Histol Histopath (1991) 6: 459-467

Histology and Histopathology

# Morphological and histochemical pattern of response in rat testes after administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

**G.M. Rune[1], Ph. de Souza[2], R. Krowke[2], H.J. Merker[1,2] and D. Neubert[2]**

[1]Department of Anatomy and [2]Department of Toxicology and Prenatal Pharmacology, Free University of Berlin, FRG

**Summary.** Testes of rats, which had been injected with a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (0.3 µg/kg - 25 µg/kg body weight [BW]), were studied after 7 days using morphological and histochemical means.

Light and electron microscopic examination revealed that TCDD affected testicular morphology in a dose-dependent manner. TCDD led to decreased intercellular contact, indicated by wide intercellular spaces between Sertoli cells between and Sertoli cells and neighbouring germ cells. Morphological alaterations in rat testes after TCDD administration included the sloughing off of premature spermatids into the tubular lumen and numerical increase of necrotic germ cells, in particular pachytene spermatocytes. Compared with control animals, Sertoli cells of treated rats exhibited an increased amount of lipid droplets and phagolysosomes. Vacuolization of the cytoplasm and fragmentation of the Sertoli cells occurred frequently. Examination of the different spermatogenic stages revealed that no stage was specifically susceptible to TCDD.

In Leydig cells a decrease in enzyme activity of 3ß- and 17ß-hydroxysteroid dehydrogenases became evident by histochemical investigation. This effect on steroidogenesis was already found at a dose of 1 µg/kg BW TCDD, whereas morphological effects were seen in the germinal epithelium for the first time at 3 µg/kg BW.

**Key words:** 2,3,7,8-tetrachlorodibenzo-p-dioxin, Rat, Testis

## Introduction

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent toxicant which is generated as an undesired by-product in the manufacturing of the phenoxyherbicides. Among the great variety of toxic effects which have been described (Poland and Knutson, 1982), it has been reported that TCDD induces endocrine changes (Mebus et al., 1987; Astroff and Safe, 1988; Umbreit et al., 1988) and in particular affects male reproductive fertility (Chahoud et al., 1989). Decreases in the amount of testicular microsomal cytochrome P450 and in serum testosterone (Tofilon and Piper, 1982; Moore et al., 1985) have been found after exposure of rats to TCDD, and it could be demonstrated (Mebus et al., 1987) that decreased testosterone production is related to decreased activity of microsomal cytochrome P-450 dependent enzyme 17-hydroxylase and 17,20-lyase.

Morphological data, available in this context, include testicular hypoplasia (Allen and Lalich, 1962) in chickens and a decreased number of primary and secondary spermatocytes and spermatids in chickens, monkeys and rats, whereas Leydig cells and Sertoli cells appeared unaffected (Norback and Allen, 1973).

Ultrastructural studies, which describe precisely the effects of TCDD at various doses during the seminiferous cycle of the rat are so far missing as are histochemical investigations, which could deliver more insight into the endocrine function of testis after TCDD exposure. For this reason we investigated the activity of enzymes, which are related to steroidogenesis (3ß- and 17ß-hydroxysteroid dehydrogenase) besides the morphological lesions due to TCDD.

In order to detect a broad spectrum of effects for this basic study, we decided to investigate animals at lower doses (e.g. 0.3 µg/kg BW TCDD) up to a dose of 25 µg/kg BW TCDD. Furthermore, we investigated rats after a single dose, since it is known that chronic application leads to decreased food consumption and body weights. Thus, functional suppression of reproductive organs could be due to an indirect toxic effect associated with the poor physical condition of the animals, rather than being due to specific toxicity to the reproductive tract (Kociba et al., 1976).

*Offprint requests to:* PD Dr. Gabriele M. Rune, Institut für Anatomie, FU Berlin, Königin-Luise-Str. 15, D-1000 Berlin 33, FRG

## Materials and methods

### *Animal maintenance*

Wistar rats (Bor:spf, TNO; Winckelman, Borchen, FRG; 300-350 g) were kept at a constant day/night cycle (light from 9:00 a.m. to 9.00 p.m.), at a room temperature of 21 ± 1° C and 50 ± 5% relative humidity. They received a standard pellet (Altromin $^{R}$ 1324) and tap water ad libitum. The animals were adapted to the conditions of our animal quarters for 3 weeks before starting the experiment. They were kept singly in Macrolon$^{R}$ type 3 cages.

### *Treatment*

TCDD was given subcutaneously (into the back) to assure absorption and to minimize contamination of the cages and exposure of the personnel. For injection the substance was dissolved in DMSO and diluted with castor oil to obtain a mixture of 1 (DMSO) plus 3 (castor oil). From this solution a volume of 1 ml/kg body weight was applied using a Hamilton microsyringe.

$^{14}$C-TCDD was used for all studies in order to check possible contamination of the surrounding. The substance was purchased from Cambridge Isotope Lab. (Mass, USA). It had an indicated activity of 33 mCi ($1.22 \times 10^{9}$ Bq)/mmol. It was dissolved in DMSO and kept in the dark. $^{14}$C-TCDD was diluted with cold TCDD to a specific activity of 3.9 mCi ($1.44 \times 10^{8}$ Bq) mmol (100 dpm = 3.73 ng TCDD).

Twenty-eight rats were treated with TCDD at doses of either 300, 1000, 3000, 5000, 8000, 10000, or 25000 ng/kg BW TCDD. For each dose two non-treated animals and two, injected with the vehicle, were used as control.

All animals were injected for changes in appearance at least twice a day and sacrificed after 7 days by cervical dislocation. Testes were weighed, cut into small pieces and specimens were immediately fixed according to Karnovsky (1967) or frozen in liquid nitrogen.

