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The Selective Removal of Pachytene Spermatocytes Using Methoxy Acetic Acid as an Approach to the Study *In Vivo* of Paracrine Interactions in the Testis

J.M.S. BARTLETT,* J.B. KERR,† AND R.M. SHARPE*

From the MRC Reproductive Biology Unit, Centre for Reproductive Biology, Edinburgh, Scotland, and the Department of Anatomy, Monash University, Melbourne, Australia†*

Testicular weight and morphology, serum gonadotropins, intratesticular levels of testosterone and ABP levels in testicular interstitial fluid were studied in adult rats at intervals of 1 to 70 days after a single oral dose of 650 mg/kg methoxy acetic acid. At 3 days, this treatment resulted in the selective loss or depletion of pachytene and later spermatocytes from seminiferous tubules at all stages other than VIII to XI of the spermatogenic cycle. At later times this lesion was expressed as an absence mainly of round (14 days) or elongated (21 days) spermatids from the majority of seminiferous tubules. Other than these changes, spermatogenesis did not appear to be affected by treatment and was qualitatively normal in all tubules at 70 days after treatment. As deduced from cell counts at 3 days posttreatment, the initial action of methoxy acetic acid was restricted to late zygotene spermatocytes (stage XII) and pachytene spermatocytes at all stages other than early- to mid-stage VII. Levels of FSH in serum and those of ABP in testicular interstitial fluid indicated that Sertoli cell function was altered in rats treated with methoxy acetic acid. Both were increased at 1 to 3 days posttreatment, returned to normal at 7 to 14 days but were increased again at 21 days before finally returning to control levels at 28 days. In contrast, the levels of testosterone in serum, isolated seminiferous tubules and testicular interstitial fluid were unaffected by treatment, as also were the serum levels of LH. The two periods of increase in FSH and ABP levels coincided with the times of greatest decrease (~ 20%) in testicular weight, and may be related either to the type of germ cell missing from the affected tubules and/or to the stage of the cycle of the affected (or unaffected) tubules. These data suggest that chemicals such as methoxy acetic acid may prove useful in the study of paracrine interactions *in vivo*.

Key words: Seminiferous tubule, Sertoli cell, pachytene spermatocytes, round spermatids, elongate spermatids, testosterone, LH, FSH, ABP.

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Studies on paracrine interactions in the testis have concentrated mainly on isolation of the various testicular cell types, namely Leydig cells, Sertoli cells, peritubular and germ cells, their purification and subsequent culture *in vitro*, either alone or together. Such studies combined with investigations of seminiferous epithelial function using isolated short lengths of rat seminiferous tubules have provided most of our *in vitro* information regarding the nature and type of interactions between the various cell types (Parvinen, 1982; Sharpe, 1986). However, since the presumed effect of paracrine interactions is to coordinate the function of all of the various cell types in the testis (Sharpe, 1986), the study of isolated testicular cells *in vitro* obviously has limitations, particularly in relating paracrine events to subsequent changes in fertility. In the present study we

Reprint requests: R.M. Sharpe, Ph.D., MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, Scotland, United Kingdom.

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have therefore attempted to use a different approach that does not have these limitations. Instead, we have investigated the consequences of removing *in vivo* a specific germ cell population, thus leaving intact the majority of testicular cell types and their presumptive interactions. To achieve this objective, we have used a germ cell-specific toxin, methoxy acetic acid, which, in low doses, has been shown in previous toxicologic studies to act on pachytene primary spermatocytes (Foster et al, 1983, 1984; Creasy et al, 1985). The aim of the present study was to relate the damage caused by this toxin to intratesticular function and to determine whether this approach might have more general application in the investigation of cell to cell interactions in the testis and, ultimately, their relationship to fertility.

Materials and Methods

Animals and Treatment

Animals were from our own colony of Sprague–Dawley rats and were aged 75 to 85 days when treated. They were housed under standard conditions. A total of 88 rats (approximately 350 g body weight) received a single dose of methoxy acetic acid (650 mg/kg; Aldrich U.K. Ltd) which, after adjustment to pH 7.0 with NaOH, was administered by gavage in 0.9% w/v NaCl in a volume of 4 ml/kg. Rats were killed in groups by inhalation of CO_2 at 1, 3, 7, 14, 21, 28, 42, or 70 days after treatment and subjected to the procedures described below. Control rats (N = 27) received an equivalent volume of vehicle and were killed in four groups at 3, 14, 21, and 70 days after treatment. Since none of the parameters measured in the controls differed significantly among these four groups, data for the four groups were pooled.

