X-Ray Studies on Phospholipid Bilayers. XII. Interactions of Pentachlorophenol with Myelin

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Pentachlorophenol (PCP) is a widely used pesticide, particularly for the preservation of wood. Given its high toxicity and resistance to degradation it has become a dangerous environmental pollulant. Due to its high lipophilicity, PCP is able to partition into the lipid bilayer of cell membranes disrupting several vital functions. The present research was concerned with the effects that the chronic administration of PCP could produce *in vivo* to the sciatic nerve of rats. X-ray diffraction patterns obtained from freshly dissected and dried sciatic nerves of PCP treated rats did not show significant differences in their reflections with respect to those present in the patterns from untreated animals. However, morphological studies performed by optical and electron microscopy showed degenerative changes in about 10% of the A and B type of nerve fibers.

Introduction

Pentachlorophenol (PCP) and its sodium salt are widely used fungicides, particularly for the preservation of wood. Given their high toxicity and resistance to degradation they have become dangerous environmental pollulants [1–4]. PCP is readily absorbed through the skin and the digestive and respiratory systems. As it is strongly lipophilic it is able to partition into the lipid bilayer of cell membranes disrupting several functions. Thus, it uncouples the oxidative phosphorilation [5], alters the microsomal electron transport system [6] and inhibits the amino acid transport across cell membranes [7]. As a result of treatment with sublethal doses of PCP, the lipid bilayers of cell mammalian membranes became destabilized [8].

Structural studies on the interaction of PCP with phospholipid bilayers have been performed in this laboratory by X-ray diffraction techniques [9]. Multibilayers built up of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), phospholipids that are respectively present in the outer and inner monolayers of cell membranes, were used. It was observed that PCP strongly perturbed DMPC and DMPE molecular structures in a hydrophobic as well as in a hydrophilic medium. In view of these results, it was considered of interest to study the effects that the chronic administration of PCP could produce *in vivo* to the structure of cell membranes. For this purpose, rats were allowed to drink *ad libitum* aqueous solutions of PCP for 90–120 days. This paper presents the results of the X-ray studies performed on freshly dissected and air-dried sciatic nerves of rats treated and untreated with PCP water solutions. Optical and electron microscopy observations were also made on these specimens.

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Materials and Methods

Male Wistar albino rats of 300–350 grams of body weight were divided into four groups. The first was given PCP (Sigma, lot 127 F 3439) 1.0 mM for 90 days; the second, 3.0 mM for 120 days; the third received 10.0 mM PCP until the rats showed the symptoms of intoxication and the fourth was a control group. Each animal was killed by decapitation, both sciatic nerves were removed and kept under slight tension in sample holders. The X-ray diagrams were obtained in a Warhus camera provided with a 0.38 mm diameter pinhole collimator. One of each pair of freshly dissected nerves was immediately set in the camera and X-ray diffracted at 18 °C and about 100% of relative humidity. This was attained by bubbling air first through two water-containing gas-washing bottles. The other nerve was dried in the ambient atmosphere by

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keeping it for several days at 15 °C and then X-ray diffracted under vacuum. Sample-to-film distances were either 8 cm or 29 cm. Ni-filtered Cu Kα radiation from a Philips PW 1140 generator was used. The relative intensities of the reflections were measured from the X-ray films using a Joyce-Loebl MK III CS microdensitometer connected to an Acer 915 microcomputer.

For the electron microscopy observations, the nerves were inmediately fixed in 2.5% glutaraldehyde in 0.15 M sodium phosphate buffer for 30 min and post fixed with 2% osmium tetroxide for 1 h. After a short rinsing with buffer, the specimens were dehydrated in graded ethanol series, embedded in Epon and processed for ultra thin section using a MT2 Porter Blum ultramicrotome (Du Pont Instr., Sorvall). Sections for electron microscopy were stained with a 2% aqueous solution of uranyl acetate and lead citrate stain. Thick sections for light microscopy were stained with toluidine blue. Transmission electron microscopy observations were made in a Philips 200 EM, while a Leitz Orthoplan was used for optical microscopy.

