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REPRODUCTIVE TOXICITY OF 2,4-TOLUENEDIAMINE IN THE RAT. 3. EFFECTS ON ANDROGEN-BINDING PROTEIN LEVELS, SELECTED SEMINIFEROUS TUBULE CHARACTERISTICS, AND SPERMATOGENESIS

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In previous studies we demonstrated reduced fertility, arrested spermatogenesis, and diminished circulating testosterone levels in rats fed 0.03% 2,4-toluenediamine (TDA) for 10 wk. These studies were extended in three experiments by determining TDA effects on androgen-binding protein (rABP) production and on seminiferous tubule

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structure, and on early changes in testes morphology and spermatogenesis. In the first experiment, rats fed 0.03% TDA for 10 wk showed a 7- to 9-fold increase in rABP content in testicular cytosol or in media of cultured seminiferous tubules, a 4-fold increase in serum rABP, but a two-thirds decrease in epididymal rABP levels. Testes examination by transmission electron microscopy revealed degenerative changes in Sertoli cells with, where present, normal spermatocytes and spermatids. In the second experiment, 0.03% TDA fed for 4, 6, or 8 wk resulted in a doubling of testes/body weight ratios and a highly correlated 2.5- to 2.9-fold increase in seminiferous tubule fluid volume. An approximately 50% decrease in epididymal sperm reserves was found after 6 or 8 wk of TDA exposure. After 10 wk of exposure to 0.03% TDA, testicular weight was the same as in control-fed rats but seminiferous tubule fluid volume was still elevated. These changes in testicular characteristics indicate TDA effects on Sertoli cell function, on rABP release from the testes (and epididymides), and possibly on tubular fluid transport. In the third experiment, rats fed 0.06% TDA for 1 wk showed a 25% decrease in epididymal sperm content, reduced epididymal weight, and minor structural changes in Sertoli cells. After 3 wk of 0.06% TDA feeding, sperm counts were further reduced, and were accompanied by a dramatic increase in testes weight, intense fluid accumulation, and ultrastructural changes in Sertoli cells. No significant changes in serum testosterone levels were noted in the TDA-treated rats. The results of this third experiment demonstrate TDA toxicity on testicular spermatogenesis within 3 wk of TDA feeding. The findings in this study suggest that the early inhibition of spermatogenesis by TDA is mediated through Sertoli cell damage.

INTRODUCTION

The industrially important compound 2,4-toluenediamine (TDA) has been implicated in reducing human fertility (Ahrenholz and Meyer, 1980; Centers for Disease Control, 1981). Its site or mechanism of action is unknown. In animals toluenediamine isomers lead to Leydig cell lipid depletion (dogs; Slotwinski, 1930), interstitial cell tumors (rats; Greene et al., 1981) and reduced testicular DNA synthesis (mice; Cardy, 1979). In studies using male rats we found TDA fed for 10 wk to reduce fertility, to moderately depress testosterone production, and to strongly inhibit spermatogenesis (Thysen et al., 1985a, 1985b). The effects persisted after an additional 11 wk on a normal diet and, in addition, profound testicular atrophy was noted. The data indicated effects on both testicular spermatogenesis and endocrine function.

This communication presents the results of further studies of the action of TDA on spermatogenic tissues. Effects on androgen-binding protein (rABP) production, on ultrastructural changes in seminiferous tubules, and on testicular weights were evaluated and early effects of TDA administration on spermatogenesis and testicular morphology were determined.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, Mass.), 60–70 d old, 300–325 g, were randomly distributed into dietary groups and caged singly at 24°C on a 12-h light-dark cycle,

with lights on from 0600 to 1800 h. After acclimatization in 8 × 6 1/2 × 10 1/2 in metabolism cages for a 2-wk period in our animal quarters, the rats were placed on diets of ground Purina Lab Chow containing 0, 0.03, or 0.06% TDA (98% purity, Aldrich Chemical Co., Milwaukee, Wis.). The number of weeks on the diet and the number of rats per dietary group are stated under each experiment. Food and water were available ad libitum.

All rats were killed with CO_2 gas; testes and epididymides were removed and weighed. Just prior to killing, blood was collected under CO_2 anesthesia by cardiac puncture in experiments where serum rABP or testosterone concentrations were to be determined. Samples were collected between 0900 and 1100 h to avoid any confounding effects of circadian variation. Testes, when retained for later rABP analysis, and all epididymides and all sera were immediately frozen and stored at −20° C.

Testes were analyzed for (1) rABP production into medium during culture, (2) rABP content in homogenates, (3) volume of seminiferous tubule fluid, or (4) ultrastructural changes. rABP production in culture was determined by dispersing seminiferous tubules, obtained by gentle teasing of decapsulated testes, in 8 ml Dulbecco's modified Eagle's medium, Ham's F-12, HEPES buffer medium, pH 7.4, and maintaining preparation at 36°C under air/CO_2 (95:5) for 24 h. Media was collected and stored at −20°C until assay for ABP content. rABP concentrations were also measured in testicular cytosol, prepared by homogenizing minced testis tissue in 10 m*M* Tris, 2.5 m*M* EDTA, 10% glycerol buffer, pH 7.4 (tissue/buffer ratio 1:2) with three 10-s bursts of a Brinkman Polytron and centrifuging sequentially at 800 × g and 105,000 × g and stored at −20°C until assay. In one experiment only (Experiment 2), the volume of seminiferous tubule fluid was determined by the method of Turner et al. (1984). When testes were to be evaluated for ultrastructural changes by transmission electron microscopy, small segments were placed into 0.1 *M* phsophate-buffered 2.5% glutaraldehyde, pH 7.4, dehydrated in graded alcohols, embedded in Epon, and sectioned with a Porter-Blum MT2-B ultramicrotome at 700–800 Å. Specimens stained with uranyl acetate and lead citrate were examined with a Philips 300 electron microscope.

Epididymal cytosol was analyzed for rABP content by the same method as testes cytosol, except that the tissue/buffer ratio was 1:9. Sperm reserves in homogenized cauda epididymides were determined in quadruplicate by hemocytometric counting of spermatogoa heads (Robb et al., 1978).

rABP levels were measured by radioimmunoassay as described by Gunsalus et al. (1978). Immunoassayable rABP is expressed in terms of milliliter equivalents (mleq), which is equal to 1000 times that of a standard rat epididymal cytosol preparation (GMB-E-1). The standard was

run in every assay (Gunsalus et al., 1978). Serum testosterone levels were determined in duplicate by RIA using reagents purchased from Diagnostic Products, Los Angeles, Calif.

