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Effect of Lindane on the differentiation of embryonic chick gonads in culture

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The embryonic chick ovary and testis in organ culture were used to test the direct effect of a chlorinated organic pesticide, Lindane. The ultrastructural alterations in the three main cell populations allowed the authors to characterize the Lindane action and its basic mechanism in affecting the germ and somatic cells in the gonads.

Key words: Lindane, ovary, testis, organ culture, chick embryo, ultrastructure.

During the last decade the agricultural intensification was connected with the administration of various kinds of herbecides and insecticides which resulted in entrance and accumulation in biosphere of stable chemical compounds noxious for humans and animals. Lindane, the gamma-isomer of benzenehexachloride, holds an unique place among chlorinated organic insecticides. It is widely used because of its good solubility in water and lipids and because of its wide spectrum of action.

The uptake of Lindane into the body can occur mainly with water and food of animal and vegetable origin. It is known that the Lindane is quickly metabolized in the liver and the main excretion products are water-soluble conjugates of glucuronic acid and of sulphuric acid, free phenols etc. [6]. The metabolism of Lindane counteracts the accumulation of residues, and may be considered an important factor for maintaining an equilibrium between the uptake of Lindane and the excretion of its metabolites.

In warm-blooded animals, pesticides and their metabolites affect almost all organs and systems especially liver and reproductive system. Some data point to an augmentation of abortions in pregnant animals as well as of mortality of the offspring in regions with intense chimization [12]. A delayed sexual maturity and a diminished fertility in laboratory animals were also observed. Experimental studies revealed a gonado- and embryotoxic effect of some pesticides such as Lindane and sevin [9, 10].

In Mammals, Lindane is able to cross the placenta into the embryo [3]. In Birds, Lindane is accumulated in egg yolk, the 80-85% of the total quantity being detected in its lipovitellin fraction [3].

On the basis of these observations, the question of harmful influences on the fetus and on the reproductive system is of particular interest. It is very important to know if

Lindane effects directly developing germ cells and gonads or its metabolic products induce the histopathological alterations.

The present study was undertaken with aim to investigate the dirrect effect of Lindane on embryonic gonads during ontogenesis. The chick embryo was chosen as an experimental model, taking into consideration the fact that avian egg develops outside the influences of hormonal and other controlling factors coming from the maternal organism, in natural conditions as those neighbouring humans and animals.

Material and methods

Male and female gonads explanted in organ culture, were used. Our experiments concern 2 critical periods in chick embryo development: 1) 9th embryonic day (ED), immediately after the gonadal sex differentiation (stage of 7,5-8th ED) when indifferent gonad development is already oriented in male or female directions; 2) 15th ED, a stage when considerable differences in the development of male and female germ cells are observed: in the ovary, cortical oogonia enter the meiotic prophase, but in the testis an active spermatogonial proliferation is present.

When Lindane was given orally in rats, LD_{50} was 125μg/g body weight but during longterm administration, doses between 25 and 50 μg/g did not have pathological effect [8]. On the basis of these results, in our experiments the action of 3 doses of Lindane was studied: high dose of 100 μg/ml of culture medium, a concentration near to the LD_{50}; middle dose of 60 μg/ml, and low dose — 40 μg/ml (the latter is effectless during in vivo administration).

The left testes and ovaries, explanted from 9- or 15-day old chick embryos, were cultured on celloidin membranes covered with a thin layer of 1% agar [5]. A culture medium containing yolk dialysates [4] and 25% calf serum, was applied. Lindane was added in final concentrations of 100, 60 and 40 μg/ml. A part of explants served as controls. After a 24h incubation, the organs were fixed in Carnoy's fluid or in 2,5% glutaraldehyde — 1% osmium tetraoxide, and embedded in paraffin or epon, respectively. The observations were carried out on a light microscope Opton, as well as on an electron microscope Opton 109.

Results and discussion

Using the mentioned above experimental technique for organ culture, we observed, as described previously [5], that the development of ovarian and testicular explants corresponds to the same process in vivo.