### *Morphology*

The fixed specimens were then thoroughly washed in cacodylate buffer (0.1 M, pH 7.4) and postfixed for 1 h in cacodylate-buffered (0.1 M) 1% $O_SO_4$ at pH 7.4. After dehydration in acetone they were embedded in Epon 812. Sections were cut on ultrathin sectioning microtome (Reichert-Jung, OmU 3, Vienna, Austria).

Contrast of the ultrathin sections was enhanced with uranyl acetate followed by lead citrate (Reynolds, 1963). The sections were then examined on a Zeiss electron microscope (EM 109, EM 10, Oberkochen, FRG). «Thick» sections (1 µm) were mounted on slides and stained with methylene blue/azure II according to Richardson et al. (1960).

### *Histochemistry*

Frozen testes were cut on a cryostat (Reichert-Jung, Frigocut 2800, Vienna, Austria) at -20° C. The sections (8 µm) were fixed in acetone at -25° C for 5 min and then incubated for 3ß- and 17ß-hydroxysteroid dehydrogenase as described by Bergmann (1987) and Haider (1988) with dehydroepiandrosterone or androstendione respectively as substrate. Controls were performed by omitting the substrate. The sections were finally mounted in glycerine jelly.

## Results

None of the animals died during the week after administration not even at a dose of 25 µ/kg BW TCDD. However, these animals appeared inactive and lost weight in contrast to animals having received lower doses: 300 ng-10 µg/kg BW TCDD. The average body weight of the controls, vehicle-treated, and experimental groups after lower doses (300 ng-10 µg/kg BW TCDD) increased up to 106% of the initial value with no significant difference between the three groups. A decrease in body weight (average 5% when compared with controls) could already be observed after one week in animals of the 25 µg/kg BW TCDD group. No weight loss of the testes under TCDD influence could be found. The absolute weights were no lower in treated rats when compared with controls. In contrast, at high doses (25 µg/kg) the relative weight of the testes increased due to the loss of body weight.

### *Morphology*

Except for animals which had been treated with less than 3 µg/kg BW TCDD, with all other doses morphological alterations could already be seen at the light microscopic level. The qualitative character of the effects was similar at all doses equal or less than 3 µg/kg BW TCDD. However, the frequency of occurrence was clearly dose-dependent. All developmental stages were affected. However, at the highest dose (25 µg/kg BW TCDD) a small proportion of tubules could not be determined, because only few cells were still lining the basement membrane, whereas most of the cells had floated into the tubular lumen.

Extensive descriptions of normal rat spermatogenesis are given by Leblond and Clermont (1952) and Bröckelmann (1963). In our investigation the morphology of non-treated and vehicle-injected animals and those which had been injected with an equal or lower dose than 3 µg/kg BW corresponded to their findings.

#### Light microscopy

Generally, TCDD led to a dissolution of the germinal epithelium, indicated by wide gaps between