The results of Experiments 2 and 3 were analyzed by two-way ANOVA for homogeneity of mean responses to changes in TDA dosage, length of administration, and their interaction. Any set of means showing significant lack of homogeneity ($p < .05$) was analyzed by the Mann—Whitney *U*-test for paired samples (Experiment 2) or by Dunn's multiple comparison technique for all planned comparison among means (Experiment 3) (Kirk, 1968). The statistical significance of differences in rABP or testosterone mean values between experimental and control groups was evaluated by Student's *t*-test and the Wilcoxon rank-sum test (Snedecor, 1956).

RESULTS

Experiment 1. rABP Production and Sertoli Cell Morphology after 10 Weeks of 0.03% TDA Feeding

Groups of 9 rats were each fed a 0 or 0.03% TDA diet for 10 wk, and serum, medium of seminiferous tubule cultures, and testes and epi-

TABLE 1. Androgen Binding Protein (ABP) Concentrations in Sera, Media of Cultured Seminiferous Tubules, and Testicular and Epididymal Cytosols from Rats Fed 0.03% TDA for 10 Weeks[a]

Experiment	Tissue source	Control diet (mleq ABP/unit)[b]	0.03% TDA diet (mleq ABP/unit)[b]
1A	Serum (per millileter)	0.118 ± 0.025(9)	0.41 ± 0.166(9)
	Culture medium (per testis)	2.30 ± 0.42 (8)	16.0 ± 8.6 (8)
	Testis cytosol (per gram)	1.17 ± 0.24 (9)	10.5 ± 1.88 (8)
	Testis cytosol (per testis)	1.90 ± 0.44	16.1 ± 4.91
	Epididymal cytosol (per gram)	3.58 ± 0.97 (9)	1.17 ± 0.97 (9)
	Epididymal cytosol (per cauda epididymis)	2.26 ± 0.63	0.53 ± 0.41
1B	Serum (per milliliter)	0.115 ± 0.041(9)	0.246 ± 0.112(9)
	Culture medium (per testis)	2.57 ± 0.55 (8)	14.4 ± 6.0 (8)
	Testis cytosol (per gram)	1.63 ± 0.31 (9)	15.8 ± 5.5 (8)
	Testis cytosol (per testis)	2.68 ± 0.59	24.1 ± 13.4
	Epididymal cytosol (per gram)	4.01 ± 1.08 (9)	0.53 ± 0.23 (9)
	Epididymal cytosol (per cauda epididymis)	2.76 ± 0.65	0.31 ± 0.27

[a]Rats were fed 0 or 0.03% TDA diet for 10 weeks, and testes and epididymides were removed. Seminiferous tubules were incubated in DMEM, Ham's F-12, HEPES buffered medium, pH 7.4, at 36°C under air/CO_2 (95:5) for 24 h. Cytosols were prepared by homogenizing minced testes with 2 volumes 10 m*M* Tris: 2.5 m*M* EDTA, 10% glycerol buffer, pH 7.4, or epididymides with 9 volumes of the same buffer, and centrifuging at 105,000 × g. Sera, culture media, and cytosols were analyzed for rABP content by radioimmunoassay (Gunsalus et al., 1978). The experiment was carried out twice (Experiments 1A and 1B). Values are means ± SD, with number of rats in parentheses. All ABP values from TDA-fed rats were significantly different ($p < .01$) from their paired control group.

[b]Values are expressed as mleq ABP per milliliter (for serum), per testis (for culture medium and testis), and per gram wet weight (for testis and epididymal cytosol).

didymal cytosol were analyzed for rABP content. Serum rABP and testicular cytosol concentrations from treated rats were respectively 3.8- and 8.9-fold higher than from control rats; media from cultures of TDA-exposed seminiferous tubules contained 7 times as much rABP as those of control tubules (Table 1). When expressed as "per whole testis," the medium and cytosol rABP contents of testes from individual rats correlated very closely (for Experiment 1A, $r = .98$, $p < .01$; for Experiment 1B, $r = .84$, $p < .01$). In contrast, epididymal cytosol from TDA-treated rats showed a 67% decrease in rABP content. These patterns of rABP levels were confirmed in a repeat experiment (Experiment 1B, Table 1).

Examination of testicular tissue by electron microscopy showed variable degenerative changes in Sertoli cells as compared with controls (Fig. 1). Affected cells exhibited differing degrees of cytoplasmic swelling, membrane disruption, and vacuolization (Figs. 2–4). The effects were patchy in distribution. Germ cells, where present, demonstrated normal maturational changes. Leydig cells appeared normal.

Experiment 2. Testicular and Epididymal Weights, Seminiferous Tubule Fluid (STF) Volume, and Epididymal Sperm Concentrations during 4–10 Weeks of Feeding 0.03% TDA

The marked decrease in spermatogenesis and changes in seminiferous tubule morphology and in rABP release and tissue distribution after 10 wk of 0.03% TDA intake (this report, Experiment 1; Thysen et al., 1985a, 1985b) made it of interest to establish earlier effects, if any, of rABP. Accordingly, 40 rats were randomly assigned to 8 groups of 5 animals each; 4 groups were placed on 0.03% TDA diet, and the other 4 groups on control diet (Purina Lab Chow). At 2-wk intervals (i.e., after 4, 6, 8, and 10 wk on this diet), one experimental and one control were sacrificed and testes and epididymides were examined (Table 2).

Intake of TDA for 4, 6, or 8 wk nearly doubled testes/body weight ratios. Part of this increase reflected slower weight gains and therefore lower body weights in the treated rats. However, the absolute testes weights were also 1.4- to 1.8-fold greater than control testes. The absolute increase was significant at 6 ($p < .05$) and 8 wk ($p < .01$) of feeding. The testes weight gain correlated closely with the highly significant 2.5- to 2.9-fold increase in seminiferous tubule fluid volume ($r = 0.75$, $p < .01$). At 10 wk of feeding, the testicular weight differences disappeared, and the STF volume was reduced to 1.5 times that of the control. It must be noted that one or two testes in each group fed TDA was much less affected by the dietary exposure than the others. This explains the large variances seen in most experimental groups (Table 2).

