ANTIPYRIN METABOLISM AND HEPATIC ULTRASTRUCTURE OF RATS DURING ISOLATED AND COMBINED EXPOSURE TO TRICHLORODIPHENYL AND DIOCTYL PHTHALATE

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An extensive literature is devoted to the problem of chlorinated diphenyls. Because of their high temperature stability they possess the properties of dielectrics and are used in the electrical engineering industry for the manufacture of capacitors and as plasticizers and lubricants. One of the first reactions of the body on contact with polychlorinated diphenyls is the induction of the liver microsomal system with acceleration of metabolism of endogenous compounds and xenobiotics [6].

The basic problems of the use of chlorinated diphenyls are connected with their accumulation in man and animals and in nature, and with the formation of their exceptionally stable and more toxic products (dioxins and furanes) from them during fires [5, 9]. As substitutes for polychlorinated diphenyls, phthalate plasticizers (diesters of orthophthaleic acid) have begun to be used in several branches of industry, especially the manufacture of electrical capacitors [2]. Changes in the liver of animals following injection of di-2-(ethylhexyl)phthalate, including an increase in the content of cytochrome P-450 with weakening of cytochrome–c-oxidase activity [7, 8], have been reported.

The aim of this investigation was to compare the hepatotoxic action of small doses of trichlorodiphenyl (TCD) and dioctyl phthalate (DOP) under experimental conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male albino rats. TCD and DOP were administered through a gastric tube, dissolved in sunflower oil, 5 times a week in doses of 0.1 mg and 50 mg/kg body weight respectively, for 3 months. This dose was chosen because of the desire to bring it as close as possible to concentrations observed in industry.

One group of animals was subjected to the combined action of these compounds. The control group received sunflower oil in the same volume (0.2 ml/100 g body weight). To assess the hepatotropic action of the compounds, antipyrin metabolism and characteristics of the hepatic ultrastructure were used as parameters.

Antipyrin was injected into the rats' stomach in a dose corresponding to 10 mg/kg body weight.

Antipyrin metabolism was judged from elimination of its metabolites 4-hydroxyantipyrin and norantipyrin with the urine. Antipyrin metabolites in the urine were determined 1, 2, and 3 months after the beginning of the experiment.

Determination of 4-hydroxyantipyrin and norantipyrin in the urine was carried out by thin-layer chromatography [3]. One animal was killed at these same times for a study of the hepatic morphology. Liver tissue for electron-microscopic investigation was fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the EM-1200 EX electron microscope, with a magnification range of between 5000 and 50,000.

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TABLE 1. Content of Antipyrin Metabolites in Rats' Urine following Isolated and Combined Administration of TCD and DOP (in % of injected dose)

Experimental groups		After 1 month	After 2 months	After 3 months
Control	4-Hydroxy-antipyrin	18,4±1,15	13,2±1,7**	10,7±0,68**
	Noranti-pyrin	6,2±0,45	6,5±0,5	5,4±0,35
TCD	4-Hydroxy-antipyrin	22,2±2,4	17,3±1,55	24,5±2,7*
	Noranti-pyrin	7,9±0,65*	13,0±2,3*	18,5±3,5*
DOP	4-Hydroxyantipyrin	18,9±0,8	23,9±3*	17,7±2,7*
	Norantipyran	7,1±0,75	12,0±2,25*	11,1±2,4*
TCD + DOP	4-flydroxy-antipyrin	27,4±2*	18,7±2,0*	22,2±1,25*
	Noranti-pyrin	9,4±0,7*	14,1±2,45	14,3±1,9*

Legend. *$p < 0.05$, Difference from control is significant; **$p < 0.05$, significant difference between control group and value after 1 month.

EXPERIMENTAL RESULTS

The results of investigation of antipyrin metabolites in the urine are given in Table 1.

As early as 1 month after injection of TCD a significant increase was found in norantipyrin excretion with the urine, and after 3 months it was almost 4 times greater than the value determined in the control group and more than twice that observed in the same group after the first month. The 4-hydroxyantipyrin concentration was significantly higher than the control only 3 months after the beginning of TCD administration. In animals receiving DOP, after 2 months increased excretion of both antipyrin metabolites with the urine was observed, and after 3 months their urinary levels were reduced, although they were still significantly higher than in the control group.

In response to combined administration of TCD and DOP, the increase in excretion of antipyrin metabolites with the urine was observed as early as 1 month after the experiment began, but the difference in norantipyrin excretion was more highly significant.

It can be concluded from the results described above, allowing for a considerable difference in the dose given, that DOP has a weaker hepatotropic action, in particular, on the microsomal oxidase system, than TCD. Changes induced by it in antipyrin metabolism differ somewhat from those of TCD: whereas TCD mainly causes an increase in norantipyrin secretion, under the influence of DOP an increase in the excretion of both metabolites was observed, although it was less marked. There are indications in the literature of differences in the action of drugs and xenobiotics not only on the rate of biotransformation of antipyrin, but also on the spectrum of its metabolites [1].