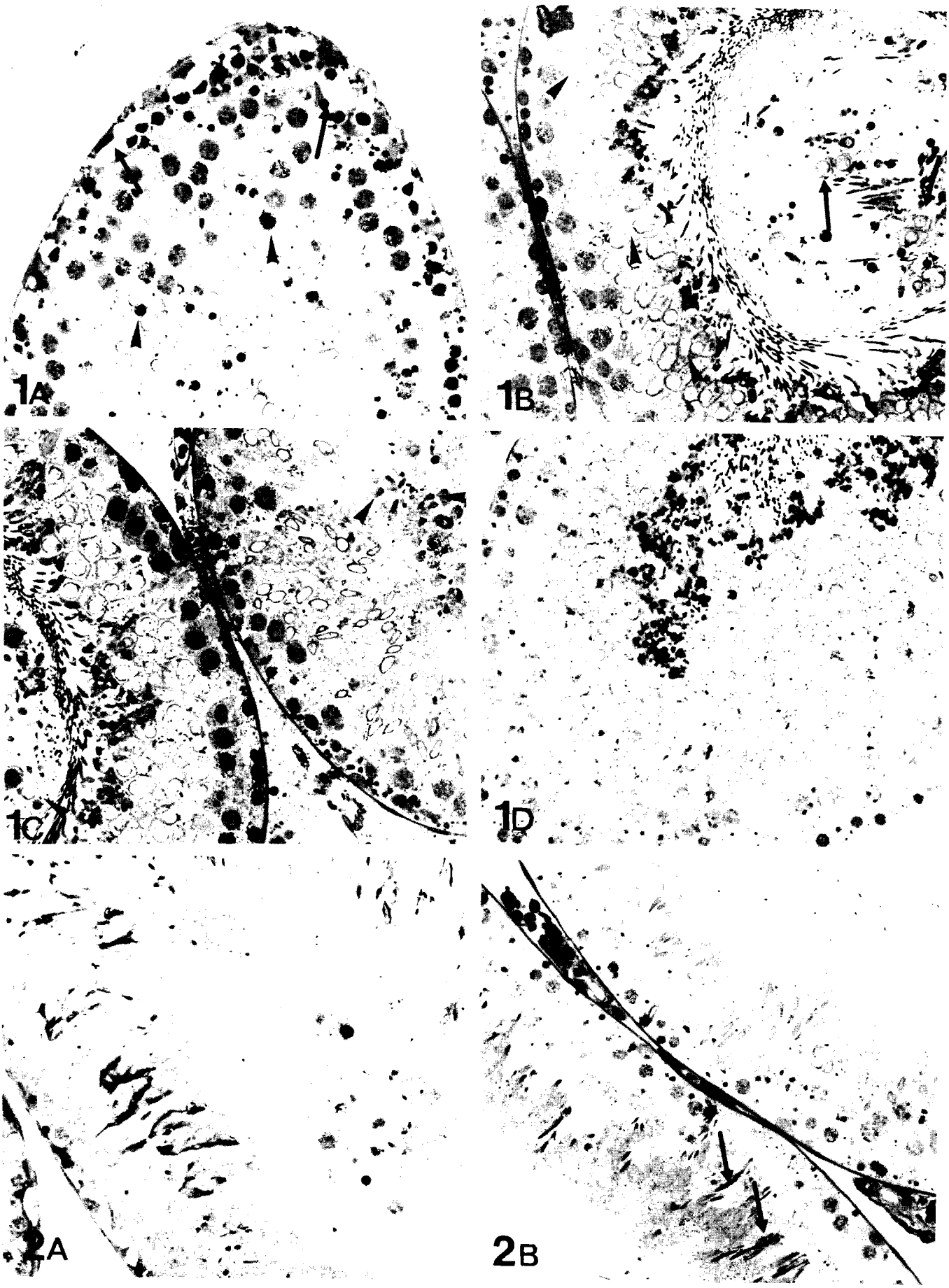



**Fig. 1.** Semithin sections. **A.** 10 μg/kg BW TCDD. Typical disruptions of the germinal epithelium are seen in the basic compartment of the seminiferous epithelium (arrows). Degenerated germ cells are frequent (arrowheads). × 350. **B.** 25 μg/kg BW TCDD. At this dose intercellular spaces are not only seen in the basic but also in the adluminal compartment (arrowheads). Premature spermatids are perceptible in the tubular lumen (arrow). × 370. **C.** 10 μg/kg BW TCDD. Tubular stage VII (left) and XI (right). Premature spermatids are found in the tubular lumen at both stages (arrowheads). The germinal epithelium dissolves particularly in the basic compartment. × 350. **D**: Control. Stage VII of the seminiferous cycle. Cells have close contact with each other. × 350

**Fig. 2.** Semithin sections. **A** 25 μg/kg BW TCDD. Clumping of elongated spermatids are frequently observed in stage XIII. × 350. **B.** Control. Semithin section. Stage XI (above) and XIII (below). Elongated spermatids form bundles between the older spermatocytes (arrows). × 350