Administration of the high dose of Lindane (100 μg/ml) resulted in intense degenerative changes of the three main cell populations in the gonads: germ cells, satellite cells (Sertoli cells in the testis and prefollicular cells in the ovary) and interstitial steroidogenic cells. Histopathological alterations were observed on the two stages examined (9th and 15th ED) which point's to the fact that the embryonic gonad and the differentiating cells are quite sensitive to toxic action of Lindane. In the most cases germ cells are absent, a disturbed structure and almost full degeneration of the gonads were visible.

Treatment of the cultured gonads with a concentration of Lindane at the middle rate of 60 μg/ml of culture medium resulted in more diverse alterations in the ovary and testis. In earlier ontogenic stages (9th ED) the histological structure of the gonads was disturbed. In the testis, a desorganization of the seminiferous

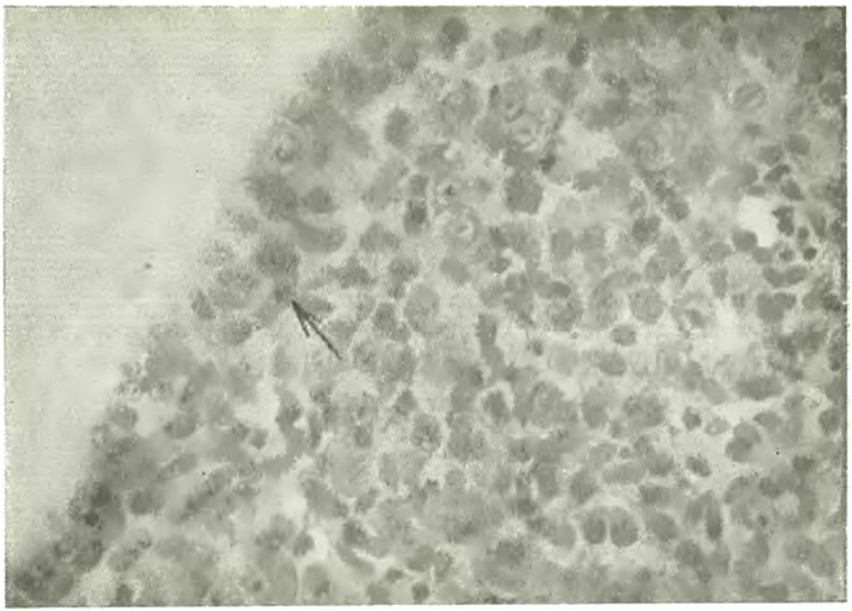

Fig. 1. A 9-day old testis incubated 24 hours with Lindane (60 µg/ml). Pseudocorticalization of the testis including the presence of a high germinative epithelium and a desorganization of seminiferous cords (↑), × 500

cords in the periphery occurred thus resembling a "pseudocorticalization" (Fig. 1). In the ovary, sometimes the typical orientation of the satellite cells around oogonia and the formation of the so-called "prafollicle" [2] was disturbed. At the beginning, degeneration affected the germ cells; later, the prefollicular cells were also damaged. Alterations in spermatogonia and oogonia were analogous; they were expressed in pyknosis and karyorrhexis, as well as in cytoplasmic vacuolization and lipid dystrophy. Ovarian interstitial cells and testicular Leydig cells were less damaged: the structure of some cytoplasmic organelles was affected, irregular lipid vacuoles and secondary lisosomes were observed.

At a dose of 40 µg/ml, ovaarian and testicular morphological changes were less expressed especially on day 9 of the embryonic development. The integrity of the membranous structures in the spermatogonia was affected. An irregular dilatation of the perinuclear space (Fig. 2) as well as of the endoplasmic reticulum was present. A destruction of the mitochondrial cristae or of the mitochondria, and a formation of myelin figures were observed. In the cytoplasm, secondary lisosomes and residual bodies were visible. The Leydig cells were more or less preserved, in some places cytoplasmic vacuoles were formed (Fig. 3). On the 15th ED the degenerative changes were more advanced. Cells more or less preserved as well as Leydig cells with damaged mitochondria and agranular endoplasmic reticulum, with myelin figures and irregular lipid inclusions were present.