The electron-microscopic investigation showed that injection of TCD into rats is accompanied by a sharp increase in the surface area of the hepatocytes due to the formation of numerous outgrowths of the plasma membrane, especially in the region of the sinusoidal pole of the cell. Widening of the intercellular spaces was observed, and a dense structureless substance is located in the widest of them. Most hepatocytes are characterized by an irregular condensation of the hyaloplasm. Near the biliary pole of the cell groups of vesicles of the smooth endoplasmic reticulum can be distinguished, with a fragmented membrane, together with solitary or multiple zones of the Golgi lamellar complex and large lysosomes with contents that are heterogeneous in their density and structure. The mitochondria are quite uniformly distributed in the cell. They are small (not more than 1 μ) and they have a dense structure with hardly distinguishable short and narrow cristae (Fig. 2). Zones of compact distribution of profiles of the rough endoplasmic reticulum are visible in many hepatocytes. They are characterized by an indistinct structure of their membrane, by focal degranulation (detachment of ribosomes from the membrane), and by unequal density of the ribosomes (Fig. 2). Among elements of the rough endoplasmic reticulum there lie many free ribosomes and polysomes. Single large fat inclusions are seen in some hepatocytes.

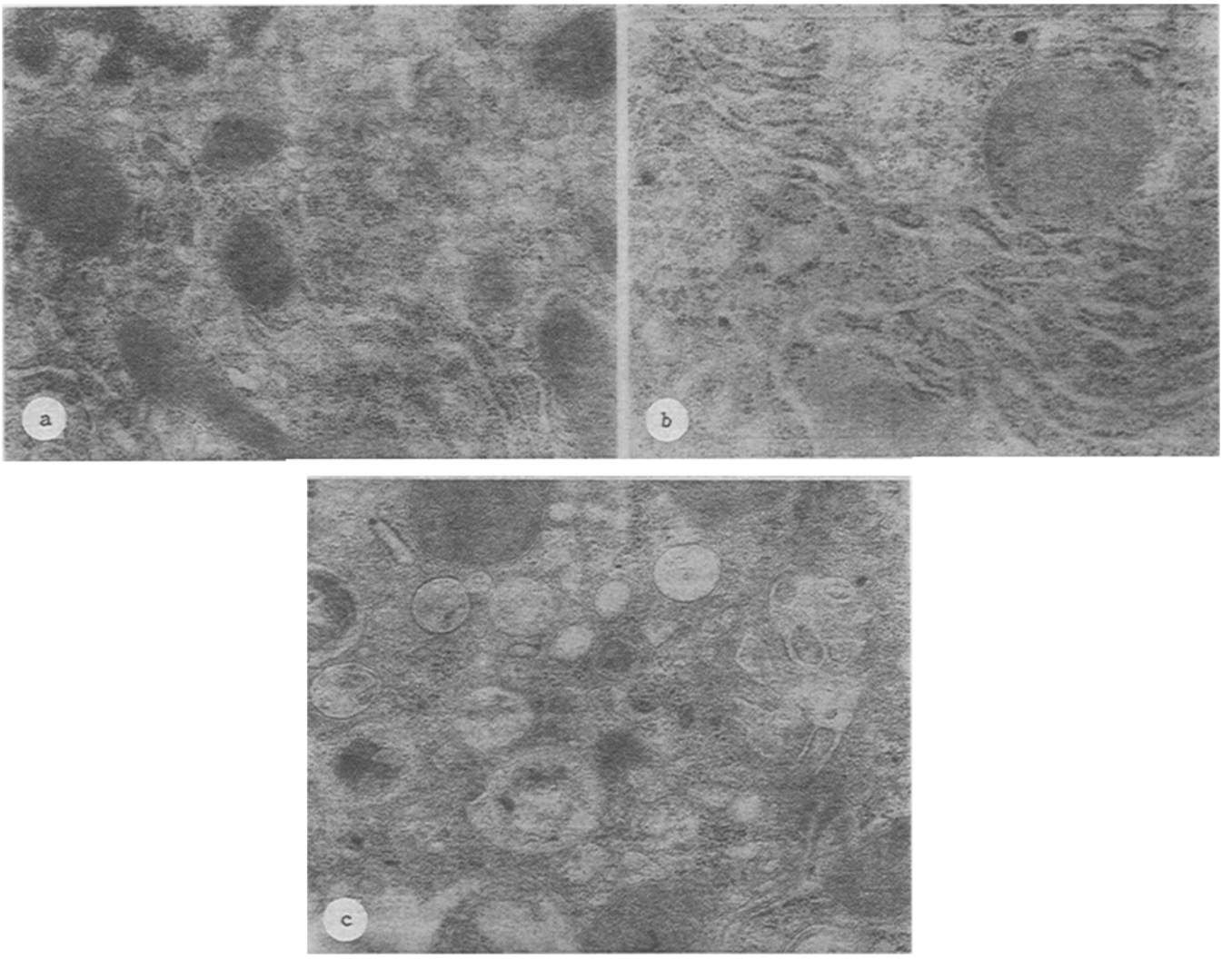

Fig. 1. Changes in hepatocytes under the influence of dioctyl phthalate: a) destruction of smooth endoplasmic reticulum and condensation of mitochondria, ×12,000; b) glycogen accumulation (arrow), ×15,000; c) lysosomes near biliary pole, ×15,000.