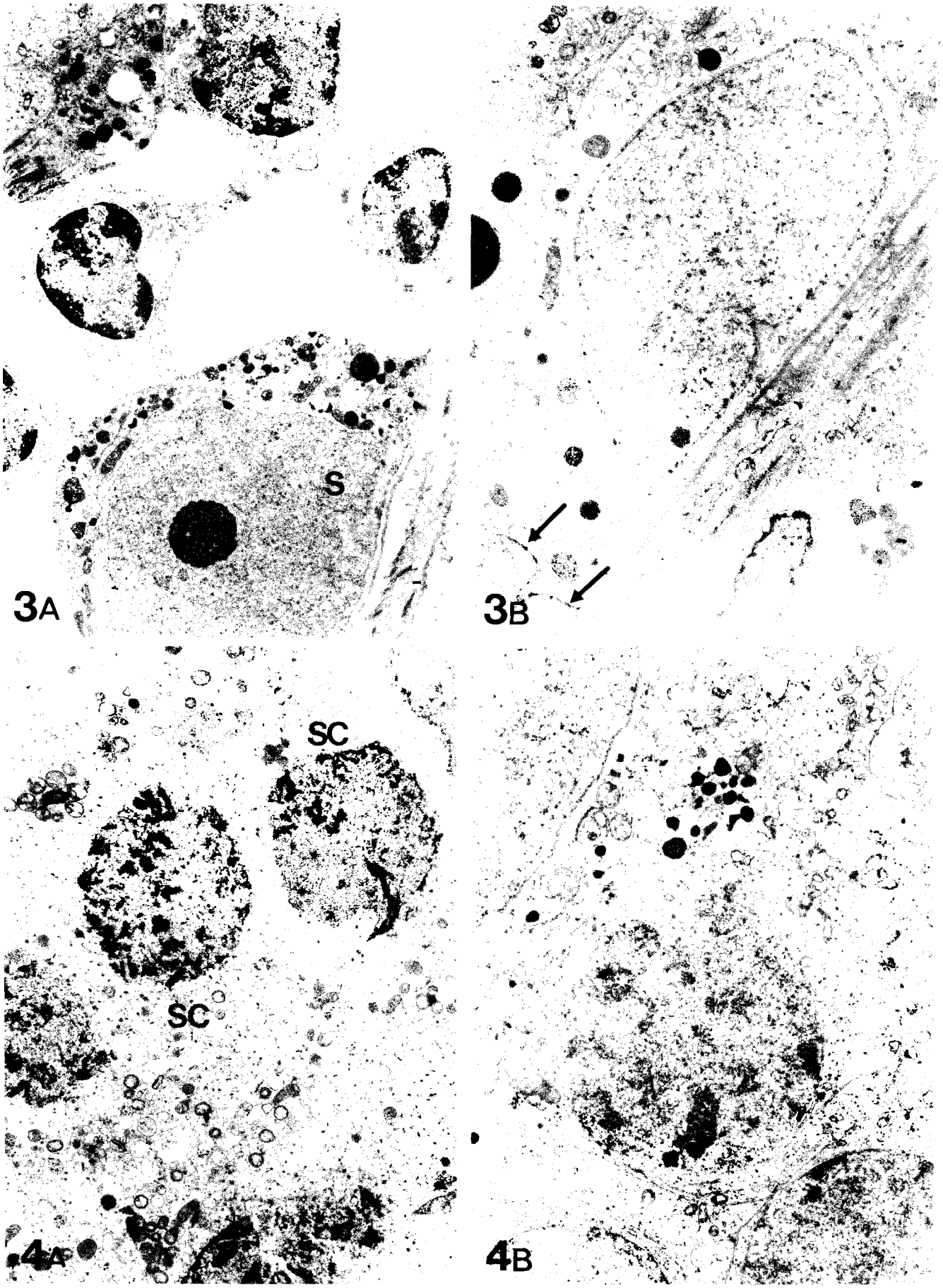
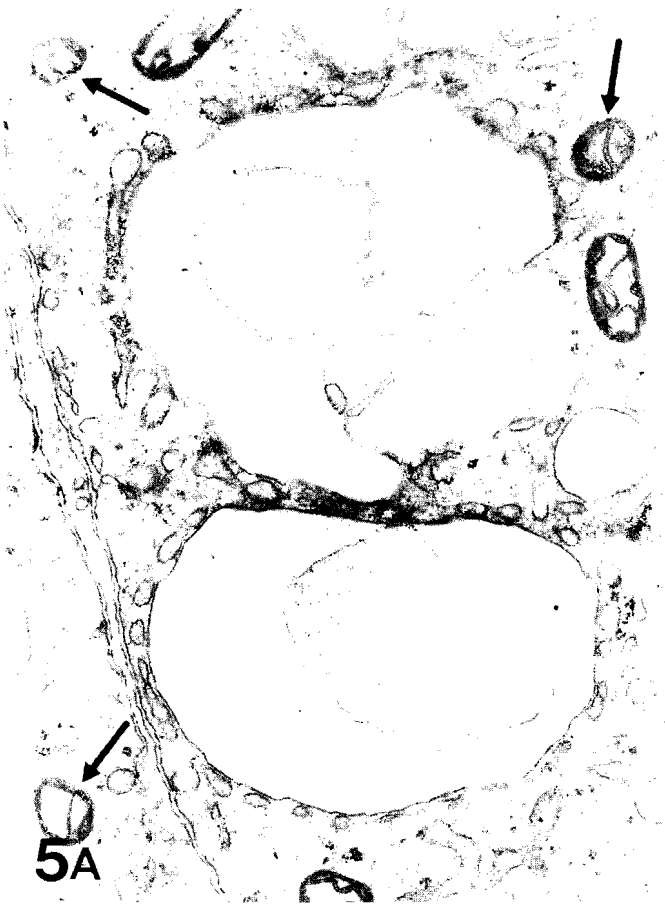
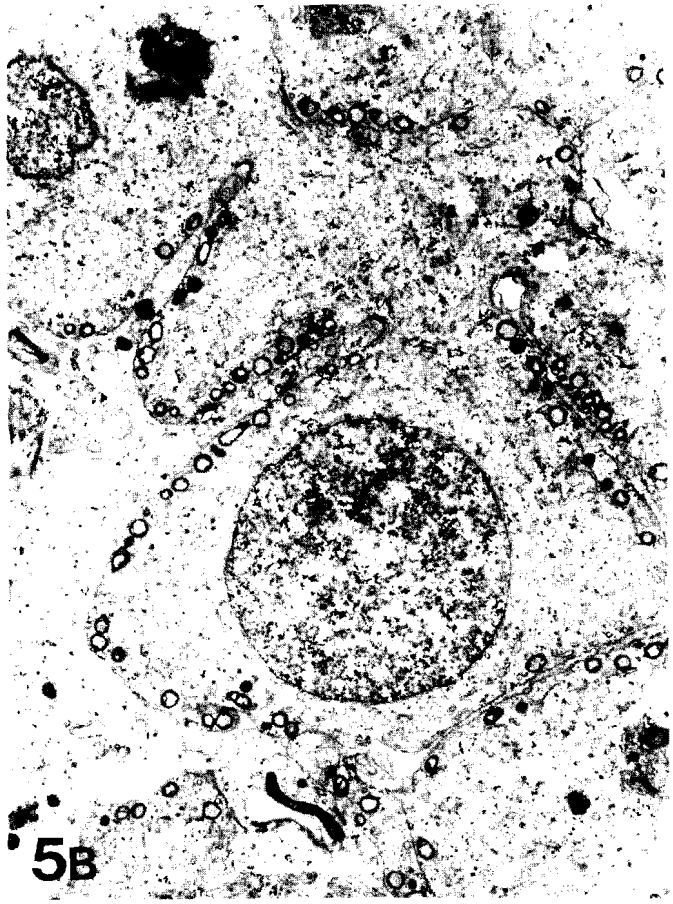
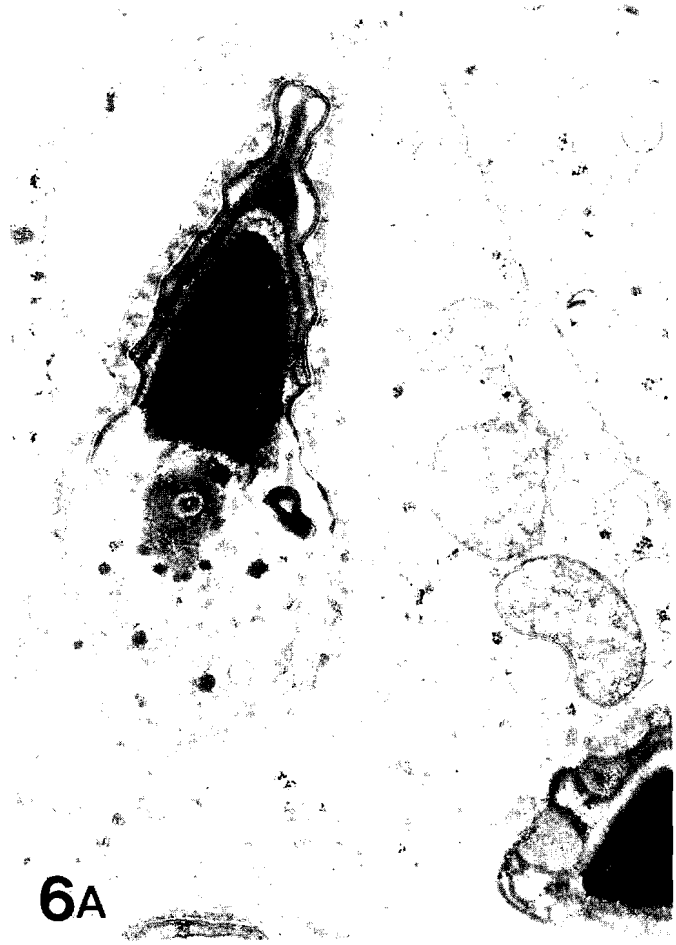



