & Ottoboni, 1956; Falkowska *et al.*, 1961; Gammarrota & Bartoli, 1964; Ursan & Suciu, 1965). The answer to the problem of the toxic effect of the lead on inner ear and the VIII nerve in man is still under discussion. Gambault (1880) was the first who demonstrated peripheral nerve changes in guinea pigs resulting from lead poisoning. The next experiment reports described the influence of lead upon the central nervous system and peripheral motor nerves (Ferraro & Hernandez, 1932; Fullerton, 1966; Kornofsky & Ridgway, 1952; and others).

The present work was undertaken to study the effect on the inner ear and VIII nerve after lead poisoning.

METHOD

Fifty healthy young guinea pigs were used for the experiment. Their weights ranged between 300 and 350 g. Ten guinea pigs served as a control for the estimation of normal lead levels in the blood. Forty animals were given repeated doses of 1% solution of lead acetate $Pb/NO_3/_2$ by intraperitoneal injections. The total dose of 300 mg/kg was administered one time every 7 days during 7 weeks. Eight animals died before the end of the experiment. All remaining guinea pigs were weak and had lost weight. During the poisoning their growth was inhibited. The loss of weight is, according to Fullerton (1966) and Causey (1965), a significant sign of serious poisoning. Only five animals were able to run slowly, but they appeared to have mild paralysis of their hind limbs. After seven weeks the animals were sacrificed, the temporal bones removed for histopathological examination and the lead level in the blood was estimated. The latter was determined in all animals

Fig. 1.

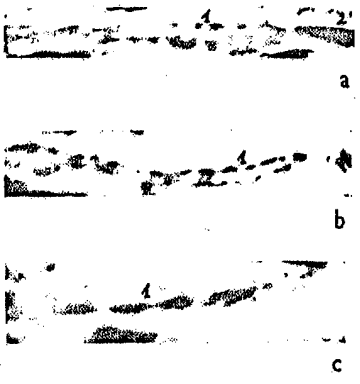

Fig. 2.

Fig. 1. The VIII nerve from the poisoned guinea pig (cross section) stained by Sudan black B to show demyelination. The bundles of demyelinated fibres are present in the center of the trunk; ×100. At the right of the figure the vestibular ganglion cells are seen.

Fig. 2. VIII nerve—single fibre from a poisoned animal stained by Heidenhain method (*a*, *b*) and Sudan black B (*c*). (1) Segmental demyelination with the ovoids of degenerating myelin. (2) Intact myelin sheath. Magnification: (a) ×1600, (b) ×2500, (c) ×3200.

after poisoning and in 10 healthy guinea pigs (control group). The estimation of lead was made by a polarographic method similar to that described by Teisinger *et al.*, (1956). The lead level in control group was 0.025 mg% – 0.33 mg%. This level in the poisoned animals increased about 10 times to 0.31 mg% – 0.42 mg%. For histopathological examinations the animals were divided into two groups. The temporal bones of 16 guinea pigs (first group) after fixation and decalcification in EDTA were embedded in celloidin, and sections were cut at 15–20 μ and stained with haematoxylin-eosin, cresyl violet and by silver impregnation method described by Gomori.

The temporal bones from the remaining 16 guinea pigs (second group) were embedded in paraffin, because the application of methods to demonstrate myelin and axon are limited by celloidin technic. Paraffin sections were stained by modified Heidenhain's method, Sudan black B and luxol fast blue for myelin sheaths and Roger-Foot's method to demonstrate axons.

RESULTS

The histological investigations showed no pathological changes in the sensory cells of the inner ear or in spiral and vestibular ganglion cells. In

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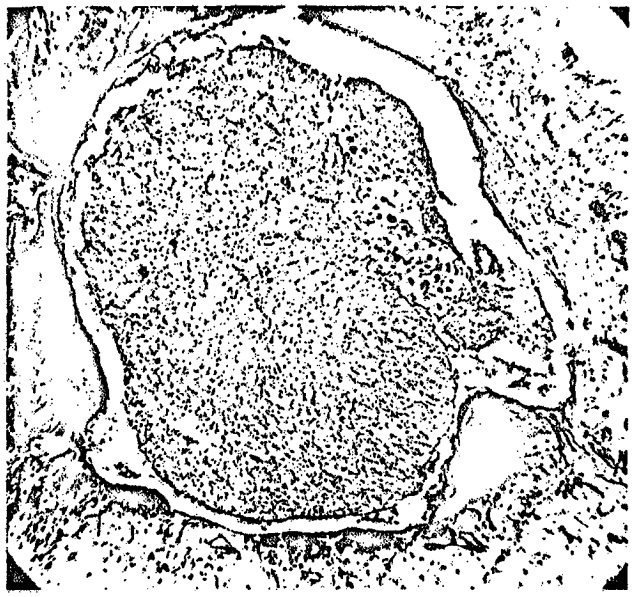

