The Effect of DDT on Spermatogenesis of the Juvenile Rat

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Dichloro-diphenyl-trichlorethane (DDT) found world-wide application as a reliably effective insecticide. Only at a later date it was recognized that the substance is very slowly broken down, and the amounts once introduced into nature are today still present in almost unvaried quantity but diffuse distribution. In the German Federal Republic its use was prohibited in 1971, but the large reservoirs justify further work in relation to its toxicological mechanisms.

Fertility disorders were first reported in pheasants (BRYSON et al. 195o), high concentrations were demonstrated in pheasant eggs. Leghorn cocks reacted to DDT with marked testicular atrophy (BURLINGTON et al. 1950). Trials on mammals, for example rat (COTTRELL et al. 1959), mouse (DEICHMANN et al. 1966), and dog (DEICHMANN et al. 1971) so far have not revealed a distinct influence of DDT on reproduction.

Therefore, we undertook feeding experiments with juvenile male rats for quantitative assessments on spermatogenesis and fertility.

Material and methods

Male rats, 4 days old, were allocated to three groups of 16 each and treated as follows: group 1 received 500 mg/kg on both the 4th an 5th day of life ("DDT acute"); group 2 received 200 mg/kg DDT daily from the 4th to the 23rd day, total 4000 mg/kg ("DDT

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chronic"); group 3 received o.1ml olive oil daily from the 4th to the 23rd day ("Control"). All treatments were by a stomach tube, with DDT dissolved in olive oil.

At defined dates, i.e. on the 6th, 12th, 18th, 26th, 34th, and 50th day of life, two animals of each group were killed by ether and the body weight determined. The testes were removed, weighed and examined histologically. The tubular diameter and tubular wall thickness were measured on 20 tubules. The number of Sertoli cells, A-spermatogonia, pachytene spermatocytes, and spermatids were determined in 10 tubular

T A B L E 1

Body weight and testes weight after administration of DDT. Significant differences against control are marked with an asterisk +. sem = standard error of mean.

Body weight (g)

Day	N	Control	DDT acute	DDT chronic
6	2	12	11	10
12	2	22	19	20
18	2	33	43	32
26	2	44	51	48
34	2	100	93	92
50	2	185	166	173
sem		10	10	10

Testes weight (mg)

Day	N	Control	DDT acute	DDT chronic
18	4	72	78	35+
26	4	176	175	119+
34	4	486	364+	410+
50	4	1062	959+	1042
sem		500	500	500

T A B L E 2

Tubular diameter and tubular wall thickness after administration of DDT. Significant differences against control are marked with an asterisk $^{+}$. sem means standard error of mean.

Tubular diameter (µm)

Day	N	Control	DDT acute	DDT chronic
6	20	67.9	56.7^{+}	57.9^{+}
12	20	71.4	62.4^{+}	62.9^{+}
18	20	92.2	91.1	82.7^{+}
26	20	135.0	134.6	127.7^{+}
34	20	190.0	188.4	183.5
50	20	259.9	255.9	251.0
sem		41.0	41.0	41.0

Tubular wall (µm)

Day	N	Control	DDT acute	DDT chronic
6	20	0.90	0.90	0.88
12	20	0.89	0.89	0.87
18	20	0.87	0.88	0.86
26	20	0.90	0.90	0.86
34	20	0.90	0.88	0.88
50	20	0.86	0.86	0.85
sem		0.03	0.03	0.03

cross sections of the stages I - IV (LEBLOND 1952). The number of Leydig cells was counted 20 times in a defined area (marked by a photo-ocular). The relation between tubular and interstitial tissue per testis section was estimated four times by means of a modified "Treffer-Methode" (HAUG 1955). After this the number of Leydig cells was expressed as counts per mm^2 of testis section.

The last four animals of each group were mated on both the 60th and 90th day of life to healthy

females an the fetuses or implantations were counted on the 18th day of gestation.

All results were compared by the means of analysis of variance.

Results

Body weight did not change compared with controls as a result of treatment, but testicular weight was reduced (Table 1).

TABLE 3

Tubular cell counts

Significant differences against control are marked with an asterisk^{+}. sem = standard error of mean, N = 20.