**Fig. 3.** EM. **A.** 5 μg/kg BW TCDD. Wide intercellular spaces between germ and Sertoli cells (S). × 6,500. **B.** Control. Close contact between Sertoli cells and germ cells is common under physiological conditions. Note: tight junctions between Sertoli cells (arrow) × 7,000

**Fig. 4.** EM. **A.** 10μg/kg BW TCDD. Degenerating pachytene spermatopcytes (SC) with an irregularly-shaped nucleus and clumped chromatin. × 9,000. **B.** Control. Intact pachytene spermatocyte. × 8,000



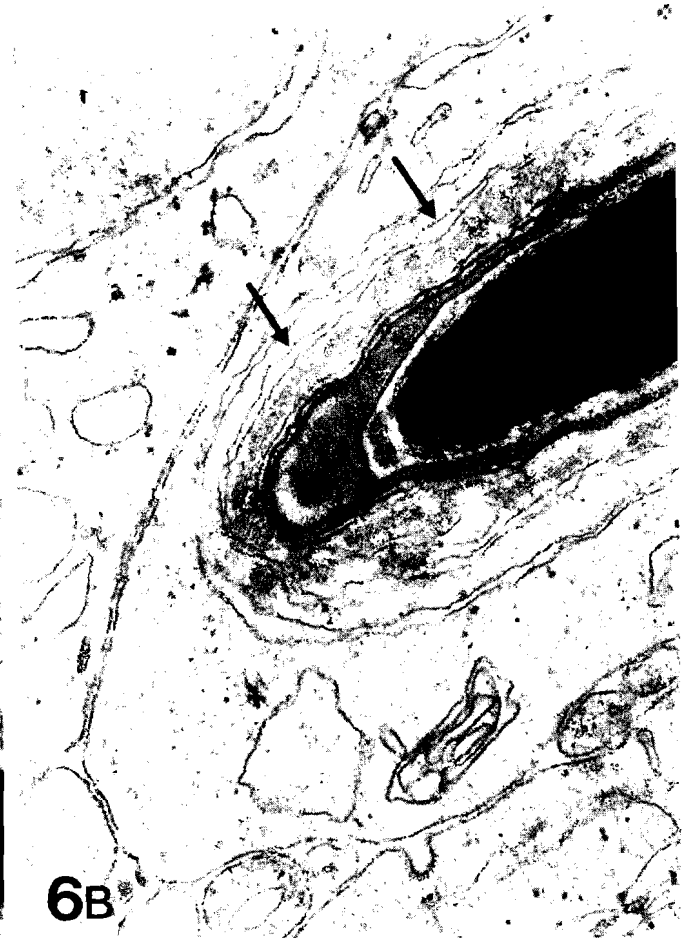




**Fig. 5.** EM. **A.** 5 μg/kg BW TCDD. Vacuoles in the cytoplasm of degenerating spermatids; note typical marginated mitochondria (arrow). × 18,000. **B.** Control. Round spermatid with the typical peripheral location of mitochondria. × 6,000

**Fig. 6.** EM. **A.** 5 μg/kg BW TCDD. After TCDD treatment a vacuole in Sertoli cell cytoplasm often appears around the sperm head instead of ectoplasmic specializations. × 26,000. **B.** Control. Typical ectoplasmic specializations (arrows) surrounding the developing sperm head. × 30,000