In the ovary on the 9th ED lipid inclusions, destroyed mitochondria, lisosomes and myelin figures in the cytoplasm were observed. Fragmentation of nucleoli and irregular chromatin distribution (condensation or dispersion) in some oogonia were found (Fig. 4). Similar degenerative changes in prefollicular cells were also present. Interstitial cells were less damaged (Fig. 5).

On the 15th ED degenerative alterations in the female gonads were more ex-

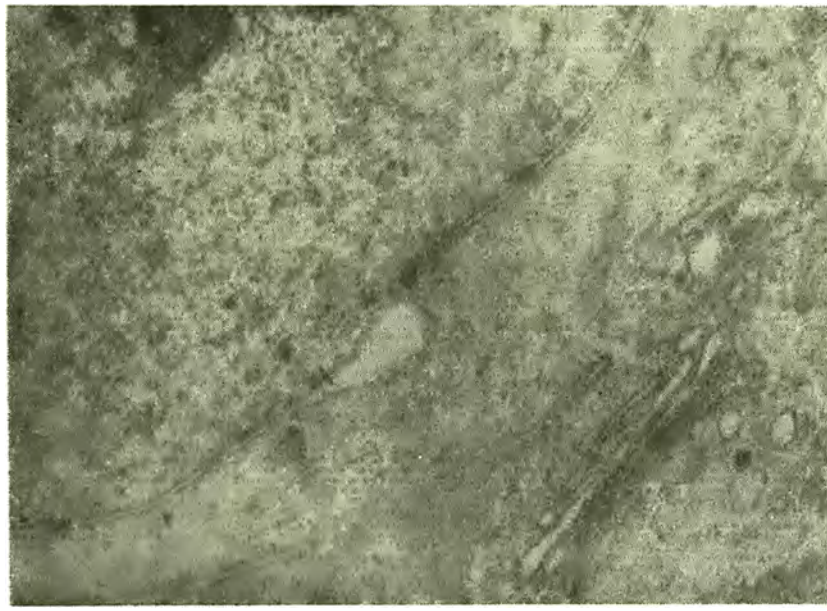

Fig. 2. Spermatogonium in a 9-day old testis treated with Lindane (40 µg/ml). Irregular dilatation of the perinuclear space is observed, × 20 000

Fig. 3. A 9-day old testis is incubated 24 hours with Lindane (40 µg/ml). Irregular mitochondrial configuration and a cytoplasmic vacuole in a Leydig cell. × 20 000

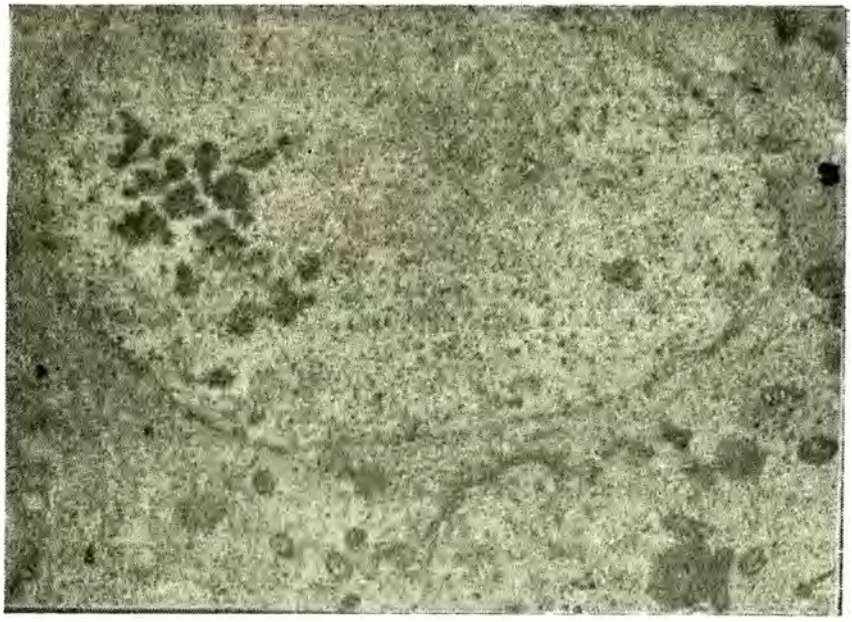

Fig. 4. Ovary from a 9-day old embryo after a 24 hours culture with Lindane (40 µg/ml). Nucleolar fragmentation and lipid inclusions in the cytoplasm of a cortical oogonium, × 20 000