Day	Treatment	Sertoli cells	A-spermatogonia	P-spermatocytes	Spermatids
	Control	32.0	1.0		
6	DDT acute	26.7^{+}	0.8^{+}		
	DDT chronic	28.4^{+}	0.9^{+}		
	Control	50.6	2.0		
12	DDT acute	44.4^{+}	2.3		
	DDT chronic	45.9^{+}	2.1		
	Control	49.9	3.2	15.5	
18	DDT acute	46.0	2.3	17.3	
	DDT chronic	47.5	2.6	10.7^{+}	
	Control	37.3	2.3	58.3	
26	DDT acute	35.9	2.4	55.7	
	DDT chronic	42.7	2.7	43.4^{+}	
	Control	31.8	3.3	78.8	98.6
34	DDT acute	29.8	3.4	63.6^{+}	101.0
	DDT chronic	30.7	3.0	72.0	84.1
	Control	26.4	2.8	64.3	156.0
50	DDT acute	24.8	3.2	67.4	155.5
	DDT chronic	25.7	2.9	66.8	147.1
sem		4.3	0.8	9.4	17.3

Tubular diameters were initially reduced but after the end of the treatment period they approached control values. Tubular wall thickness was not influenced (Table 2).

The following changes of tubular cells were found (Table 3). Statistically significant decreases on different days were found in the number of Sertoli cells and A-spermatogonias also, but to a smaller extent, in pachytene spermatocytes and spermatids.

The number of Leydig cells decreased in interstitial tissue (Fig. 1).

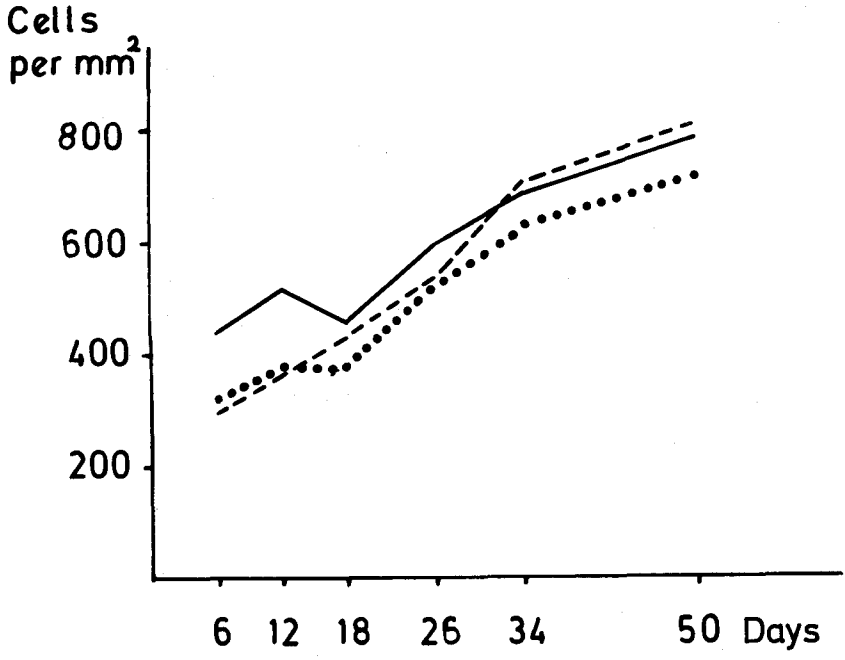

Fig. 1 : Leydig cells per mm^2 of testis section after administration of oil (——), DDT acute (----) and DDT chronic (····) treatment. Significant differences are seen on day 6 and 12 between control and the two other groups, on day 18 between control and DDT chronic. (N = 4)

The reproduction test showed considerable deviations from the controls (Table 4).

T A B L E 4

Average number (N = 4) of fetuses and implantations in healthy females mated to treated males. Significant differences against control are marked with an asterisk $^{+}$.

Day	Control	DDT acute	DDT chronic
60	1o.25	7.o^{+}	o.5^{+}
90	11.75	10.5	7.o^{+}

Discussion

Our results indicate that DDT during the period of application had a damaging effect on spermatogenesis, which in part persisted and was still observable at a later time. The action of DDT was different, however, from that of certain cytostatics (HEESE et al. 1973; HILSCHER et al. 1971; De ROOIJ et al. 1968) in that A-spermatogonia remained almost un affected. Thus, the effects seen cannot be explained by a damage of this cell type passed on to the daughter generations.