Epididymal weights were significantly increased after 4 wk ($p < .02$) and significantly decreased after 10 wk ($p < .05$) on TDA diet. No effect

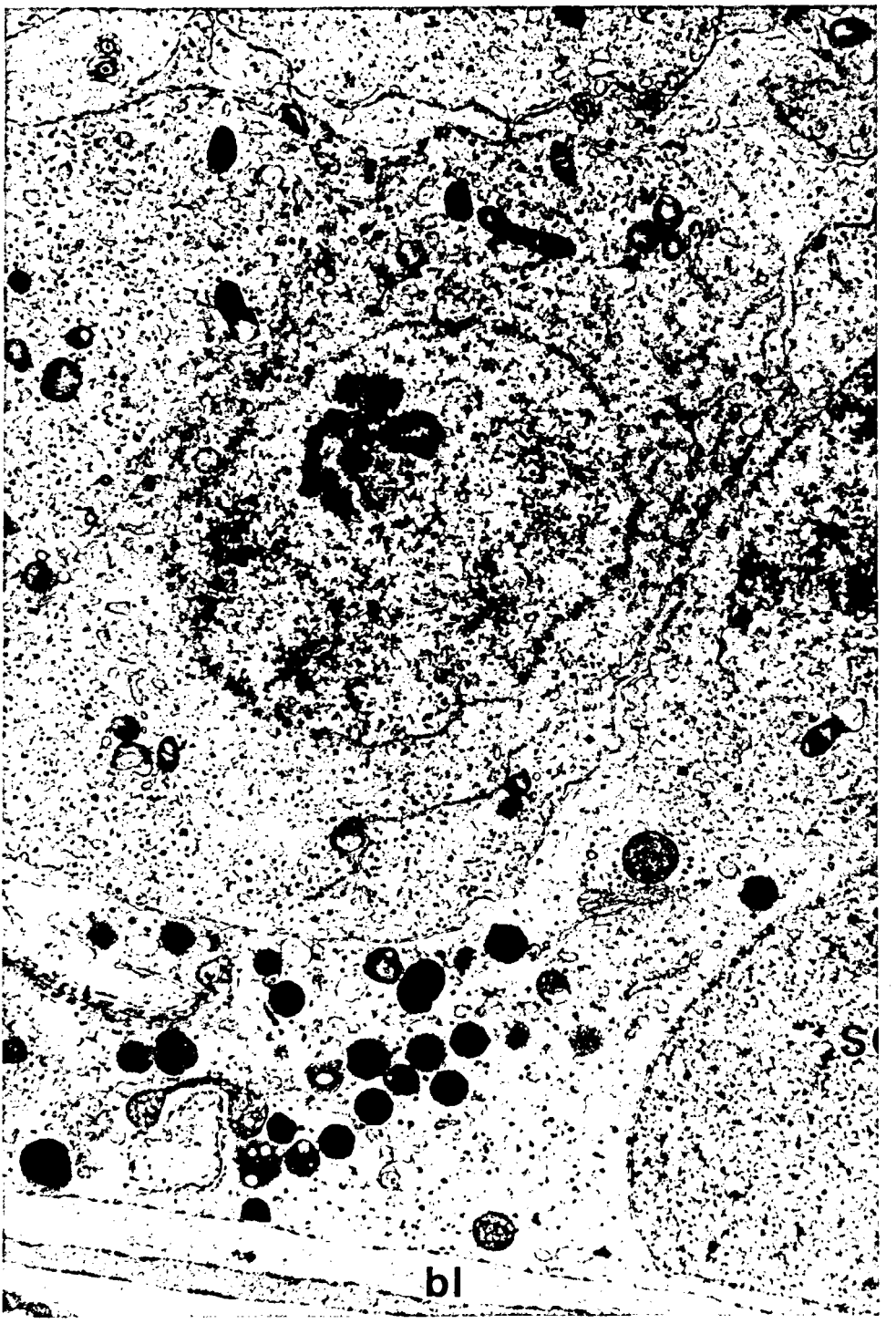

FIGURE 1. Control testis (10 wk) showing normal Sertoli cell (SC) with regular nuclear border and cytoplasm containing scattered polyribosomes, strands of rough endoplasmic reticulum, and small lipid droplets. Note the thin, delicate basal lamina (bl). ×10,400.

