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EFFECT OF TRIORTHOCRESYL PHOSPHATE POISONING ON INTENSITY OF INCORPORATION OF [2-^{14}C]ACETATE INTO PHOSPHOLIPIDS AND CHOLESTEROL OF THE GUINEA PIG BRAIN AND SPINAL CORD

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A severe form of chronic triorthocresyl phosphate poisoning was induced in guinea pigs by a single intradermal injection of this compound and the intensity of incorporation of [2-^{14}C]acetate into lipids of the spinal cord and brain stem was investigated in vivo. In the paralytic stage of the disease incorporation of the label into phospholipids and cholesterol was clearly reduced; inhibition of synthesis of these lipids was observed not only in the most vulnerable lumbosacral region of the spinal cord, but also in the brain stem, evidence of the systemic character of the disturbance of lipid metabolism in the CNS and of changes in the metabolism of the oligodendroglia.

KEY WORDS: phospholipids; cholesterol; brain and spinal cord; triorthocresyl phosphate poisoning.

Chronic poisoning in man by certain organophosphorus compounds (OPC) widely used in agriculture and industry [1, 3, 4], leading to severe damage to the nervous system with the development of permanent pareses and paralyses of the limbs, has been described in the literature [9, 11]. The neurotoxic effects of these compounds are unconnected with their anticholinesterase properties [10]. Clinical and morphological studies have shown that the neuroparalytic action of these OPC is connected with their demyelinizing action [2, 10], but the mechanism of this phenomenon still remains virtually unstudied.

Existing fragmentary investigations of the lipid components of myelin are contradictory and do not give a complete picture of the action of the demyelinizing OPC on the lipid composition of nerve tissue.

The object of this investigation was to study disturbances of phospholipid and cholesterol metabolism of nerve tissue in vivo by the use of [2-^{14}C]acetate as radioactive precursor of their synthesis during chronic triorthocresyl phosphate (TOCP) poisoning.

EXPERIMENTAL METHOD

An experimental model of chronic poisoning was created in adult male guinea pigs weighing 350-450 g by intradermal injection of TOCP (the industrial oily mixture, containing 37% of the ortho isomer) in a dose of 2.0-2.2 ml/kg body weight. [2-^{14}C]acetate in a dose of 100 μCi/100 g body weight was injected subcutaneously 27-33 days after injection of TOCP. The animals were decapitated 2 h later, and the brain stem and lumbosacral region of the spinal cord were removed.

Extraction of lipids from the tissue homogenate, isolation of cholesterol and the total phospholipid fraction from the total lipid extract, and also their quantitative determination was carried out by the method de-

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TABLE 1. Specific Radioactivity of Phospholipids, Cholesterol, Homogenates, and Acid-Soluble Fractions of Brain and Spinal Cord of Normal Guinea Pigs and of Guinea Pigs Poisoned with TOCP (M ± m)

Fraction	Phospholipids, cpm/mg		Brain	
	control (9)*	TOCP (8)	control (8)	TOCP (8)
Phospholipids, cpm/mg	115±8	96±8	123±7	105±4†
Cholesterol, cpm/mg	68±8	38±7†	39±2	28±3†
Homogenates, cpm/mg dry weight of tissue	270±9	400±47†	423±22	668±79†
Acid-soluble fraction, cpm/mg wet weight of tissue	42±4	71±8†	51±9	80±8†

*Number of animals shown in parentheses.
†$P < 0.05$.

scribed in [6, 7]. To estimate the reserves of radioactive precursors of lipid synthesis, the acid-soluble fraction was extracted from a separate weighed sample of tissue with 5% TCA [8].

The radioactivity of the homogenate, of the acid-soluble fraction, and of the phospholipids and cholesterol was measured by means of a Protok gas-flow counter. The difference between the mean values was taken to be significant at the $P \leq 0.05$ level.

EXPERIMENTAL RESULTS

In all the animals manifestations of acute poisoning were observed during the first week after injection of TOCP (disturbance of functions of the gastrointestinal tract, worsening of the general condition, sharp decrease in weight), from which some of the animals (about 16%) died. The late neurotoxic action of TOCP appeared after 2-3 weeks of the recovery period: permanent pareses and paralyses of the limbs developed, a further decrease in the body weight by 15-20% took place, and the animals' general condition worsened. The severity of the lesions was assessed from the neurological symptoms, using the criteria developed by Zil'ber [3] and Dvorkin [1].

The results given in Table 1 show that the intensity of incorporation of labeled acetate into cholesterol of the brain and spinal cord of the guinea pigs was significantly lower than into phospholipids, in good agreement with modern views regarding the comparative metabolic inertness of cholesterol, which is localized mainly in myelin sheaths.

In the paralytic stage of the disease produced by TOCP the specific radioactivity of phospholipids was virtually unchanged in the spinal cord ($P > 0.1$), and in the brain it was only slightly reduced (by 14.7%). The specific radioactivity of cholesterol was clearly reduced in both spinal cord (by 44.1%) and the brain (by 28.3%). Meanwhile a sharp increase in specific radioactivity was found in the homogenate of spinal cord (by 50%) and brain (by 58%), evidently as a result of an increase in the reserves of radioactive precursors of lipid synthesis, as was confirmed by a sharp increase in the specific radioactivity of the acid-soluble fraction (by 55-70%). Accordingly to assess the changes in the rate of lipid synthesis, just as previously, the writer used the relative specific radioactivity of the carbon of the lipids, rather than the specific radioactivity, compared with the specific radioactivity of carbon of the homogenates, which was taken as 100 [7].

The results of the corresponding calculations showed that chronic TOCP poisoning causes a sharp decrease in the intensity of synthesis of phospholipids (by 40-45%) and cholesterol (by 52-62%) both in the spinal cord and in the brain. Considering that incorporation of other substrates (1,2-^{14}C]choline bromide, [1,2-^{14}C]-ethanolamine-HCl, L-[3-^{14}C] serine) into phospholipids of the cat spinal cord in vitro is unchanged in TOCP poisoning, this suggests that the inhibition of phospholipid synthesis observed in the present experiments was due to inhibition of synthesis of fatty acids.

Quantitatively, the decrease in intensity of synthesis of phospholipids and cholesterol in the brain and spinal cord was about equal, evidence of the systemic character of the disturbance of lipid metabolism in the CNS following exposure to demyelinizing OPC, and also, as the writer showed previously, in radiation sickness [5] and demyelinization of allergic origin [7]. The action of different pathogenic factors thus leads to similar disturbances of lipid metabolism in nerve tissue. Moreover, the similar changes in the specific radioactivity of the homogenate and of the acid-soluble fractions in these three pathological states indicate that the action of the noxious factors is not only aimed at the system of lipid metabolism, but also causes changes in other metabolic systems [12].

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