The lesions are observed at many cell types in the sequence of generations of spermatogenesis and may be due to a synchrone damage to these cells. The pattern of damage is additionally blurred by the physiological degenerations of spermatogenetic cells. An A_1-spermatogonia does not result in 128 spermatids but only in about 75. When physiological degeneration becomes less, other damages can be compensated (ROOSEN-RUNGE 1955).

Since DDT tends to cumulate and is only very slowly eliminated, it is understandable that its action

outlasts the period of application. Further, since it concentrates in lipid rich tissues, it is also stored in the testis (AN DER LAN 1969).

The decreased Leydig cell count in combination with the decreased tubular diameter indicates damage of these cells. KUPFER (1969) could demonstrate that DDT modifies the steroid metabolism of the liver by accelerating microsomal hydroxylases. Also in the reproductive organs (prostate, testes) these enzymes were influenced by DDT (SMITH et al. 1972). Apparently an interaction with zinc plays a role here (FEASTER et al. 1972).

Furthermore, DDT appears to have an anti-gonadotrophic action. This was demonstrated by GELLERT and SWERDLOFF (1972). It is estrogen-like and in female animals induces a decrease of both serum LH and FSH. The latter could possibly explain the reduction of some spermatogenetic cells. LH decrease would partly explain the decrease of Leydig cells in our experiments.

Refering to these mechanisms, it seems to be evident that the disturbance of the spermatogenesis is due to a lack of local testosterone.

In conclusion it should be noted that the dosage of DDT we selected is very high, and is hardly to be expected in nature. This does not, however, lessen a potential danger of this substance to the propagation of species.

Summary

Juvenile male rats of Wistar/Han strain were fed DDT suspended in olive oil. They received either 5oo mg/kg at the 4th and 5th or 2oo mg/kg daily at the 4th till 23rd day of life. After certain inter-

vals the testes were examined histologically, and at the 6oth and 90th day of life fertility was proved. The spermatogenetic cells showed an increase of the physiological degeneration in the course of spermatogenesis and a decrease of their total number. The number of Leydig cells was diminished. In parallel, the litters of normal female rats mated to treated males were smaller than those of controls. The hypothesis is suggested that the damage of the seminiferous epithelium can be explained by a lack of local testosterone.

References

AN DER LAN, H., Zbltt. Bakt. I. Abt. 210:234(1969)

BRYSON, M., DRAPER, Ci, HARRIS, J.R., BIDDULPH, C. Adv. Chem. Ser. I, 232 (1950)

BURLINGTON, H. and V.F.LINDMANN, Proc. Soc. Exp. Biol. Med. 74: 48 (1950)

COTTRELL, T.L.C. and N.J.HECKEL, J.Urol. 81:551 (1959)

DEICHMANN, W. and M.C.KEPLINGER, 5th Ann. Meet. Soc. Toxicol. Virginia 1966

DEICHMANN, W., MacDONALD, W.E., BEASLEY, A.G. and D. CUBIT, Ind, J. Med. 40:10 (1971)

FEASTER, I.P. and D. STEVENS, J. Nutri. 1o2:523 (1972)

GELLERT, D.J., HEINRICHS, W.L. and D.S.SWERDLOFF, Endocrin. 91: 1095 (1972)

HAUG, H., Zschr. Anat. Entw. Gesch. 118:302 (1955)

HEESE, K., STRÖDTER, D. und D. SCHRECK, Inaug. Diss. Gießen 1973

HILSCHER, W. and B. HILSCHER, Morph. Asp. Androl. 1:17 (1971)

KUPFER, D., Ann.N.Y.Acad.Sci. 160:244 (1969)

LEBLOND, C.P. and Y. CLERMONT, Am. J. Anat. 90: 167 (1952)
De ROOIJ, D.G. and M.F.KRAMER, Zschr. Zellforsch. 92: 400 (1968)
ROOSEN-RUNGE, E. and D. ANDERSON, Zschr.Zellforsh. 41:221 (1955)
SMITH, J. and F. THOMAS, Toxicol. Appl. Pharm. 23: 159 (1972)