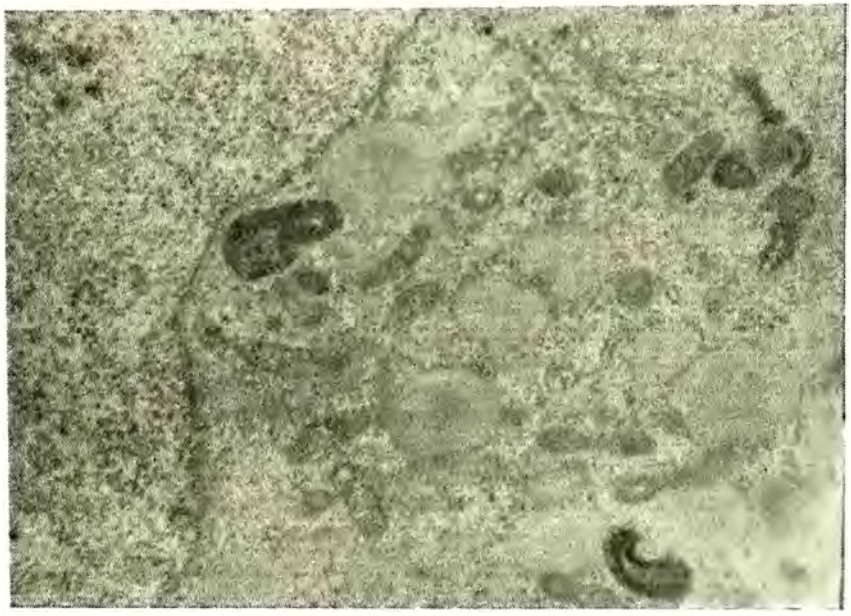

Fig. 5. Interstitial cell in a 9-day old ovary treated with Lindane (40µg/ml). Degenerative changes in mitochondria and myelin figures in the cytoplasm are present, × 12 000

tended. Some cortical oocytes were almost fully destroyed but other oogonia, prefollicular cells and interstitial cells with normal structure were also present.

The results obtained by us allow the following conclusions to be made:

1. The germ cells in both ovary and testis are the most sensitive ones to the direct effect of Lindane. The lower dose induced histopathological alterations of the membrane components and mitochondria, as well as of the genetic apparatus of the cell. When higher doses were administrated, both the germ cells and the somatic cells in the gonads were damaged.

2. During earlier stages of ontogenesis, the histological structure of the gonads is also affected (for instance, pseudocorticalization of the testis).

3. The induced changes are dose-dependent.

Our investigations supplement and extend the previous communication of Baleva et al. [1]. They present a more complete characterization of the Lindane action on the main gonadal cells populations and their ultrastructure. It was established that even in low doses, the pesticide induce pathological ultrastructural changes in differentiating germ and somatic cells. The observed alterations in the membranous structures are morphological characteristics of the toxic Lindane action. Recently, it was demonstrated that the interaction of pesticides with protein components of the interne mitochondrial membrane which causes an inhibition of electron transport and a decrease of the ATP synthesis in mitochondria, is a basic mechanism of toxic pesticide effect [11]. Our results confirm these data. The observations mentioned above suggest that the in vivo induced alterations in gonads are also due mainly to direct action of Lindane. On the other hand, the effect of Lindane on spermatogenesis, oogenesis and during the perinatal stages of development indicates the possibility of the induction of chromosomal abberations [7], which are also connected with the affected embryo development and survival.

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