Results

Fig. 1 and 2 show the high-angle X-ray oriented diagrams of dry sciatic nerves respectively obtained from normal and PCP fed rats. A comparison of their interplanar spacings, orientation of the reflections and their intensities, including those obtained from low-angle X-ray diagrams, are presented in Table I. As it can be observed, there are no significant differences between the patterns of both nerves. About the same results were obtained when freshly dissected sciatic nerves were exposed to X-ray diffraction. These patterns, however, showed fewer reflections and they were more diffuse than those obtained from the dry specimens. Table II shows a comparison of the spacings and intensities of fresh nerves from PCP untreated and treated rats. These results, obtained from dry and fresh nerves, were essentially the same independent of whether the rats were fed with PCP free water or 1, 0, 3.0 or 10.0 mM PCP solutions.

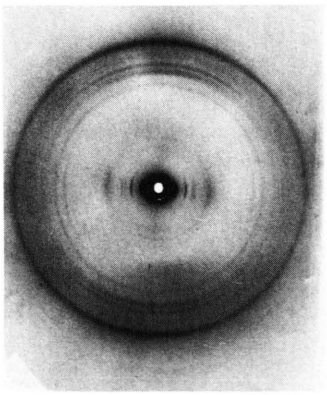

Fig. 1. High-angle X-ray diagram of a dry sciatic nerve of a rat without treatment with PCP. D = 8 cm.

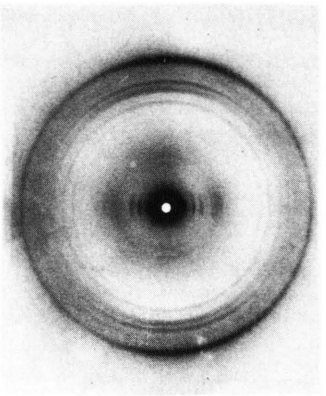

Fig. 2. High-angle X-ray diagram of a dry sciatic nerve of a rat treated with 10 mM PCP for 120 days. D = 8 cm.

Observation made by optical and electron microscopy showed, however, different results. In fact, the sciatic nerves of rats fed with 1.0 mM PCP for 90 days and 3.0 mM for 120 days presented degenerative changes in about 10% of the A and B type of nerve fibers. As it can be observed in Fig. 3 to 6, the damage to the neural components consisted in various degrees of ultrastructural degenerative changes of the myelin sheath. These changes vary from fibers with incipient and sectorized alterations of the neuroglial coat (Fig. 4) to fibers presenting a total disruption of the classical lamellar and periodical structure of myelin (Fig. 5). In addition, a severe loss of neurofilaments, neurotubules, vesicles and other axoplasmic components were observed when compared with normal myelin fibers (Fig. 6).

Discussion

X-ray diagrams were obtained from the sciatic nerves of rats that drunk PCP containing water whose concentration ranged between 1.0 and 10.0 mM for 90 to 120 days. They were compared with those diagrams obtained from rats that drunk

Table I. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of dehydrated sciatic nerves of rats untreated and treated with PCP[1].

Dry sciatic nerve of rats untreated with PCP			Dry sciatic nerve of rats treated with PCP		
do [Å]	Io rel	Orientation	do [Å]	Io rel	Orientation
152.3*	47	equatorial	152 *	37	equatorial
76.2*	88	equatorial	76.2*	84	equatorial
63.1*	100	equatorial	64.0*	100	equatorial
43.7*	62	equatorial + diagonal	43.7*	36	equatorial + diagonal
33.5	5	equatorial	32.7	7	equatorial
31.6	2	equatorial	31.2	3	equatorial
21.0	12	equatorial	20.9	10	equatorial
16.8	3	equatorial	16.7	6	equatorial
15.9	4	equatorial	16.0	4	equatorial
14.0	2	equatorial + meridional	13.8	3	equatorial + meridional
12.7	1	equatorial	12.7	4	equatorial
11.4	23	equatorial + meridional	11.4	15	equatorial + meridional
6.9	2	equatorial	6.9	3	equatorial
6.3	5	equatorial	6.3	6	equatorial
5.8	6	unoriented	5.8	10	unoriented
5.2	9	meridional	5.2	6	meridional
5.1	4	meridional	5.1	3	meridional
4.9	11	meridional	4.9	13	meridional
4.6	2	meridional	4.6	1	meridional
4.19	34	meridional	4.19	81	meridional

[1] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and (*) 29 cm.

Table II. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of freshly dissected sciatic nerves of rats untreated and treated with PCP[1].