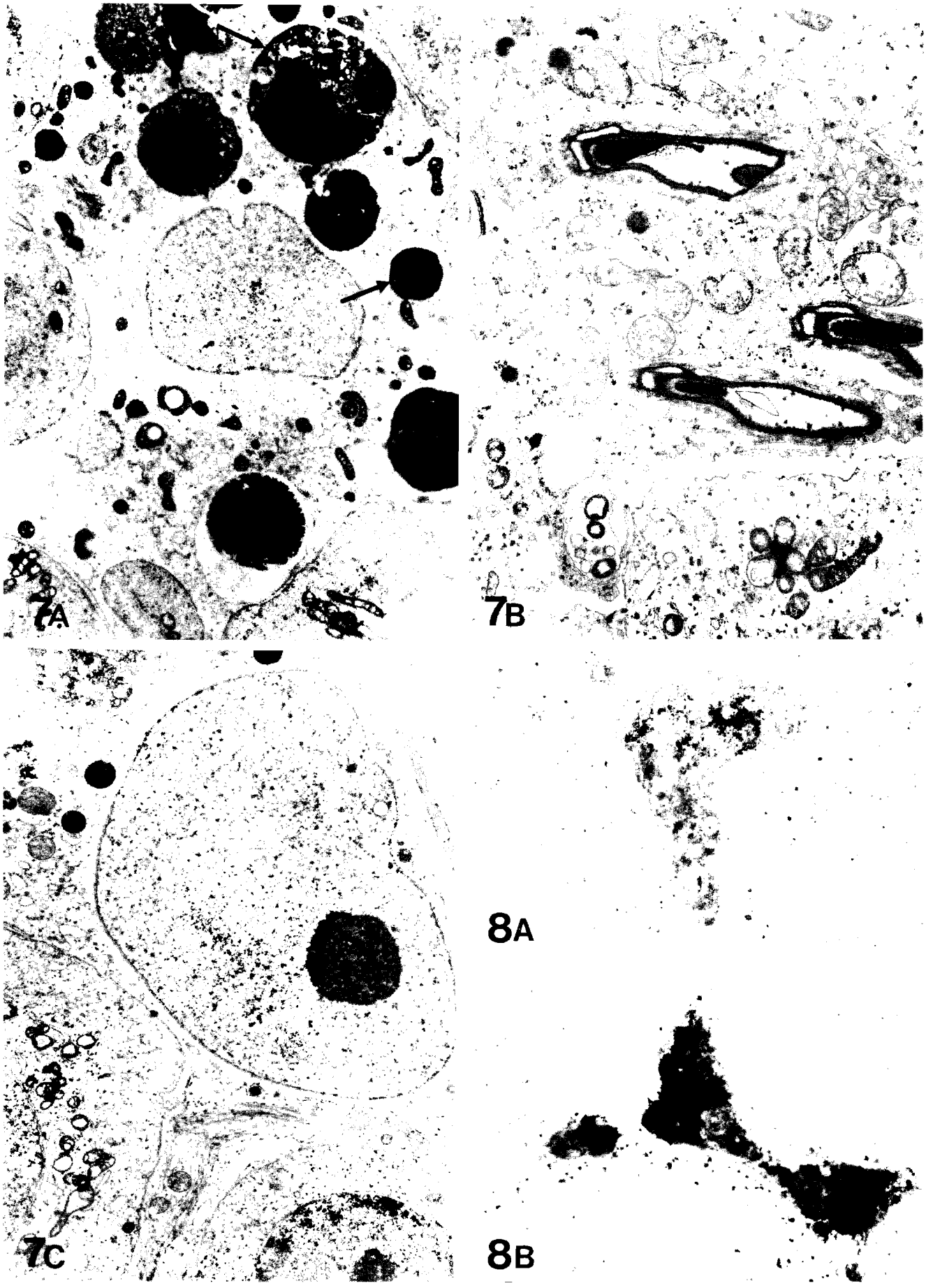



**Fig. 7.** EM. **A.** 10 μg/kg BW TCDD. Sertoli cell cytoplasm is filled with large phagolysosomes and lipid droplets (arrows). × 4,500. **B.** 25 μg/kg BW TCDD. Altered Sertoli cell with cytoplasmic vacuoles and disintegrated mitochondria. × 14,000. **C.** Control. Intact Sertoli cell with smooth endoplasmic reticulum and few lipid droplets. × 8,000

**Fig. 8.** 3ß-HSD. **A.** 1000 ng/kg BW TCDD. Activity has considerably decreased in comparison with the control testis. × 350. **B.** Control. Activity is clearly seen in Leydig cells. × 350

neighbouring cells and an enlargement of the intercellular spaces. The number and size of these spaces, which resulted in loosened intercellular contact, was clearly dose-dependent and was predominantly observed in the basic compartment of the seminiferous tubule. This compartmentalisation is based upon the existence of tight junctions between adjacent Sertoli cells, which prevent the diffusion of blood-borne substances into the lumen of the tubules (for details see: Waites and Gladwell, 1982). Thus, only cells having contact to the basal lamina (i.e. spermatogonia and preleptotene spermatocytes) are often completely isolated from neighbouring germ cells. However, at high doses wide intercellular spaces could also be seen in the adluminal compartment.

The sloughing off of premature spermatids into the tubular lumen was a common feature in all rats which had been injected with 3 µg/kg BW TCDD or more, but was most pronounced in animals which had received 25 µg/kg BW TCDD. Round spermatids in cap phase and those which had started to elongate could be observed in the tubular lumen. Round spermatids appeared in the tubular lumen preferentially at stages V and VI, whereas those which had started to elongate were visible in the tubular lumen at stages IX and X. In comparison with control testes, TCDD led to an increase of damaged germ cells. Necrotic germ cells occurred with highest frequency in tubules at stages V, VI and VII (Fig. 1).

Under physiological conditions stages XI, XII, and XIII were characterized by the fact that spermatids formed bundles between the older spermatocytes, the tip of the bundle being directed towards the Sertoli cell nucleus. Under TCDD influence it appeared that single spermatids could no longer be recognized, only clumps of elongated spermatids being seen (Fig. 2).

Electron microscopy

Electron microscopic inspection again showed the wide intercellular spaces between Sertoli cells and Sertoli cells and germ cells (Fig. 3). Consequently, tight junctions between adjacent Sertoli cells were rarely found after TCDD treatment.

Altered germ cells (Figs. 4, 5), which were already perceptible with light microscopic means, were a common phenomenon at all developmental stages, but appeared particularly at stages V-VII. Damaged spermatogonia were rare and mostly concerned spermatogonia type A. Irregularly-shaped vacuoles appeared in the cytoplasm before the nucleus became irregularly formed and finally pyknotic. Within the group of spermatocytes, pachytene spermatocytes were primarily affected. The nucleus of these cells was irregularly shaped instead of being round, and showed accumulations of clumped chromatin. The cytoplasmic matrix exhibited accumulations of dense granular masses, myelin figures, and clumped groups of disintegrated organelles. Affected round spermatids (preferentially step III to VII) frequently showed large vacuoles in the cytoplasm, sometimes filled with membranous material, which occupied the whole cytoplasm.

Besides the sloughing off of premature spermatids into the tubular lumen, which was already perceptible with light microscopic means, elongated spermatids often appeared deformed under TCDD influence, either the characteristic arrangement of the middle piece was not seen or the acrosome was surrounded by a single large vacuole of the Sertoli cell instead of the numerous ectoplasmic specializations of Sertoli cells that are typical for this developmental stage (Fig. 6).

