Influence of DDT, DDVP and Malathion on FSH, LH and Testosterone Serum Levels and Testosterone Concentration in Testis

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We previously reported damage to spermatogenesis in mice and juvenile rats which were treated with DDT (dichlordiphenyltrichlorethane), DDVP (dichlorvos) or malathion for several days (KRAUSE et al. 1974; 1975;1976). By histological methods, these insecticides were shown to induce damage to the spermatogenetic and the Leydig cells. It was suggested that the main damage could be a biochemical one, since these substances inhibit many enzymes. Androgen metabolism could be especially affected, and a resulting lack of testosterone would cause disturbances of spermatogenesis. Other authors described metabolic effects of insecticides (KUPFER 1969;FEASTER and STEVENS 1972), and endocrine alterations were also found after application of DDT and homologues (GELLERT et al. 1974).

Consequently, we repeated our experiments including measurement of testosterone levels in serum and testes

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tissue.

Material and Methods

Animals: Adult male Wistar rats weighing about 300 g were kept on a 12^h - 12^h light-dark schedule and fed standard feed (Altromin) and water ad libitum. Groups of 6 animals each received the following treatments orally by a stomach tube:

Group 1 DDVP 10 mg/kg, dissolved in ol. arachnidis in a concentration of 5 mg/ml, every second day for two weeks

Group 2 DDVP 5 mg/kg (5 mg/ml) three times a week for 3 weeks

Group 3 DDT 200 mg/kg (100 mg/ml) every second day, 2 weeks

Group 4 DDT 100 mg/kg (100 mg/ml) three times a week, 3 weeks

Group 5 20 mg/kg (10 mg/ml) malathion every second day, 2 weeks

Group 6 0.2 ml ol. arachnidis, three times a week, 3 weeks

At the end of the treatment animals at 17^h were sacrificed by an overdose of ether; body weight, weight of testes and seminal vesicles were recorded. Blood was taken through an incision of the abdominal aorta, stored in the refrigerator overnight, centrifuged and serum stored at -20^o. One testis was examined histologically without counting cells, the other homogenized.

Separation of testosterone: A piece of the testis weighing about 500 mg was homogenated in a potter with 5 ml of physiological saline solution. 1 ml of the mixture after addition of 450 cpm ^3H-testosterone was extracted twice with 5 ml ethylether ("for analysis" Merck), the ether evaporated to dryness and the residues redissolved on 0.2 ml dichlormethanemethanol (3:11). 100 µl therefrom were placed on a thin layer chromatographic plate (Silicagel 60, Merck) development for 60 min with, in 30 ethylacetate, 90 Benzene.

In each chamber an additional plate was run with testosterone standard (0.2 mg), and after chromatography the spots were stained in iodine gas.

Related areas from the other plates were extracted twice for 10 min with 1 ml dichlormethanemethanol

(3.11). Both extracts were mixed, 1 ml dried in a counting vial, and the radioactivity measured for yield evaluation. The yield had an average of 78%. The other part of extract, 1 ml was evaporated to dryness and immediately before radioimmunoassay dissolved in 1 ml PBS-buffer. Steroid free areas of the plates were treated in the same manner and served as controls.

Radioimmunoassays: FSH and LH were measured in serum by the aid of kits supplied by the NIAMD. Details of the method are previously described (KRAUSE and SCHWAEBE 1976). Testosterone in serum and in testis extracts was determined with a commercial kit of the S.O.R.I.N. This method was also previously described (KRAUSE and SCHWAEBE 1976). The levels of FSH and LH are expressed in ng/ml, those of testosterone in serum in ng/100 ml and testosterone concentrations in ng/g testis tissue wet weight.

Means and standard derivations of the values were calculated on a PDP. Significances of the differences of mean were estimated with the t-test.

Results

Table 1 summarizes body weight, testis and seminal vesicles (filled). The number of animals as indicated in the column "N" is sometimes lower than 7, the lost animals died within the experiment. No significant differences can be seen.

TABLE 1

Body, testis and seminal vesicle weight

Treatment*	N	Day weight (g)	Testis (mg)	Seminal vesicles (mg)
DDVP 10 mg	2	360 ± 20	2990	1445
DDVP 5 mg	7	363 ± 51	3114	1061
DDT 200 mg	3	330 ± 17	3255	1077
DDT 100 mg	7	358 ± 24	3458	917
Malathion 20 mg	4	352 ± 26	3179	1506
Oil	7	298 ± 25	3162	740

*For details of the treatment see "methods"

Figures 1 - 4 show the mean levels of FSH, LH and testosterone in serum and testosterone concentration in testis. FSH levels after application of Malathion are

significantly increased against controls (t = 2.28. p = 0.05).