Under the influence of DOP the general characteristics of the ultrastructure of the rat hepatocyte closely resembled that following injection of TCD into the animals. Numerous microvilli were formed, enlarging the cell surface. This was expressed to the greatest degree also on the sinusoidal surface of the cell. In the widened intercellular spaces, individual fibrillar structures appeared within the masses of dense material. The hyaloplasm of the hepatocytes was unevenly condensed, and in places it was loose in structure and edematous. The smooth endoplasmic reticulum close to the biliary pole and in other zones of the cell resembled vesicles with a destroyed membrane (Fig. 1a). Small mitochondria (under 1 μ in diameter) were concentrated near the nucleus, whereas larger mitochondria, at different stages of division, were located nearer to the sinusoidal surface of the cell. All mitochondria were characterized by a dense matrix, masking the few short cristae (Fig. 1a). Under these circumstances, both mitochondrial membranes preserved their structural integrity. Unlike TCD, exposure to DOP was accompanied by the formation of glycogen depots among the mitochondria (Fig. 1b). Just as in the previous experiment, zones of compact arrangement of the narrow tubules of the rough endoplasmic reticulum could be seen in the hepatocytes. They were characterized by an indistinct membrane and by moderate degranulation. The free ribosomes distributed here formed polymorphic groups, differing in density and size. Regions where the tubules of the rough endoplasmic reticulum were somewhat dilated, and, to a lesser degree, their membrane was degranulated, were observed less frequently. The polysomes near these tubules were rosette-shaped and more homogeneous in their density. In

Fig. 2. Changes in hepatocytes under the influence of TCD-disorganization of the rough endoplasmic reticulum and condensation of mitochondria, ×25,000.

those hepatocytes the rough reticulum, located in the perinuclear region, preserved a normal structure. In many hepatocytes, polymorphic lysosomes could be seen near a biliary capillary (Fig. 1c).

The electron-microscopic investigation of the liver showed that injection of TCD and DOP into rats leads to changes in structure of the hepatocytes and to disturbance of intercellular junctions. In virtually all the hepatocytes the mitochondrial structure was modified, and under the influence of DOP, glycogen depots were formed in many cells. This is evidence of a change in the energy metabolism of the cell. Under these circumstances fatty infiltration of the cell was not observed. The protein-synthesizing structures of the cell (rough endoplasmic reticulum, free ribosomes and polysomes) were characterized by an opposite reaction. On the one hand, an indistinct structure of the membrane was accompanied by focal degranulation, evidence of depression of functional activity. On the other hand, accumulation of free ribosomes and polysomes was discovered, reflecting the presence of regenerative processes in the cell. In all hepatocytes studied destruction of the membrane of the smooth endoplasmic reticulum was noted. Activation of the lysosomal system was observed in many cells: a Golgi lamellar complex and numerous lysosomes, differing in size, density, and character of their contents, was located close to the biliary pole. The structural characteristics of the lysosomes are evidence that these were more likely to be secondary lysosomes than primary, and it reflects the active metabolic background, with a catabolic tendency.

Thus under the influence of TCD and DOP changes indicating disturbance of various types of metabolism in the hepatocytes develop in the rat liver, including in the energy metabolism of the cell and the protein synthesizing function. Hyperplasia of the lysosomal apparatus and moderate activation of the intracellular regenerative process were observed in many hepatocytes.

The results of these investigations are in agreement with data in the literature on the inducing effect of TCD on the microsomal system of the liver DOP, in the dose studied (50 mg/kg), had a similar action. The combined effect of these two compounds is synergic.

The hepatotropic action of the above-mentioned substances was not confined to activation of cytochrome P-450-dependent enzymes, but was manifested as disorganization of energy and protein metabolism and of hepatocyte organelles.

The results are evidence of the hepatotoxic action of small doses of chlorinated diphenyls and of esters of orthophthaleic acid.

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FEBRIFUGAL ACTIVITY OF ACUPUNCTURE AND ITS POTENTIATION BY PROPRANOLOL

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With the increasingly widespread use of acupuncture in clinical medicine, the elucidation of its anatomical and physiological basis assumes great importance [6, 11, 13]. There have now been many investigations into the neurophysiological and neurochemical mechanisms of acupuncture analgesia [5, 8, 10, 12]. Many workers have noted the involvement of adrenergic structures in its realization [4, 14, 15]. Yasnetsov [9] reports potentiation of the analgesic effect of acupuncture by propranolol in a dose of 1.5 mg/kg. In studies by other workers [1] propranolol weakened acupuncture analgesia. On the whole, the problem of the oriented action of drugs on various aspects of the therapeutic action of acupuncture has received comparatively little study. There are only isolated reports of the effect of benactyzine on the antipyretic effect of acupuncture, which depends on the dose of the cholinolytic [7].

The aim of the investigation described below was to study the relationship between the febrifugal effect of acupuncture and the β-adrenoblocker propranolol (anaprilin).

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