In additon to the rare occurrence of tight junctions, as a consequence of the disruptions in intercellular contact, Sertoli cells underwent further morphological alterations (Fig. 7). Under TCDD influence Sertoli cells exhibited a dose-dependent increase in phagocytic activity. Multiple forms of phagolysosomes, sometimes of striking size, could be observed. Moreover, the number of lipid droplets had increased in Sertoli cells. The increase in lipid content was also often seen in Sertoli cells of spermatogenic stages, where lipid content is normally low (Kerr et al., 1984). Other Sertoli cells had become extremely vacuolated. The occurrence of these cytoplasmic alterations was not specifically related to any tubular stage. This process of alteration finally resulted in a total disintegration of the Sertoli cells, where only cellular fragments with damaged cell organelles were perceptible.

Most of the Leydig cells showed less smooth endoplasmic reticulum than in Leydig cells of non-treated animals and frequently exhibited mitochondria which were devoid of tubules.

*Histochemistry*

Activity of 3ß- and 17ß-hydroxysteroid dehydrogenase (HSD), which was found in Leydig cells, had clearly decreased dose-dependently in animals which had been treated with 1000 ng/kg - 25 µg/kg BW TCDD (Fig. 8). At a dose of 25 µg/kg BW TCDD activity was no longer found. The sections were comparable with those wich had been treated without substrate. No difference in the amount of dye compared with the control testes could be observed at a dose of 300 ng/kg BW TCDD.

## Discussion

The morphological and histochemical data clearly demonstrate that the environmental toxicant TCDD is able to induce morphological lesions and effects on steroidogenesis in rat testes.

Morphological lesions and histochemical changes were observable after doses by which the animals were in a normal physical condition as judged by their general appearance and no testicular hypoplasia was found. The pattern of morphological alterations was principally identical at all doses investigated after one week of exposure. This result may indicate that the

described morphological and histochemical effects are probably due to direct effects of TCDD on the testis.

Leydig cells are the cellular source for androgen i.e. testosterone, which is necessary for the regulation of spermatogenesis in mammals. Effects on Leydig cells due to TCDD that are indicated histochemically by decreased activity of 3ß- and 17ß- HSD corresponded to decreased serum testosterone levels in rats, which was described by Moore et al. (1985). Consequently it has to be asked whether the decline of testosterone is due to decreased levels of gonadotropins as regulatory hypophyseal proteohormones or whether the toxicity of TCDD can be regarded as a form of hypophysectomy. However, the morphological pattern of the tubular lesions after hypophysectomy is different from that found after TCDD treatment. After hypophysectomy Russell and Clermont (1977) found degenerated mid-pachytene spermatocytes and spermatids at stage VII and degenerated spermatogonia type A at stages XI to I, indicating that morphological effects after hypophysectomy were stage-dependent. Similar results were reported when the hormonal axis was interrupted by the application of clomiphene citrate, cyproterone acetate, and estradiol (Flickinger, 1977, Dym and Madhaw Ray, 1977; Russell et al., 1981). These results led to the conclusion by Russell et al. (1981) that there is a uniform change in the morphology of the testis after interruption of the hormonal axis. The pattern of degeneration after TCDD-treatment does not correspond to that described above and was not specifically stage-related. Therefore it is not likely that the response of rat testis after TCDD treatment is solely due to effects on FSH/LH metabolism or release (Nistal et al., 1983; Schulze, 1984; Viehberger et al., 1984; Bergmann, 1987). In contrast, it appears that TCDD acts directly on Leydig cells and that decreased testosterone levels are due to decreased activities of the testicular microsomal cytochrome P-450 dependent enzymes 17-hydroxylase and 17,20 lyase in the study of Mebus et al. (1985) and 3ß- and 17ß-HSD in our investigation.

Sertoli cells showed marked morphological alterations, particularly at a dose of 25 µg/kg BW TCDD, suggesting profound functional changes after TCDD treament. These morphological alterations were associated with an accumulation of lipid droplets and phagolysosomes. An increase in lipid and phagolysosome content has been frequently described during tubular degeneration by different causes (de Krester et al., 1981; Bergh, 1983; Schulze, 1984). In our investigation it seems probable that the increase in lipids is derived mostly from phagocytosed degenerative germ cells, which frequently occurred after TCDD exposure. Such a mechanism has already been proposed by Schulze (1984) to appear in man.

Furthermore, we found in our investigation disruptions of the seminiferous epithelium which at low doses appeared predominantly in the basic compartment but which were also seen in the adluminal compartment at the high dose of 25 µ/kg BW TCDD. This result corresponds to the finding that a decreased number of tight junctions could be observed under TCDD influence. Under physiological conditions these tight junctions interconnect Sertoli cells only in that area of the germinal epithelium where we found wide intercellular spaces. From this it may be suggested that TCDD, presumably in a dose-dependent manner, affects the blood-testis barrier. The disappearance of tight junctions leading to a break-down of the barrier seems only to be possible when the Sertoli cells have reached an immature or inactive stage (Bergmann, 1987). Thus, it appears from the decreasing number of tight junctions and the occurrence of obviously damaged Sertoli cells that these cells are target cells for TCDD.

By the sloughing off of spermatids into the tubular lumen it appears likely that not only the interaction between Sertoli cells but also the one between Sertoli cells and germ cells may be disturbed under the influence of TCDD. This hypothesis is supported by the fact that the typical ectoplasmic specializations of Sertoli cells surrounding maturing spermatids (Russell, 1980) were rarely perceptible after TCDD injection, but sperm heads were instead often surrounded by a single large Sertoli cell vacuole, which probably resulted from the confluence of these ectoplasmic specializations. As shown in man (Nistal et al., 1984; Nistal and Paniagua, 1984) and in *Phodopus sungorus* (Bergmann, 1987) the disruptions of ectoplasmic specializations between Sertoli and germ cells induce the sloughing off of premature spermatids into the tubular lumen.