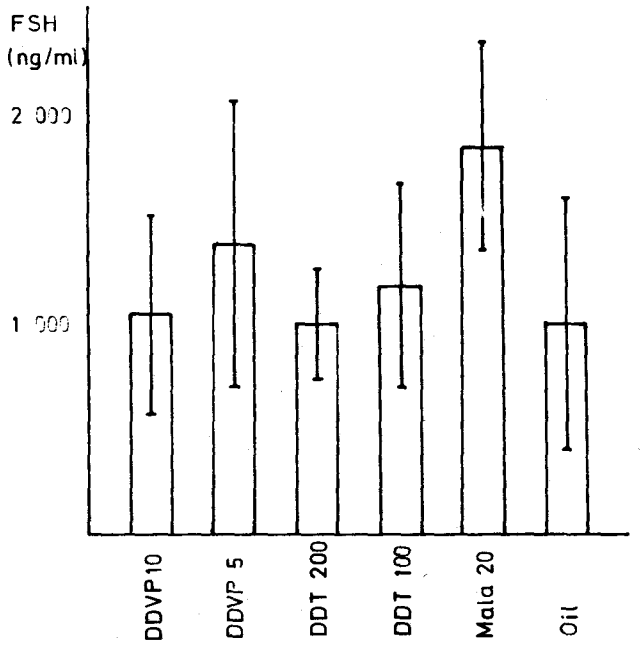

Fig. 1 Mean FSH serum levels in rats treated with DDVP 10 or 5 mg/kg, DDT 200 or 100 mg/kg, malathion (Mala) 20 mg/kg, or oil. For details of the treatment schedule see "methods." Number of animals is indicated in Table 1.

LH levels show no significant differences, as do the testosterone levels in serum.

Testosterone concentrations in testis of the DDT treated group are decreased against controls ($t = 1.77$; $p = 0.08$). Histological examination did not demonstrate any disturbances of the spermatogenetic epithelium in the testis.