Fig. 3. VIII nerve from normal guinea pig stained by Roger-Foot method to demonstrate axons. × 60.

the first group (celloidin embedding) VIII nerves from 12 of the 16 poisoned animals were histologically abnormal. Focal changes with destruction of the nerve were seen in specimens stained by hematoxylin–eosin and by the silver method. There was individual variation in the degree of these changes. Detailed information about the character of these pathological changes was obtained by histopathological examination with staining by methods demonstrating myelin sheaths and axons. The VIII nerve of the 5 lead-poisoned animals in the second group was normal (Fig. 3). The VIII nerve of the remaining 11 guinea pigs *showed both demyelination and axonal degeneration.*

On the cross section of the VIII nerve we observed characteristic changes when the nerve was stained by Sudan black B (Fig. 1). The demyelination spread to involve the nerve fibers in the centre of the trunk. Only residual fragments of myelin sheaths can be seen here. The various stages of segmental demyelination in a single fibre were observed also in remaning parts of the nerve. On the longitudinal sections (Fig. 2) several ovoids of degenerating myelin can be seen.

Process of axonal degeneration in the same nerve is shown in Fig. 4. The degree and extent of these changes varied in different parts of the same nerve. This distribution of the lesion prompts the suggestion that *in lead-poisoned guinea pigs segmental demyelination and axonal degeneration of the VIII nerve occur.*

Fig. 4. Axonal degeneration in VIII nerve from poisoned animal. Roger-Foot stain. (a) At the right of the figure is the region of total destruction of the nerve. ×400. (*b, c*) Axonal degeneration in single fibre at higher magnification. ×3200.

DISCUSSION

A full understanding of the pathogenesis of neuropathological lesions produced by lead has not emerged. The theory which was supported for many years is that of porphyrin metabolic deficiencies. Some authors have stressed damage to the vascular epithelium, suggesting that the neuropathological lesions may be secondary to the vascular (Cumings, 1959; Ursan & Suciu, 1965). The predominant changes in the central nervous system were found by other workers.

In previous experimental works on chronic lead poisoning, demyelination of the nerve fibers was found (Fullerton, 1966; Glees, 1961). This toxic effect is probably the result of disturbance to the enzymatic content of myelin sheath. The degeneration of myelin may also be the result of the degeneration of the axis cylinder. These biochemical lesions produced by heavy metals were stated by Glees (1961).

The striking resemblance of the early neurological symptoms of chronic lead poisoning in man to those seen in other demyelinating syndromes suggest that this is the samc etiological agent. For this reason some authors have suspected lead as a possible cause of multiple sclerosis (Campell, 1950; Cone *et al.*, 1934; Falkowska *et al.*, 1964).

Our work showed that lead poisoning in guinea pigs might produce chronic, demyelinating neuropathy of the VIII nerve with axon degeneration. The sensory cells of the inner ear have been found to be most resistant to the toxic effect of lead. The degree of the histopathological changes is not necessarily the same in different animals. This finding suggests the individual sensitivity to lead intoxication and confirm other experimental and clinical observations.

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The results of our experiment in guinea pigs are relevant with reference to lead intoxication in man, but may suggest the pathogenesis of VIII nerve lesions.

ZUSAMMENFASSUNG

Die Untersuchungen wurden an 40 gesunden, jungen, weissen Meerschweinchen durchgeführt. Die Meerschweinchen wurden mittels intraperitonealer Injektionen von Bleiazetat allmählich vergiftet. Nach einer 7 Wochen lang andauernden Vergiftung wurden die Tiere getötet und das Innenohr sowie der VIII. Hirnnerv histologisch untersucht. Bei allen vergifteten Tieren und bei 10 gesunden Meerschweinchen, die als Kontrollgruppe dienten, wurde der Bleigehalt im Blut bestimmt. Die Sinneszellen des Innenohres und die Nervenzellen des vestibulären Ganglions sowie des Ganglio spirale cochleae waren normal. Bei der Mehrzahl der vergifteten Tiere wurde im VIII. Hirnnerv eine segmentäre Demyelinisation und eine Axondegeneration festgestellt. In der vorliegenden Arbeit wurden gleichfalls die bestehenden Auffassungen über die Ätiopathogenese der neuropathologischen Veränderungen bei Vergiftungen dargelegt.

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