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PHOSPHOLIPIDS AND CHOLESTEROL OF THE BRAIN AND SPINAL CORD OF GUINEA PIGS POISONED WITH TRICRESYL PHOSPHATE

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The content of phospholipids and of total, free, and esterified cholesterol in the brain and spinal cord of guinea pigs with severe neuroparalytic signs of poisoning caused by intradermal injection of tricresyl phosphate (TCP) was investigated. No change was found in the content of phospholipids and total cholesterol in either the brain or the spinal cord, but accumulation of cholesterol esters — the characteristic degradation products of myelin sheaths — was observed.

KEY WORDS: *tricresyl phosphate; phospholipids; cholesterol; demyelination; spinal cord.*

Certain organophosphorus compounds (OPC) widely used in agriculture and industry have a marked neurotoxic action. In particular, tricresyl phosphate (TCP), in cases of chronic poisoning, leads to the development of permanent pareses and paralyses of the limbs and to disability [1, 4, 5]. Despite many clinical and histological investigations, the fundamental problems of the pathogenesis of demyelination produced by OPC still remain unsolved, possibly because changes in the content of the lipid components of the myelin sheaths have received little study.

The object of this investigation was to produce an experimental model of chronic TCP poisoning in mammals with the development of neuroparalytic signs and to investigate the content of phospholipids and cholesterol in the brain and spinal cord, the main site of myelin sheaths.

EXPERIMENTAL METHOD

Experiments were carried out on adult male guinea pigs weighing 350-450 g into which TCP (an industrial mixture containing 37% of the ortho isomer) was injected intradermally in a dose of 2.0-2.2 ml/kg body weight. The animals with severe neuroparalytic signs of TCP poisoning were killed 25-30 days later and the brain stem and lumbar region of the spinal cord were removed for biochemical analysis.

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TABLE 1. Lipid Phosphorus Content (in mg P/g tissue) in Brain and Spinal Cord in Guinea Pigs with TCP Poisoning (M ± m)

Experimental condition	Brain	Spinal cord
Control (n = 17)	2.50±0.04	2.86±0.09
TCP (n = 15)	2.48±0.06	2.73±0.09

TABLE 2. Total, Free, and Esterified Cholesterol Content (in mg/g tissue) in Brain and Spinal Cord of Guinea Pigs with TCP Poisoning (M ± m)

Experimental conditions	Total cholesterol	Free cholesterol	Cholesterol esters	Cholesterol esters (% of total cholesterol)
Brain stem				
Control (10)	26,2±1,1	26,3±1,1	0	0
TCP (11)	25,4±0,6	23,9±0,7	1,43±0,13*	5,46*
Lumbosacral region of spinal cord				
Control (10)	31,1±1,7	31,0±1,8	0,06±0,06	—
TCP (11)	31,0±0,95	27,9±0,9	3,13±0,26*	10,1*

*P < 0.05 compared with control.

Lipids were extracted from the nerve tissue homogenate with a mixture of chloroform and methanol as described previously [2]. Phospholipids were determined quantitatively as lipid phosphorus by Bartlett's method [6] in 0.05 ml of lipid extract. The level of total and free cholesterol was determined in 0.1 ml of the lipid extract after treatment with saponin by the method of Davison et al. [9] with the additional use of digitonin. The content of cholesterol esters was calculated as the difference between total and free cholesterol.

EXPERIMENTAL RESULTS

To solve the problems set, an experimental model of chronic TCP poisoning was produced in guinea pigs [3, 4] in which a depot of this compound was formed by a single intradermal injection of a technical mixture of TCP. The gradual slow absorption of TCP simulates chronic poisoning.

During the first 7-8 days after intradermal injection of TCP into the animals signs of acute poisoning were observed: disturbance of the functions of the gastrointestinal tract and a loss of weight of 15-20%. During the next 2-3 weeks the general condition of the animals improved and their weight recovered. The appearance of neuroparalytic signs of TCP poisoning 21-25 days after intradermal injection was accompanied by a secondary decrease in weight and worsening of the general condition. Animals with moderately severe and severe forms of poisoning were chosen for biochemical analysis on the basis of the following features: a loss of weight of up to 20% of its initial value, lowering of the tone of the intercostal muscles; disappearance of digital splaying (often the digits were flexed into a fist); the length of the freely hanging hind limbs was increased to a maximum; marked paresis or paralysis of the hind limbs (frequently culminating in death). Depending on the neurological signs, the most vulnerable part of the spinal cord — the lumbosacral region — was chosen for biochemical analysis. For analysis of the brain the brain stem was chosen as the most highly myelinized part of it.

The results given in Table 1 show that the phospholipid content both in the brain and in the spinal cord was unchanged in chronic TCP poisoning.

No change in the phospholipid content likewise could be found during an investigation of the sciatic nerve of hens following administration of TCP and the development of paralyses as the result of demyelination of the peripheral nervous system [7, 12, 13]. The small loss

of phospholipids directly in the foci of demyelination was perhaps not detected on analysis of the tissue homogenate [2]. On the other hand, a possible cause of the absence of changes in the lipid phosphorus content in TCP poisoning may have been penetration of the TCP into the membrane structures of the nervous system [8]. In that case it becomes clear why an increase (by 8%) was found in the lipid phosphorus content in the cervical region of the spinal cord of cats following exposure to TCP [14].

The results in Table 2 show that the total cholesterol content was unchanged both in the brain and in the spinal cord during TCP poisoning.

Whereas normally practically all the cholesterol of nerve tissue is in the form of free cholesterol, in moderately severe and severe forms of poisoning caused by the neurotoxic action of TCP a considerable quantity of cholesterol esters (10.1%) was found not only in the lumbosacral region of the spinal cord but also in the brain stem (5.46%). In hens with TCP poisoning either no changes are found in the sciatic nerve [15], or cholesterol esters are found with no change in the total cholesterol level [7], or no cholesterol esters are found but the total cholesterol is increased by 25% [12]. The contradictory nature of these results can evidently be attributed to the fact that animals of different ages were investigated, different doses of TCP were used, the effects were studied at different times after administration, and most important of all, there was no quantitative assessment of the degree of severity of the lesions.

As a rule cholesterol esters accumulate in foci of demyelination of varied etiology: for example, in disseminated sclerosis and allergic encephalomyelitis [2, 10, 11]. Consequently, the accumulation of cholesterol esters observed in the present investigation can be taken as biochemical evidence that in chronic TCP poisoning destruction of myelin takes place in the spinal cord and brain; in the lumbar region of the cord, moreover, degeneration of myelin sheaths takes place more intensively than in the brain stem.

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