Morphologic Studies

At 3, 7, 14, 21, and 70 days after treatment with methoxy acetic acid, two rats per group plus four control rats were fixed by perfusion with buffered glutaraldehyde through the dorsal aorta and processed as described elsewhere (Kerr and Sharpe, 1985; Bartlett et al, 1986). After postfixation in osmium tetroxide, tissue blocks were embedded in Epon-araldite and semi-thin (1 μm) sections cut and stained with toluidine blue. Transverse sections of seminiferous tubules were then examined and photographed using a Zeiss photomicroscope and classified into stages according to Leblond and Clermont (1952). At 3 days after treatment, the number of zygotene and later spermatocytes was determined in round cross-sections of seminiferous tubules at each stage of the spermatogenic cycle. This time was chosen because most of the germ cell degeneration induced by methoxy acetic acid should already have occurred (see Creasy et al, 1985). A minimum of 13 (treated) or eight (controls) tubule cross-sections at each stage were counted for two control and two treated rats. Cell counts were corrected according to Abercrombie (1946) after measurement of the nuclear diameter of at least 30 pachytene spermatocytes at each stage by image analysis (Imagan 2; Leitz).

Collection of Samples and Hormone Measurements

Trunk blood was collected at death and stored for 16 hours at 4 C before separation of the serum by centrifugation for 30 minutes at 1500 g. Serum was stored at −20 C prior to the measurement of LH and FSH as described elsewhere (Fraser and Sandow, 1977), using materials supplied by the NIAMDDK, U.S.A. Serum testosterone (T) was measured by radioimmunoassay after extraction as described previously (Sharpe and Bartlett, 1985). The inter- and intra-assay coefficients of variation for all of these hormone assays were <14 and $<10\%$, respectively.

Each testis was dissected out, weighed and placed in ice. One testis from each pair was decapsulated and 3 × 10-cm aliquots of seminiferous tubules were collected by dissection of teased tubules into 0.5-cm lengths (20 × 0.5 cm) on a stage cooled to 4 C, procedures that have been shown to minimize the diffusion of T (Sharpe and Bartlett, 1985). Each aliquot was placed into 0.2 ml distilled water and stored at −20 C prior to sonication and extraction for T measurement (Sharpe and Bartlett, 1985). Interstitial fluid was collected from the contralateral testis over 16 hours at 4 C as described and validated elsewhere (Sharpe and Cooper, 1983). After removal of contaminating erythrocytes by centrifugation for 5 minutes at 1000 × g, a 20-μl aliquot of interstitial fluid was aspirated and diluted with 10 volumes M199 containing Hanks' salts and 25 mM Hepes (Flow Laboratories) and then stored at −20 C until thawed for the measurement of T (Sharpe and Bartlett, 1985) and androgen binding protein (ABP) using a radioimmunoassay. Although this assay has been well validated (Gunsalus et al, 1978), it was also demonstrated that interstitial fluid dilution curves paralleled that of the ABP standard. The inter- and intra-assay coefficients of variation were 17 and 12%, respectively.

Statistics

Data on testis weight and hormone levels were analyzed using one-way analysis of variance (ANOVA) and Dunnet's *t*-test to determine differences from control values. The latter comprised four groups killed at 3, 4, 21, and 70 days, but since ANOVA revealed no significant differences among the four control groups for any of the parameters measured, the results were pooled for comparison with rats treated with methoxy acetic acid.

Data on cell counts were analyzed by 2-factor ANOVA to establish that there was an overall significant difference between control and treated rats.

Results

Testicular Morphology

Compared with control rats, in which each cross-section of seminiferous tubule contained all of the germ cells appropriate to the particular stage of the spermatogenic cycle (Fig. 1a), the majority of seminiferous tubules in rats treated with methoxy acetic