Sciatic nerve of rat untreated with PCP			Sciatic nerve of rat treated with PCP		
do [Å]	Io rel	Orientation	do [Å]	Io rel	Orientation
157*	28	equatorial	157*	15	equatorial
88*	66	equatorial	86*	50	equatorial
58*	100	equatorial	58*	100	equatorial
44	9	equatorial	43	3	equatorial
33	2	equatorial	32	2	equatorial
16	2	equatorial	16	3	equatorial
13	31	equatorial	13	37	equatorial
4.5	11	unoriented[2]	4.5	24	unoriented[2]

[1] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and (*) 29 cm.
[2] Broad and diffuse.

PCP free water. Two type of nerves were analyzed. One that was subjected to X-ray diffraction at 18 °C in a high humidity atmosphere inmediately after being dissected. Their patterns showed about half a dozen of mostly diffuse and equatorially oriented reflections, all of them orders of a repeat period of about 176 Å except one of 157 Å (Table II). They also showed a broad and unoriented diffuse ring of 4.5 Å. This reflection, which is usually associated to a liquid-like conformation of the phospholipid hydrocarbon chains [10], indicated that they presented the L_α phase in the nerve myelin.

The other type of specimen consisted of sciatic nerves that were left do dry at 15 °C under slight tension. Their X-ray diagrams differed from those produced by the fresh samples in that the latter showed more and better defined reflections. As it has been reported, the extreme dehydration of

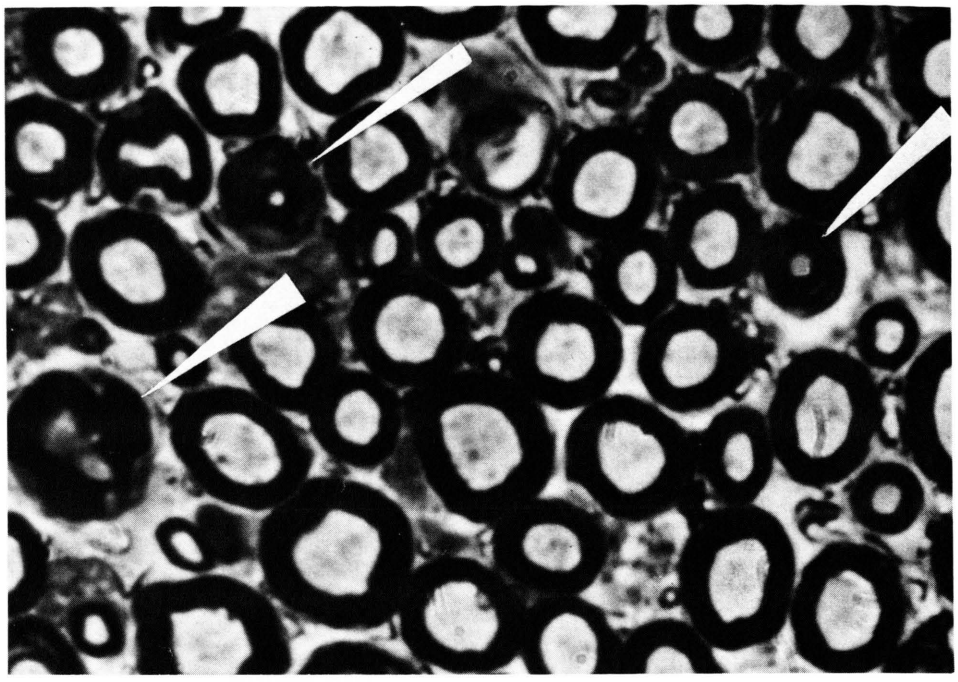

Fig. 3. Thick section of a sciatic nerve of a rat treated with 1.0 mM PCP for 90 days. Three myelinated nerve fibers showing different degrees of alteration are observed (arrows). Optical microscopy; toluidine blue staining, 845 ×. (All the micrographs correspond to the same group of rats.)