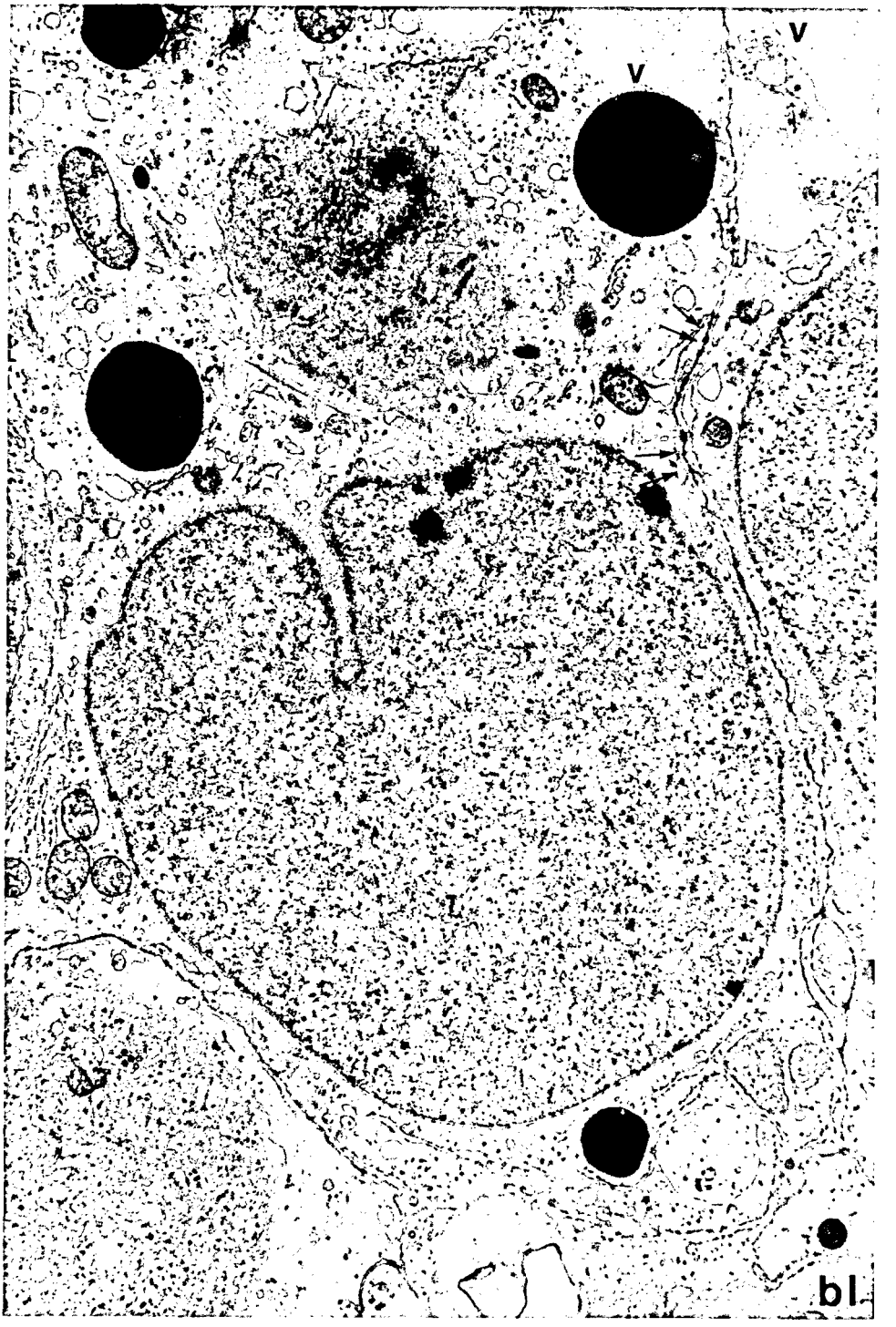

FIGURE 2. Testis from 10 wk TDA-treated animal with thickened, infolded basal lamina (b1) and early changes in Sertoli cells. Basal portions appear normal, but large vacuoles (v) are present in apical regions. Sertoli cell junctions (arrows) are intact up to the areas of cytoplasmic swelling. ×10,400.

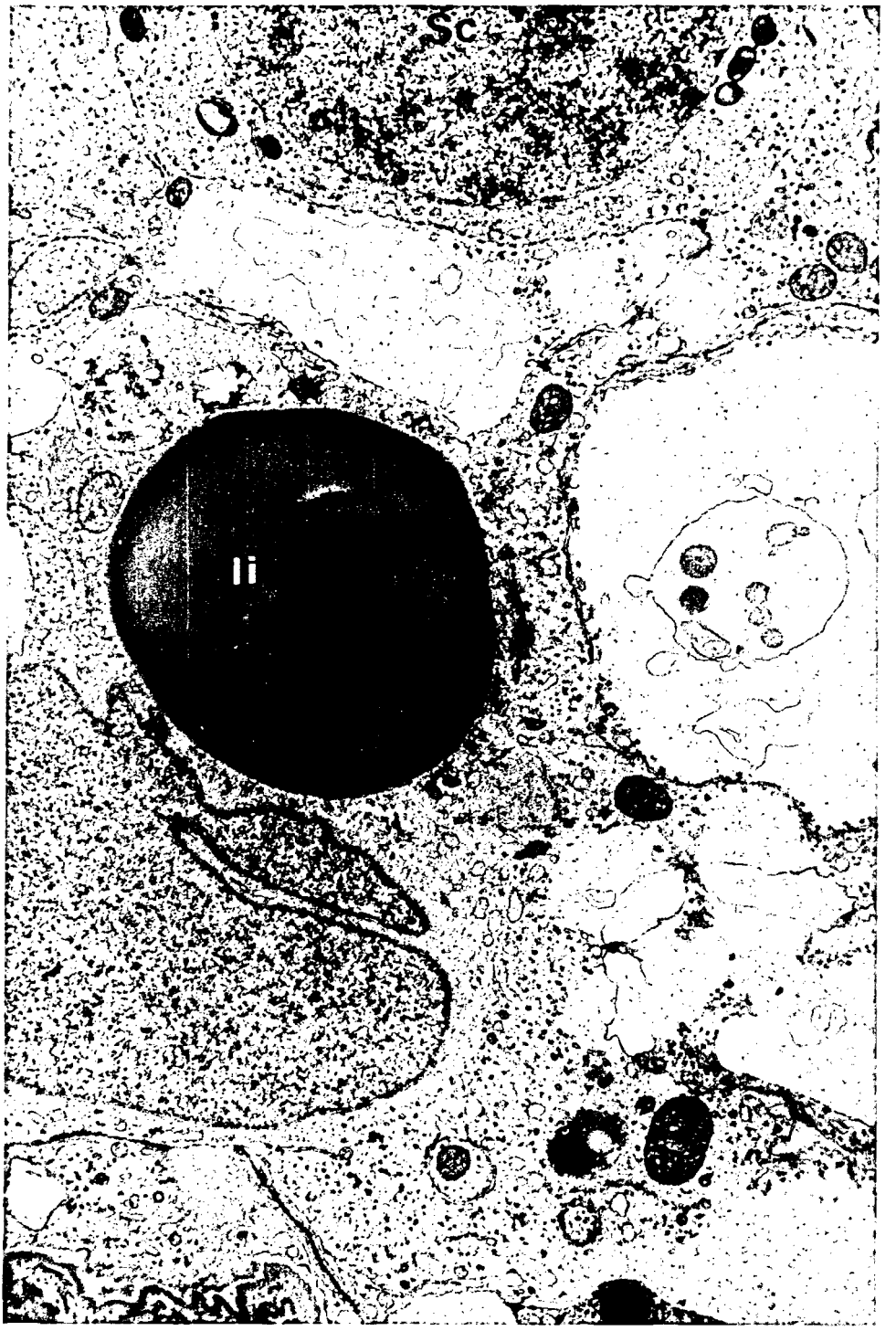

FIGURE 3. Extensive changes in Sertoli cell from 10-wk TDA-treated animal. Large vacuoles with disrupted membranes and organelles fill much of the cytoplasm. A prominent lipid (li) deposit is located adjacent to the nucleus which is distorted in outline. Spermatocytes (Sc) show no ultrastructural abnormalities and appear to be unaffected. ×10,400.