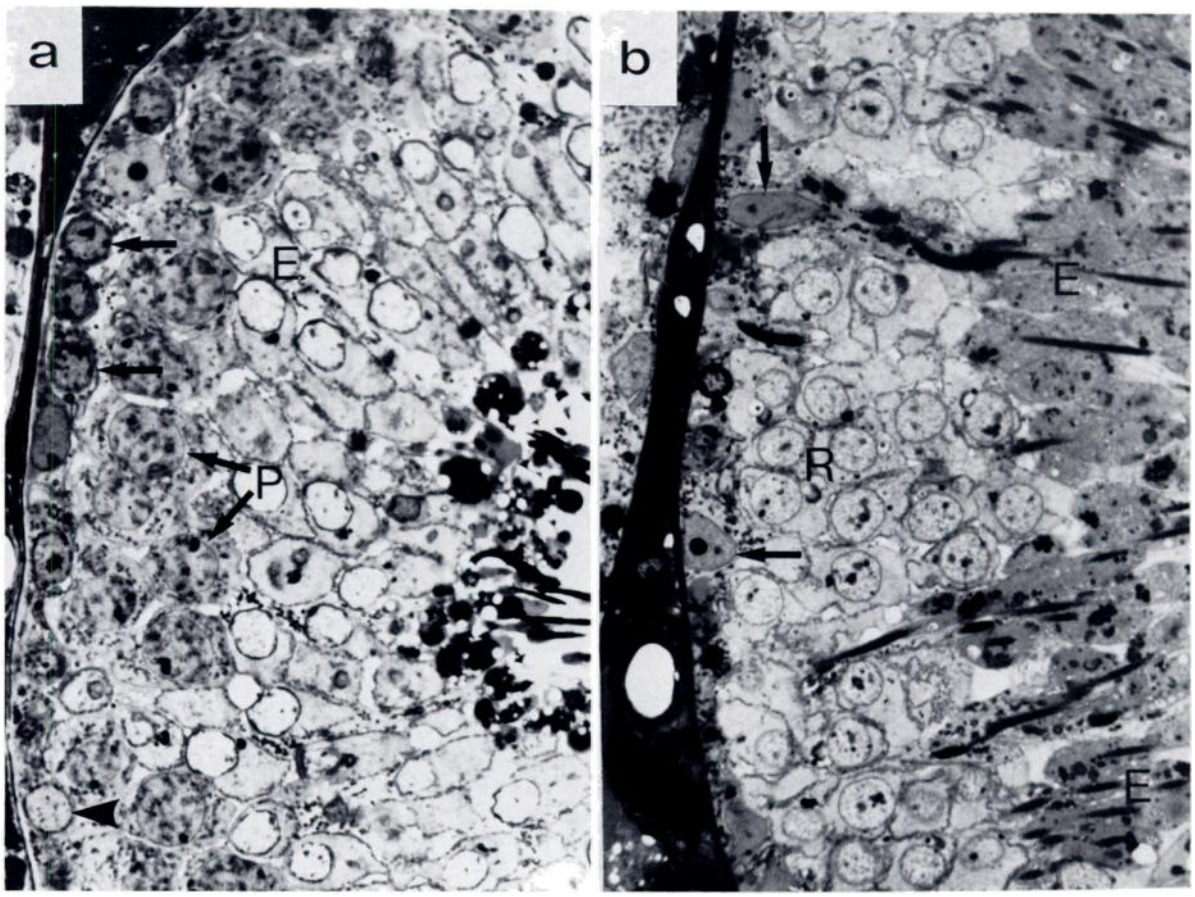


Fig. 1a–b. Vehicle-treated rat illustrating normal seminiferous epithelium at stage VIII, comprising A-type spermatogonia (arrowhead), preleptotene primary spermatocytes (arrow), pachytene primary spermatocytes (P), elongating step 8 spermatids (E) and mature step 19 spermatids projecting into the lumen (× 800). **b:** Three days after treatment with methoxy acetic acid illustrating seminiferous epithelium at stage II. Note the complete absence of pachytene primary spermatocytes but an otherwise normal-looking epithelium containing round step 2 spermatids (R) elongate step 16 spermatids (E) and Sertoli cell nuclei (arrows) (× 800).

acid showed a large reduction or complete loss of one specific germ cell type. At 3 days after treatment, tubules at all stages of the spermatogenic cycle either lacked or had severely reduced numbers of pachytene or later (diplotene, secondary) spermatocytes, the exception being stages VIII to XI, which appeared qualitatively normal. In tubules at stages II to VII, pachytene spermatocytes were absent or reduced in number but the seminiferous epithelium had collapsed leaving no spaces to indicate this loss (Fig. 1b). At other stages in the cycle (XII to I), the absence of pachytene spermatocytes was marked by the presence of a ring of spaces in the epithelium (Fig. 1c; see also Fig. 3). These spaces were not intracellular to germ cells and were not artifacts as they were clearly stage-specific (Fig. 3) and were not associated with any signs of shrinkage or swelling around the base of the Sertoli cells. The position of the spaces coincided with that of the absent pachytene spermatocytes and have been termed extracellular.