It may be concluded that the impairment of testicular function in rats after TCDD exposure is not only due to effects on the steroidogenic capacity of Leydig cells but also to effects on Sertoli cells and germ cells. The question whether the degeneration of germ cells is due to or preceedes changes in Sertoli cell morphology is open at present. An answer requires investigations where degeneration of germ and Sertoli cells are studied at a defined dose of TCDD but after different time intervals. This study is already in progress in our laboratory.

*Acknowledgements.* This investigation was supported by the Deutsche Forschungsgemeinschaft (Sfb 174)

## References

Allen J.R. and Lalich J.J. (1962). The effects of «toxic fat» on spermatogenesis. Proc. Soc. Exp. Biol. 109, 48-52.

Astroff B. and Safe S. (1988). Comparative antiestrogenic activities of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 6 methyl-1,3,8-thichlorobenzofuran in the female rat. Toxicol. Appl. Pharmacol. 95, 435-443.

Bergh A. (1983). Early morphological changes in the abdominal testes in immature unilaterally cryptorchid rats. Int. J. Androl. 6, 73-90.

Bergmann M. (1987). Photoperiod and testicular function in *Phodopus sungorus*. In: Advances in Anatomy, Embryology and Cell Biology. Vol. 105. Beck E., Hild W., Kriz W., Ortmann R., Pauly J.E. and Schiebler T.H. (eds). Springer-Verlag. Heidelberg-Berlin. New York.

Bröckelmann J. (1963). Fine structure of germ cells and Sertoli cells during the cycle of the seminiferous epithelium of the rat. Z. Zellforsch. 59, 820-850.

Chahoud I., Krowke R., Schimmel A., Merker H.-J. and Neubert D. (1989). Reproductive toxicity and pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Effects of high doses on the fertility of male rats. Arch. Toxicol. 63, 432-439.

de Krester D.M., Kerr J.P. and Paulsen C.A. (1981). Evaluation of the ultrastructural changes in the human Sertoli cell in testicular disorders and the relationship of the changes to the levels of serum FSH. Int. J. Androl. 4, 129-144.

Dym M. and Madhaw Ray H.G. (1977). Response of adult rat Sertoli cells and Leydig cells to depletion of luteinizing hormone and testosterone. Biol. Reprod. 17, 676-696.

Flickinger C.J. (1977). Effects of clomiphene on the structure of the testis, epididymis and sex accessory glands of the rat. Am. J. Anat. 149, 533-562.

Karnovsky M.J. (1967). A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 35, 213.

Kerr J.B., Mayberry R.A. and Irby D.C. (1984). Morphometric studies on lipid inclusions in Sertoli cells during the spermatogenic cycle in the rat. Cell Tissue Res. 236, 699-709.

Kociba R.J., Keeler P.A., Park C.N. and Gehring P.J. (1976). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): results of a 13 week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35, 553-574.

Leblond C.P. and Clermont J. (1952). Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann. N.Y. Acad. Sci. 55, 548-557.

Mebus C.A, Vijayapal R.R. and Walter N.P. (1987). Depression of rat testicular 17-hydroxylase and 17,20-lyase after administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem. Pharmacol. 36, 727-731.

Moore R.W., Potter C.L., Theobald H.M., Robinson T.A. and Peterson R.E. (1985). Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 79, 99-111.

Nistal M., Paniagua R. and Picazo M.L. (1983). Testicular and epididymal involvement in Fabry's disease. J. Pathol. 141, 113-124.

Nistal M., Paniagua R., Regadera J. and Santamaría L. (1984). Obstruction of the tubuli recti and ductuli efferentes by dilated veins in the testes of men with varicocele and its possible role in causing atrophy of the seminiferous tubules. Int. J. Androl. 7, 309-323.

Nistal M. and Paniagua R. (1984). Infertility in adult males with retractile testes. Fertil. Steril. 41, 395-403.

Norback D.H. and Allen H.R. (1973). Biological response of the non-human primate, chicken and rat to chlorinated dibenzo-p-dioxin ingestion. Environ. Health Perspect 5, 233-240.

Poland A. and Knutson T.C. (1982). 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. 22, 517-554.

Reynolds S.E. (1963). The use of lead citrate as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208-212.

Richardson U.E., Jarrett L. and Finke E.H . (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 335, 313-323.

Russell L.D. and Clermont G. (1977). Degeneration of germ cells in normal hypophysectomized and hormone treated rats. Anat. Rec. 187, 3347-3366.

Russell L.D. (1980). Sertoli-germ cell interactions: a review. Gamete Res. 3, 179-202.

Russell L.D., Maloje J.P. and Karpas S.L. (1981). Morphological pattern elicited by agents affecting spermatogenesis by disrupting of its hormonal stimulation. Tissue Cell 13, 3369-3380.

Schulze C. (1984). Sertoli cells and Leydig cells in man. In: Advances in Anatomy, Embryology and Cell Biology. Vol. 88. Beck E., Hild W., Ortmann R., Pauly J.E. and Schiebler T.H. (eds). Springer Verlag. Heidelberg-Berlin-New York.

Tofilon P.J. and Piper W.N. (1982). Inhibition of rat testicular heme synthesis and depression of microsomal cytochrome P-450 by estradiol benzoate. Biochem. Pharmacol. 331, 3667-3671.

Umbreit T.H., Hesse E.J., Macdonald G.J. and Gallo M.A. (1988). Effects of TCDD- estradid interactions in three strains of mice. Toxicol. Lett. 40, 1-9.

Viehberger G., Lunglmayr G. and Kratzik C. (1984). Lysosomale Aktivität der Sertoli Zelle bei idiopathischen Hodendysfunktionen. Z. Fertilitätsforch. 12, 193-199.

Waites G.M.H. and Gladwell R.T. (1982). Physiological significance of fluid secretion in the testis and blood testis barrier. Physiol. Rev. 62, 624-671.

Accepted April 3, 1991