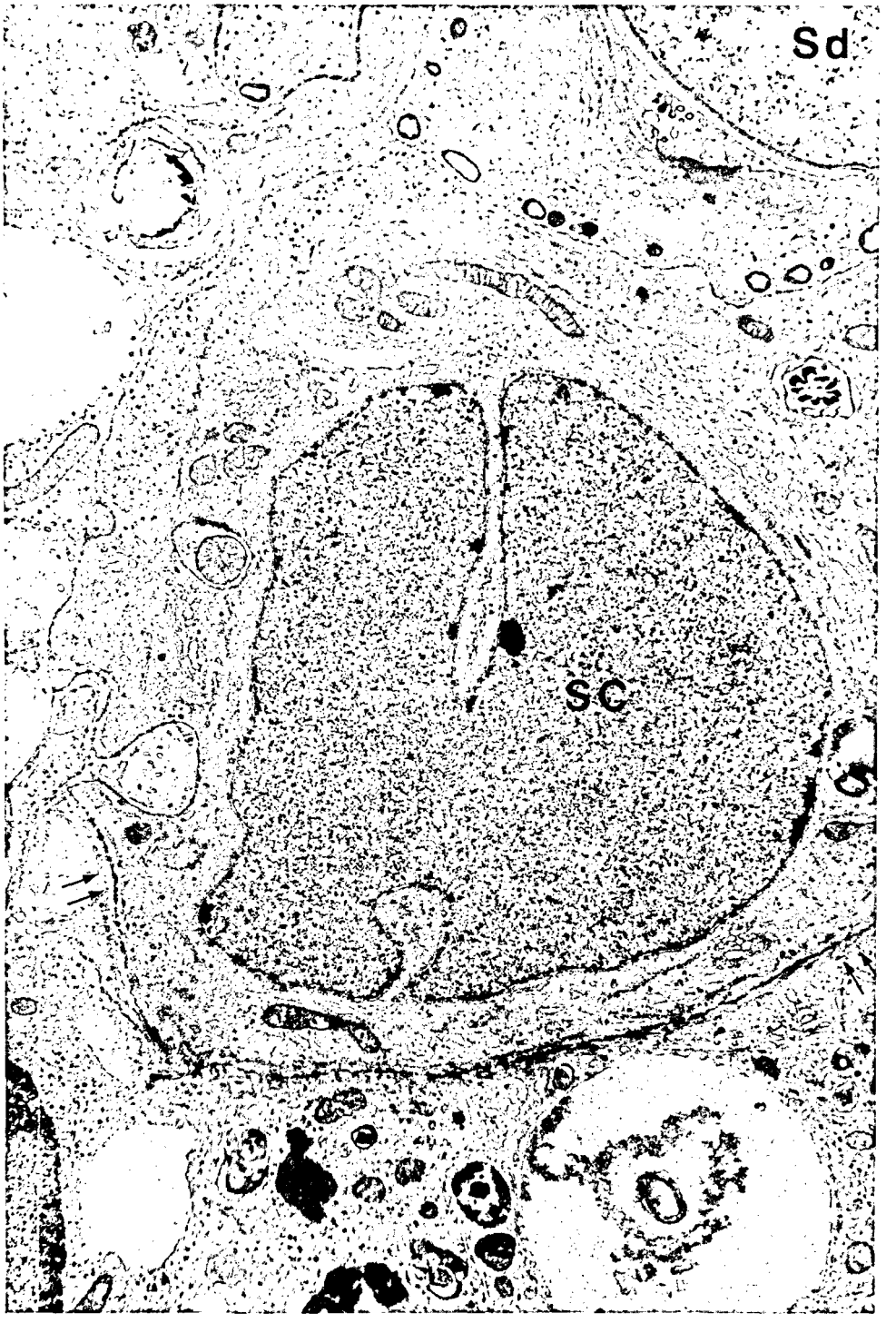

FIGURE 4. Patchy nature of the damage to Sertoli cells (10 wk) is evident in this illustration. The cell in the center (Sc) has normal nuclear and cytoplasmic appearance and retention of characteristic cell junctions (arrows). Surrounding Sertoli cells show extensive vacuolization and degenerative changes. Portions of normal appearing spermatids (Sd) are seen at upper right. ×10,400.

TABLE 2. Changes in Testicular and Epididymal Weights, Seminiferous Tubule Fluid Volume (STF),

Treatment		Body weight (g)			Testis weight	
Weeks	Dose	Initial	Final	Change	Per testis (g)	g/100 g body weight
4	0	320 ± 19	445 ± 29	125 ± 17	1.64 ± 0.12	0.37 ± 0.020
	0.03%	313 ± 14	389 ± 12	76 ± 14[b]	2.20 ± 0.55	0.56 ± 0.14[d]
6	0	298 ± 6	461 ± 22	163 ± 20	1.62 ± 0.16	0.35 ± 0.045
	0.03%	325 ± 11	442 ± 18	118 ± 16[b]	2.50 ± 0.73[d]	0.57 ± 0.16[c]
8	0	308 ± 16	489 ± 20	181 ± 14	1.66 ± 0.73	0.34 ± 0.015
	0.03%	296 ± 13	413 ± 19	117 ± 18[b]	3.00 ± 0.97[b]	0.61 ± 0.31[d]
10	0	321 ± 9	523 ± 8	201 ± 7	1.74 ± 0.13	0.33 ± 0.020
	0.03%	325 ± 8	473 ± 28	148 ± 27[b]	2.02 ± 0.97	0.43 ± 0.20

[a]Eight groups of 5 male rats each were placed on a 0 or 0.03% TDA diet. One group on each diet was sacrificed after 4, 6, 8, or 10 wk. Values are means ± SD. Testes and epididymal weights (both left only) are expressed as g/organ and g/100 g body weight, and seminiferous tubule fluid (STF) volume as total volume (μl) and as μl/g tubules.

on epididymal sperm counts was seen after 4 wk of TDA intake; the count then decreased to 37–57% of control rats when feeding TDA was extended to 6, 8, or 10 wk.