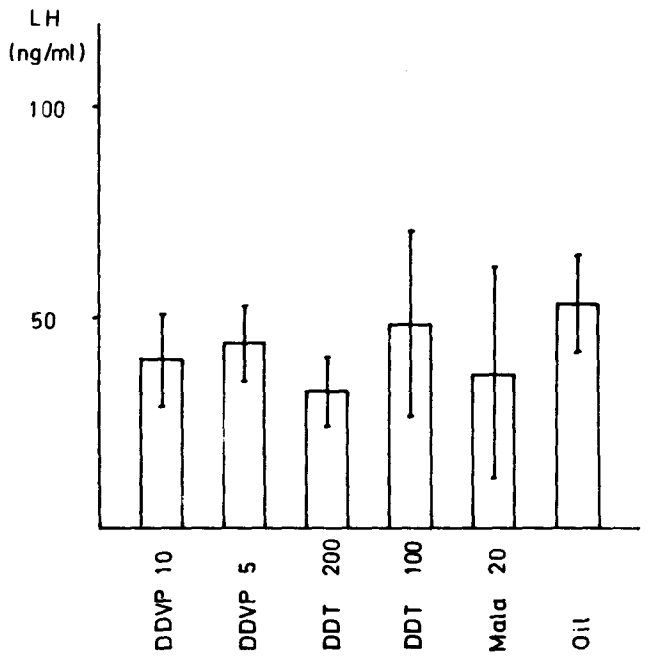

Fig. 2 Mean LH serum levels in the same animals as shown in Fig. 1

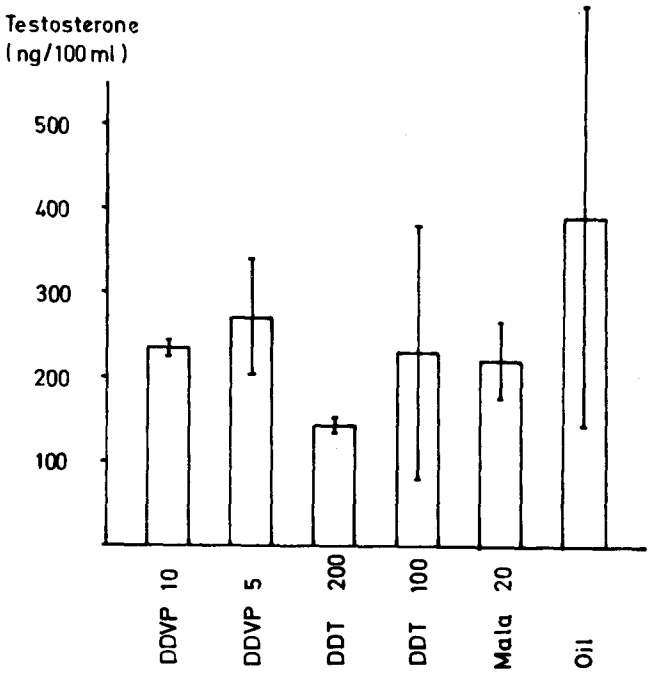

Fig. 3 Mean testosterone serum levels in the same animals as shown in Fig. 1

Discussion

As mentioned earlier, we demonstrated previously destructive effects of DDT, DDVP and Malathion on spermatogenesis in juvenile rats. The number of spermatogenetic cells, the tubular diameter and the number of Leydig cells decreased. The animals were treated

every day, in the experiments reported here every second day.

Histologic examination of testes in these experiments did not show these alterations, but we did no counting of cells. Therefore, slight effects cannot be excluded.

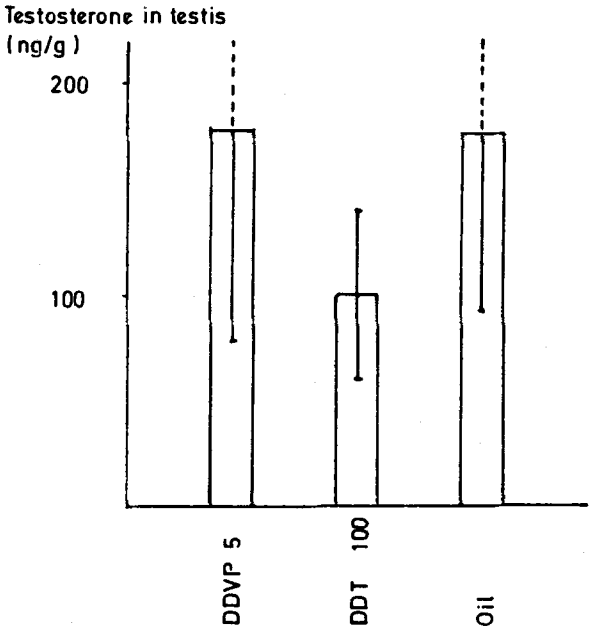

Fig. 4 Mean testosterone concentration in testis (ng testosterone per g testis tissue wet weight) in rats treated with DDVP 5 mg/kg, DDT 100 mg/kg or oil

The cellular alterations were thought to be caused by a lack of androgens. The most severe damage was seen after treatment with DDT.

The results shown here confirm these findings and demonstrate the decreased androgen production. First, testosterone levels in serum in the group treated with 200 mg/kg DDT daily for two weeks are significantly lower than those of controls.
Second, the testes of the animals treated with DDT 100 mg/kg for three weeks contain less testosterone than those of controls. This difference is just above the border of statistical significance. Since the testes weight do not differ, this result is related to an absolute decrease in testosterone concentration.

Later we will discuss whether the decreased testosterone levels, which are possibly caused by a decrease of the production, are due to a direct damage to Leydig cells or to a lowered stimulation of these cells by LH. The results of Fig. 2 suggest the first possibility. Serum LH levels are not altered by treatment with DDT, as are the FSH levels. GELLERT et al. (1974) reported estrogen-like effects of DDT and some of its analogues

on LH and FSH levels, but this was shown in the female rat, and we cannot confirm their results.

While the effects of DDT can be explained by the lowered androgen concentration, DDVP does not seem to act in the same manner. Neither serum levels of LH and FSH or testosterone nor testicular androgen concentrations are altered by a treatment with DDVP.

In our previous experiments we observed severe damage to spermatogenesis by DDVP also. Since there is no evidence for a decrease in testosterone production a direct, cytostatic effect in the spermatogenetic cells is more likely, as reported by HILGETAG and TEICHMANN (1965). The same mechanism might be suggested in the case of malathion.

Summary

Groups of adult male rats were treated with DDT, DDVP or malathion orally for two or three weeks. Body weight and testis weight did not change. FSH and LH levels in serum also remained unchanged, whereas that of testosterone was decreased by DDT. This substance also diminished the concentration of testosterone in

the testis. Therefore, the previously observed damage to spermatogenesis by DDT are assumed to be caused by a lack of androgens. Those following treatment with DDVP or malathion seem to be due to a direct cytotoxic effect.

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