Alterations of the Seminiferous Epithelium and the Leydig Cells of the Rat Testis after the Application of Dichlorvos (DDVP)

by

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Publications on fertility disturbances caused by insecticides first dealt with chlorinated hydrocarbons, e.g. DDT or Lindane (1,3). Due to their cumulation in the organism they are more likely to cause disturbances in all organs than alkylphosphates, which can be reduced more quickly. Dimethyl-2-dichlorvinylphosphate (DDVP), which is examined in this study, is quickly reduced by hydrolysis in the organism or excreted unchanged(2). JONES (1972) proved by experiments with radioactively marked substance that 80% of the DDVP intake disappears from the body within 6 hours after application. Yet the LD 50 for rats and men is about 80-100 mg/kg (13). The influence on fertility was not discussed. LÖFROTH's (1970) in vitro reports, i.e. that DDVP triggers chromosomes breaks, caused us to examine the effects on spermiogenesis.

Material and Method

42 male white mice of the NMRI/Han strain were divided into 3 groups of 14 animals each. 40 mg/kg of DDVP were administered per os to group I in one dose as a 1% solution in olive oil by means of a stomach tube. Group II was given 10 mg/kg of DDVP each for 18 consecutive days, i.e. a total of 180 mg/kg of DDVP in the above mentioned solution. For control purposes, group III was given 0.5 ml of olive oil for 18 days. The animals were kept in wire mesh bottomed cages to eliminate coprophagy as far as possible. Food and water were available ad libitum. On the 9th, 18th, 27th, 36th, 54th, and 63rd day after the experiment had been started (in accordance with the length of the spermiogenetic cycle discovered by OAKBERG (1956)),2 animals out of each group were killed. Their testes were removed and embedded in paraffin. After staining these

Bulletin of Environmental Contamination & Toxicology, Vol. 11, No. 5 © 1974 by Springer-Verlag New York Inc.

by means of the PAS-HE method, they were examined histologically. Under the microscope, the number of cross-cut tubules was determined in 4 section preparations each of one animal (i.e. 8 section preparations each day on which animals were killed). The number of tubules showing a damaged seminiferous epithelium was determined. Holes in the seminiferous epithelium, areas of desquamation, absence of various layers, and decreases in cell population were taken as evidence of damage and counted separately. The number of Leydig cells was estimated by counting them within 20 triangles formed by 3 tubules each ("interstitial triangles"). Their number was submitted to statistical comparisons by means of a variance analysis.

Results

Severe disturbances of the spermiogenesis can be observed for the test group given one single dose of DDVP as well as for the group given repeated doses of DDVP. As the experiment progressed, these disturbances become more distinct. Many cross-sections of tubules revealed areas of desquamation or decreases in cell population (Fig. 1).

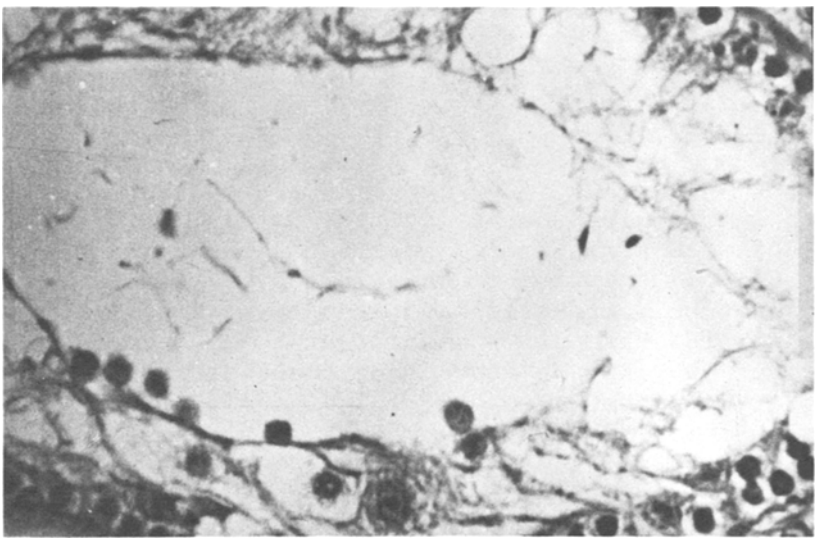

Fig. 1: Section of a seminiferous tubule showing desquamation and depletion of cell population (54 days after administration of DDVP, magnification 540 x)

Table 1 gives the average number of damaged tubules (percentage of the total number). It can be noted that - contrary to the control group - their number increased as the experiment progressed.

T A B L E 1

Mean number (in percent) of sections of damaged tubules after the administration of DDVP

Days after application	Damaged tubules after one administration	repeated	Control
9	9	5	7
18	17.5	5.5	0
27	3	4.5	3.5
36	11	13	0
54	16	18	1.5
63	33	10.5	1.5

A statistical evaluation was omitted, the test groups being rather small.
Apart from the damaged tubules, one can observe Leydig cell complexes which are bigger than normal. Table 2 gives the average number of Leydig cells per "interstitial triangle".

T A B L E 2

Mean
Mean number of Leydig cells per "interstitial triangle" after the administration of DDVP

Days after application	Leydig cells after one administration	repeated	Control
9	7.85	11.20+	7.45
18	9.85+	8.68+	5.53
27	11.20+	8.95+	5.03
36	10.83+	10.08+	7.28
54	9.85+	8.28	6.83
63	10.68+	7.55+	4.55

An increase with time is not noted. However, the figures found for the long-term treated animals as well as for those those given a single dose are significantly above the figures for the control group. This fact is demonstrated in Table 2 by means of a (+).

Discussion

The tubular damage that occurred after application of DDVP cannot just be explained as resulting from an effect on certain cell groups. Contrary to this, it is a fact that many cytostatic substances like Busulfane (14), Cyclophosphamide (6) and Procarbenicid (5), damage mainly the A1 -A_3 spermatogonia within the G_1 phase, which die during mitosis. The other cell types remain unaffected. Since the latter, however, are daughter cells of the A-spermatogonia, one can see a gap in the seminiferous epithelium which migrates from the bottom to the top layers in the course of observation. 45 days after application all cells have regenerated. Absence of certain layers is rarely observed. From the holes, areas of desquamation and decreases in cell populations, it could be concluded that the cells are washed out as well as destroyed. Since the Sertoli supporting cells are obviously damaged, it is likely that the cells just lose support.
In addition, an increase of Leydig cells is observed. This increase may be a sign of hypoandrogenism; the ensuing release of LH leads to hypertrophy. This would mean that the Leydig cells are the reason for the primary damage caused by DDVP. Since DDVP inhibits not only cholinesterase but also unspecific esterases, which occur abundantly in the Leydig cells (4,7), an effect of this kind is possible. A disturbance of steroid metabolism has also been described (9). The hypoandrogenism on its part would lead to a loss of the seminiferous epithelium. This hypertrophy of the Leydig cells may also be caused in a different way: the primary damage is exerted on the cells of the seminiferous tubules. Due to a still unknown feed-back mechanism, the Leydig cells increase their production of androgens to accelerate the regeneration of cells. The cells primarily affected in this respect are not necessarily the cells of the spermiogenesis, but can also be the Sertoli cells. In contradistinction to the germ cells, they also have esterases (11).

A decision as to which effect applies in this case cannot be made on the basis of the experiments in this study. Since there is no synchronisation between the occurrence of damage and the spermiogenetic cycle, an effect caused by DDVP on the germ cells is not likely. Besides, there is no indication of a direct hypophyseal-diencephalic action.

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