Experiment 3. Early Testicular Response to 0.06% TDA

The responsivity of tubular physiology to 0.03% TDA fed for 4 or 6 wk prompted us to test a higher TDA dose fed over a shorter period of time. We reasoned that such a protocol might amplify and localize early TDA effects with minimal growth retardation.

Accordingly, groups of 9 rats were each fed a diet of 0.06% TDA in Purina Lab Chow or Purina Lab Chow alone for 1 or 3 wk. Changes in body, testes, and epididymal weights, in epididymal sperm reserves, in seminiferous tubule ultrastructure, and in serum testosterone levels were determined.

After 1 wk of feeding 0.06% TDA, body growth, epididymal weight gain, and sperm count were decreased significantly ($p < .01$), but testis weight was unchanged (Table 3). Some Sertoli cells contained a few small cytoplasmic vacuoles, but the alterations in treated animals were limited and most Sertoli cells had a normal ultrastructural appearance. Control and TDA-fed rats had testosterone concentrations of 3.48 ± 2.09 ng/ml serum and 8.10 ± 3.79 ng/ml, respectively; the difference between the means was not significant at $p \approx .06$.

After 3 wk of ingesting 0.06% TDA, growth continued to lag behind the control group and sperm reserves were reduced by 40% (Table 3). In contrast, testis weight had more than doubled ($p < .01$) and epididymal weights were significantly greater than control rats ($p < .01$). On gross examination, the color and consistency of the seminiferous tu-

and Epididymal Sperm Concentrations with Increasing Length of Feeding 0.03% 2,4-TDA[a]

STF volume		Epididymal weight		Sperm count	
Total (μl)	μl/g	Per epidymidis (g)	g/100 g body weight	Total ($\times 10^6$)	$\times 10^6$/100 mg cauda epidymidis
178 ± 43	116 ± 32	0.52 ± 0.045	0.116 ± 0.004	265 ± 56	110 ± 9.2
609 ± 259[d]	322 ± 103[b]	0.57 ± 0.073	0.147 ± 0.024[c]	228 ± 22	100 ± 6.5
164 ± 20	130 ± 17	0.56 ± 0.038	0.122 ± 0.007	328 ± 45	119 ± 10.6
615 ± 276[b]	333 ± 80[b]	0.51 ± 0.058	0.116 ± 0.019	98 ± 38[b]	44 ± 14.5[b]
166 ± 20	168 ± 38	0.54 ± 0.051	0.111 ± 0.013	205 ± 96	109 ± 17.4
622 ± 364[d]	419 ± 119[b]	0.48 ± 0.078	0.116 ± 0.018	112 ± 45	62 ± 9.2[b]
210 ± 10	165 ± 77	0.64 ± 0.044	0.124 ± 0.010	341 ± 65	116 ± 14.2
388 ± 332	254 ± 132	0.47 ± 0.075[b]	0.095 ± 0.022[d]	116 ± 50[b]	55 ± 18.6[b]

[b]Significant difference $p < .01$ as determined by two-way ANOVA and Mann-Whitney *U*-test for paired samples.

[c]As for *b*, $p < .02$.

[d]As for *b*, $p < .05$.

bules appeared normal. By electron microscopy there were thickening of the peritubular tissue and topographical irregularities in the basal lamina separating peritubular from Sertoli cells, as compared with controls, with small vacuoles apparent in the Sertoli cell cytoplasm (Figs. 5 and 6). Serum testosterone concentrations did not differ significantly from each other, that is, 4.38 ± 1.53 and 7.82 ± 3.44 ng/ml in control and TDA-fed rats, respectively.

DISCUSSION

In previous work, we showed that feeding rats TDA for 10 wk led to impaired spermatogenesis and lowered serum testosterone levels (Thysen et al., 1985a, 1985b). The current study extends this finding by demonstrating changes in tissue rABP concentrations and Sertoli cell cytostructure in rats fed 0.03% TDA for 10 wk and reduced epididymal sperm reserves, testicular edema, and altered Sertoli cell morphology in rats fed 0.06% TDA for 1 or 3 wk. Any effects on Leydig cell testosterone production were not clearly reflected in serum testosterone levels. The influence of the agent on serum testosterone concentrations appears to depend in part on duration of treatment.

The high correlation of rABP values in media of cultured seminiferous tubules with that in testes cytosols suggests that the culture media ABP represented release of preformed rABP as well as in vitro synthesis. The tubules in culture were capable of protein synthesis as noted in the incorporation of 0.09% of added [^{35}S]methionine into TCA-precipitable protein fraction (data not shown).

In 10-wk TDA-fed rats, testicular and serum rABP levels were in-

TABLE 3. Body and Organ Weights and Epididymal Sperm Reserves in Rats Fed 0.06% 2,4-TDA for 1

Treatment		Body weight (g)			Testis weight	
Weeks	Dose	Initial	Final	Change	Per testis (g)	g/100 g body weight
1	0	389 ± 17	422 ± 23	33 ± 9	1.64 ± 0.13	0.39 ± .044
	0.06	397 ± 13	405 ± 12	8 ± 8[b]	1.50 ± 0.19	0.36 ± .059
3	0	408 ± 25	493 ± 32	85 ± 16	1.64 ± 0.15	0.33 ± .030
	0.06	404 ± 11	422 ± 20	18 ± 16[b]	3.44 ± 0.40[b]	0.82 ± .073[b]

[a]Four groups of 9 rats each were placed on a 0 or 0.06% TDA diet. One group on each diet was sacrificed after 1 or 3 wk. Values are means ± SD.

creased while rABP concentrations in the cauda epididymides were markedly diminished. Ultrastructurally, Sertoli cells exhibited degenerative changes of differing extent with germ cells appearing normal. These TDA effects, together with reduced caudal epididymal reserves, germ cell destruction, and lowered circulating testosterone levels (Thysen et al., 1985a, 1985b), demonstrate marked changes in testicular activity.