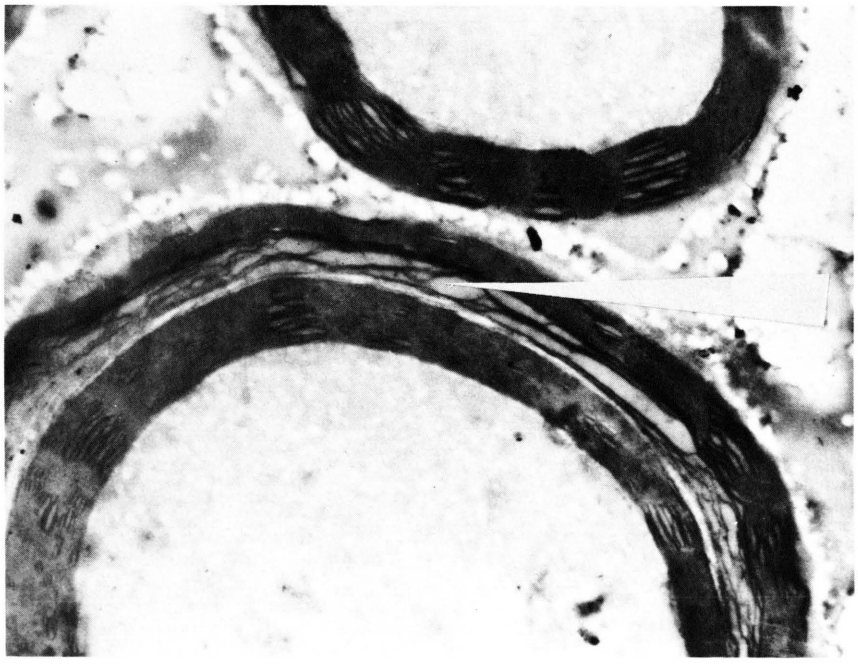

Fig. 4. Initial degenerative state of myelinated nerve fiber. Middle myelin layers showing disgregation (arrow). Transmission electron microscopy, 7200 ×.

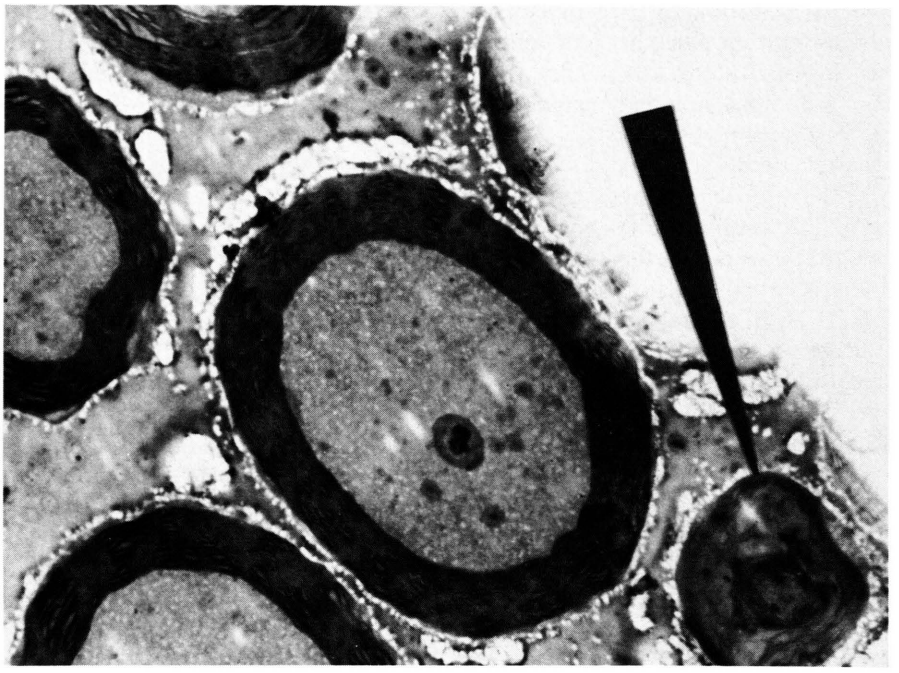

Fig. 5. Advanced degenerative state of myelinated nerve fiber (arrow). The regular appearance of the typical myelin sheath is completely lost. Transmission electron microscopy, 7200 ×.