In the rat rABP is secreted and seminiferous tubule fluid are produced by Sertoli cells. Measurements of the protein (Bardin et al., 1981) and production of the fluid (Jégou et al., 1983, 1984) have been used as indices of Sertoli cell function. The dramatically elevated testicular rABP concentrations coupled with reduced epididymal rABP and spermatozoa content may reflect diminished transport from the testes through inhibition of tubular fluid passage from the testes to the epididymides (Waites and Gladwell, 1982; Spitz et al., 1985). Inhibition of tubular flow has been reported after obstruction of efferent tubules (Gunsalus et al., 1980). Partial obstruction of the tubular lumens by exfoliated spermatogenic elements, perhaps with concomitant increased fluid secretion by Sertoli cells, could readily have occurred and would also explain the transient but profound increase in testis weight and seminiferous tubule fluid volume seen in Experiment 2 after 4, 6, and 8 wk of TDA feeding. Ligation of the efferent ducts (Smith, 1962; Laporte and Gillet, 1975) or the occluding actions of several testicular toxins on the cauda epididymides (Steinberger, 1970; Reijonen et al., 1975) or efferent ductules (Diskshith and Datta, 1972; Laporte and Gillet, 1975) can result in testicular edema, distention of seminiferous tubules, and loss of spermatozoa.

The elevated concentrations of rABP in sera may be explained by increased release of testicular rABP, either by disruption of the blood-testis barrier or facilitation of rABP release by Sertoli cells into the inter-

or 3 Weeks[a]

Epididymal weight		Sperm count	
Per epididymidis (g)	g/100 g body weight	Total ($\times 10^6$)	$\times 10^6$/100 mg cauda epididymidis
0.54 ± .046	0.127 ± .015	248 ± 35	105 ± 6.3
0.44 ± .058[b]	0.106 ± .014[b]	147 ± 33[b]	78 ± 7.5[b]
0.62 ± .049	0.126 ± .012	316 ± 42	109 ± 10.1
0.62 ± .066	0.147 ± .018[b]	160 ± 15[b]	67 ± 5.2[b]

[b]Where indicated, rats fed 0.06% TDA differed significantly from control rats ($p < .01$.)

stitial fluid, as postulated to occur with tolnidamine (Spitz et al., 1985) and progestins (Lobl et al., 1983; Mather et al., 1983). The ultrastructural changes seen in the Sertoli cells support the suggestion that Sertoli cells are one source of increased serum rABP. A second source may be a damaged (Musto et al., 1982) or regressing caput epididymis, the latter as a consequence of androgen deficiency (Becker et al., 1984).

In addition to the above mechanisms, increased rABP synthesis or destruction of spermatozoa in epididymides or testes (Jackson, 1966; Zenick et al., 1984) may have contributed to the observed changes in tissue rABP or spermatozoa contents.

Rats fed 0.06% TDA for 1 wk showed distinct reproductive toxic effects perhaps centered on the epididymis: a 25% decrease in epididymal sperm content and smaller epididymides, without gross testes effects or major Sertoli cell alterations. The reduced sperm count became more pronounced after the 3-wk feeding period and was accompanied by a dramatic increase in testes weights, which was due at least least in part to fluid accumulation. The results of 3 wk of TDA-intake were the same as those in the 4-wk group of Experiment 2, further confirming the appearance of testicular edema. The sperm counts and ultrastructural changes seen after 3 wk of dietary TDA-intake differed from those seen at 10 wk of TDA feeding only in their severity.

These data demonstrate toxic effects on sperm production within 1 wk of TDA feeding. At 3 wk, the toxicity is expressed in increased alterations in Sertoli cell ultratructure and a further reduction in epididymal sperm reserves. Since Sertoli cells provide spermatogenic cells with structural and functional support, the morphologic results, together with the changes in rABP levels, suggest the Sertoli cell as a locus of action in the spermatogenic disruption of the TDA-treated rat.

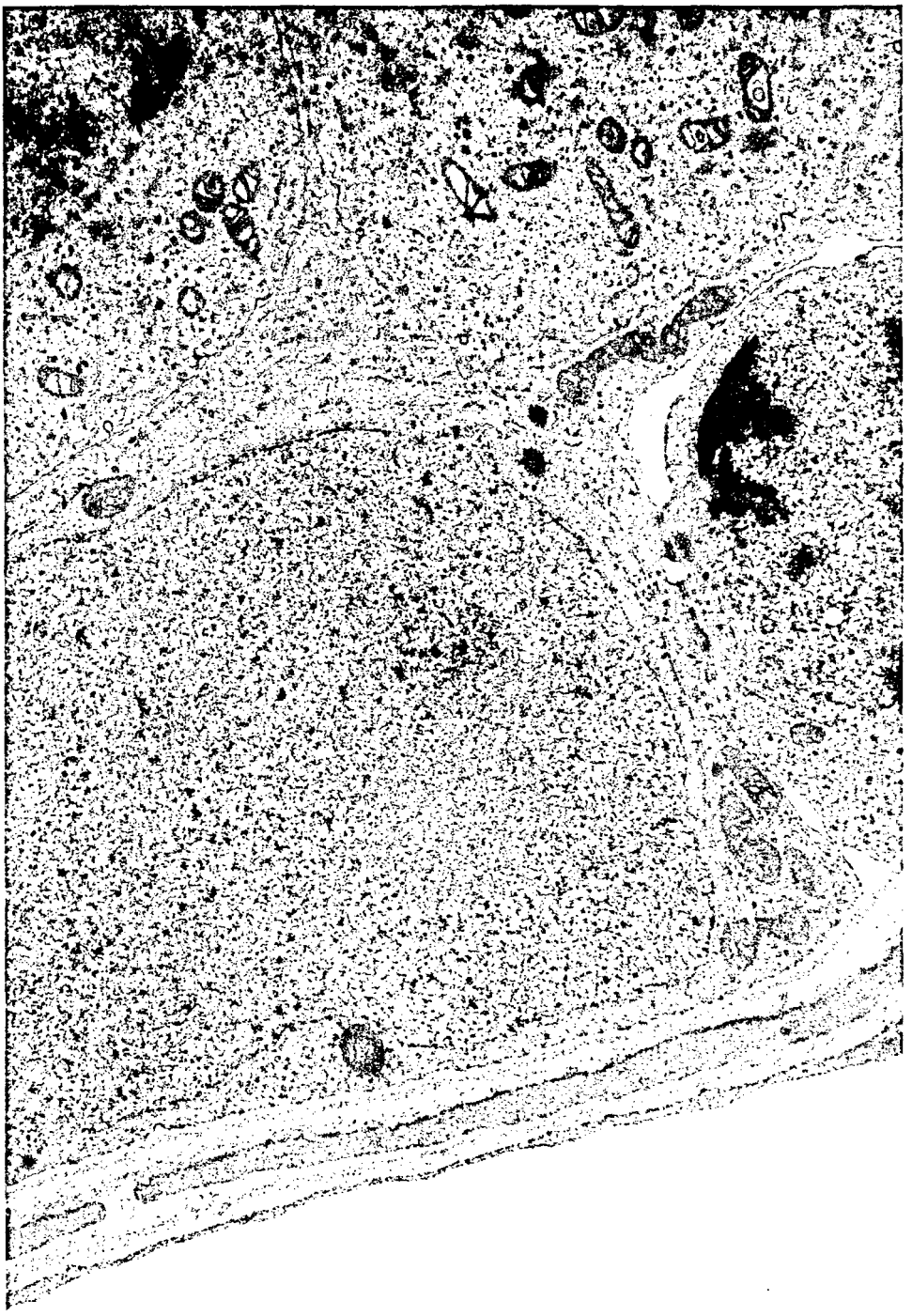

FIGURE 5. Control testis (3 wk) section shows normal Sertoli cell, adjacent type B spermatogonium, and portions of spermatocytes. Sertoli cell cytoplasm includes elongated mitochondria, strands of rough endoplasmic reticulum, and small lipid droplets. Junctional attachment plaques are seen between Sertoli cell and spermatogonium. Note the delicate peritubular tissue with smooth basal lamina. ×10,400. [This photomicrograph is identical to figure 2, published in Bloch et al. (1988).]

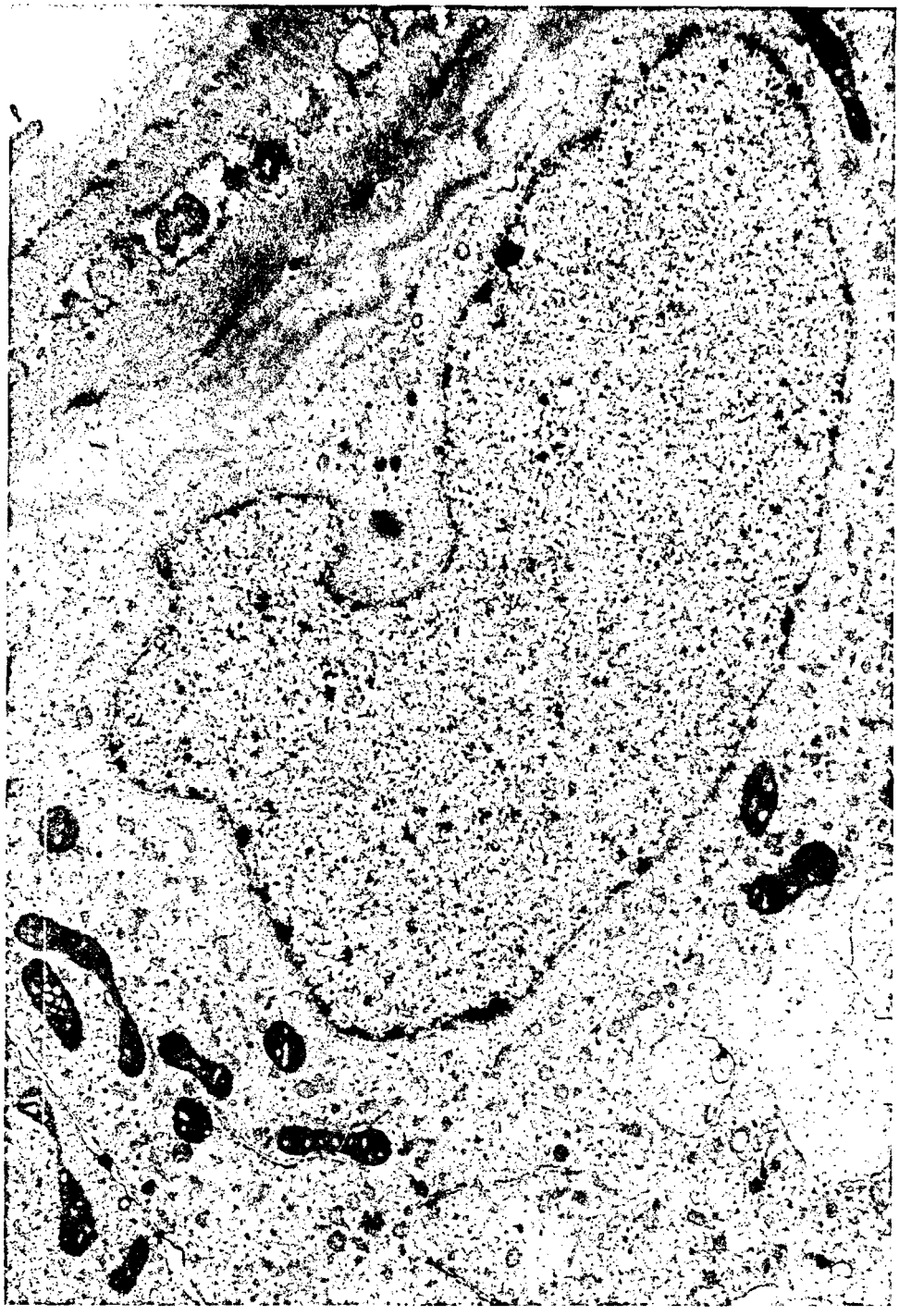

FIGURE 6. Testis from animal treated with toluenediamine for 3 wk shows thickening of the peritubular tissue and irregularity of the basal lamina. The Sertoli cell nucleus is slightly indented but otherwise unremarkable. Cytoplasm includes several small vacuoles resulting from membrane swelling and distortion, particularly in the apical region. ×101,400.

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