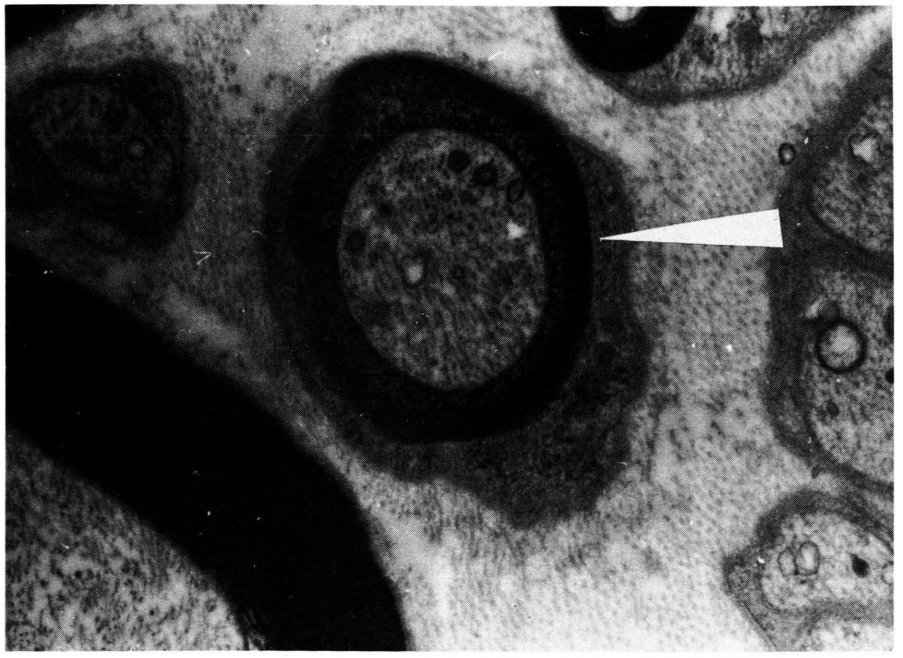

Fig. 6. Normal myelinated nerve fiber. Transmission electron microscopy, 20,000 ×.

myelin leads to an irreversible segregation of its various components [11]. The diffraction patterns from such mixed structures are difficult to unscramble. X-ray patterns from pure lipid, lipid extracts, mixture of lipids [12], dry myelin [11] and the investigation of the effects of various solvents on the structure of myelin [13] have provided the basis for the understanding of the nature of the various protein-lipid mixtures that give rise to the different repeat periods in the X-ray diffraction diagrams of the dry nerves [11]. Thus, the reflections present in Table I could be assigned in Table III to (a) a double bilayer with protein in the bilayer space with a repeat period of 152.3 Å (14); (b) a single bilayer with a 63 Å repeat; (c) a fluid-like arrangement of myelin lipids or of a lipid and lipid-protein mixture which produced the intense reflection of about 44 Å [11]; (d) a cholesterol phase that produced the 33.5 Å series, and (e) the 14 Å series, which might correspond to a form of cholesterol [11].

The comparison of the X-ray patterns obtained under the same conditions from the sciatic nerves of PCP treated and untreated rats did not show significant differences in the number, intensity and orientation of the reflections neither in their interplanar spacings. This result could easily lead to the conclusion that PCP did not affect the structure of myelin. However, the observations made by optical and electron microscopy of dry fibers indicated that PCP had indeed produced degenerative changes to some of the nerve fibers. The discrepancy between these results and those obtained by X-ray diffraction can be explained. In fact, optical and electron microscopy allowed to observe details of single fibers. Therefore, it could be detected that the 10% of the A and B type of them showed degenerative changes (Fig. 3–5). On the other hand, all the fibers contribute to the resulting X-ray diffraction patterns. Thus, the discrete reflections arise from the 90% of intact fibers whereas the remaining 10%, consisting of structurally perturbed and randomly distributed fibers, would only produce a diffuse scattering. In fact, observations made on the X-ray diagrams of the sciatic nerves of PCP treated rats, both wet and dry, revealed a higher background than those obtained from the normal rats.

Acknowledgements

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Table III. Assignment of the reflections obtained from dry sciatic nerves of rats[1].

Double Bilayer		Single Bilayer		Fluid Phase	Cholesterol		14 Å Series
do (Å)	Order	do (Å)	Order		do (Å)	Order	(do (Å)
152.3	(1)	63.1	(1)	43.7	33.5	(1)	14.0
76.2	(2)	31.6	(2)		16.8	(2)	5.8
		21.0	(3)		11.4	(3)	4.6
		15.9	(4)		6.9	(5)	
		12.7	(5)		5.2		
		6.9	(9)		5.1		
		6.3	(10)		4.9		
		4.19					

[1] Only the reflections from dry sciatic nerves of rats untreated with PCP are considered in this Table.

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