At 7 days after methoxy acetic acid, the two types of lesion described above had progressed so that tubules at stages VII to XIII exhibited a collapsed epithelium lacking or with few pachytene spermatocytes (Fig. 1d), while stages II to VI exhibited absent or greatly reduced numbers of round spermatids and a ring of extracellular spaces in the epithelium (Fig. 3a). In the latter tubules, pachytene spermatocytes from the next spermatogenic wave were now evident. Tubules at stages XIV and I appeared qualitatively normal at 7 days after treatment.

At 14 days after exposure to methoxy acetic acid, tubules at all stages other than VII and VIII lacked or had severely reduced numbers of either round or

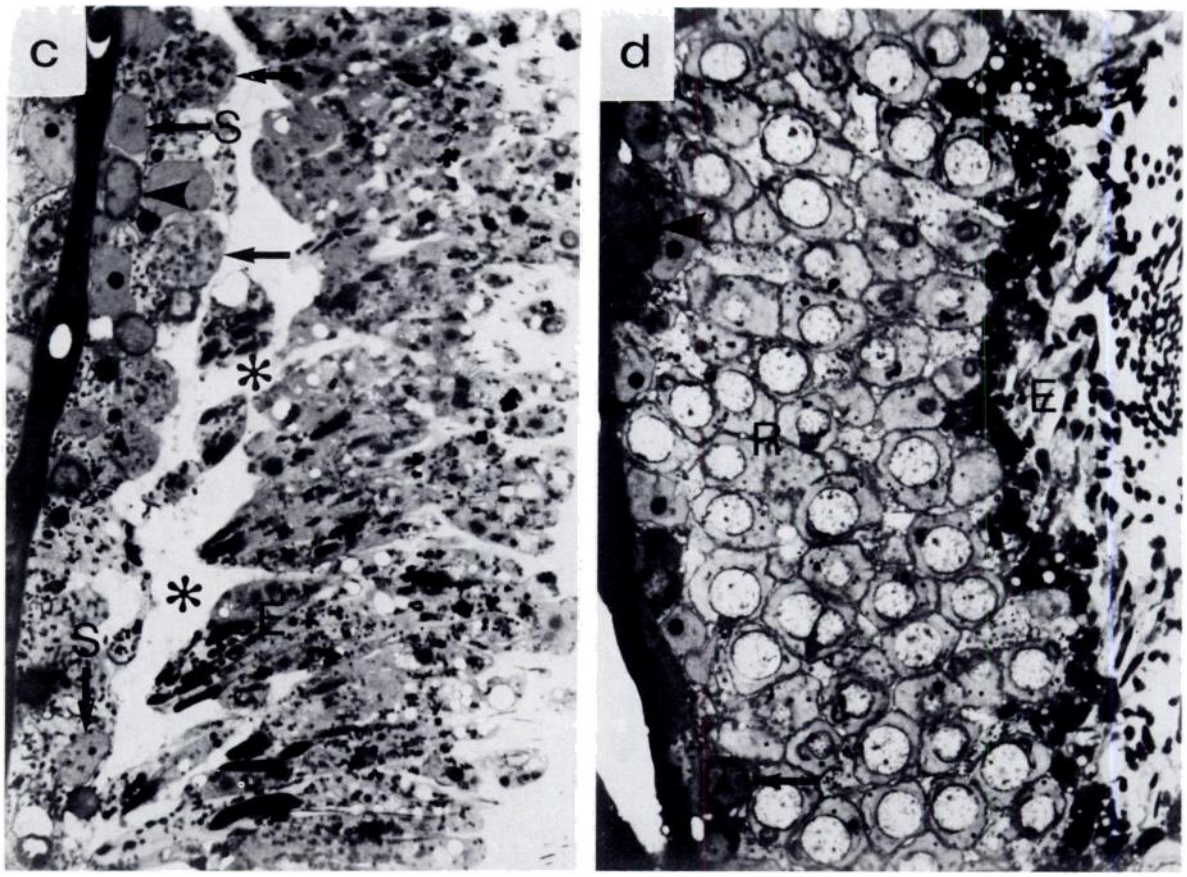
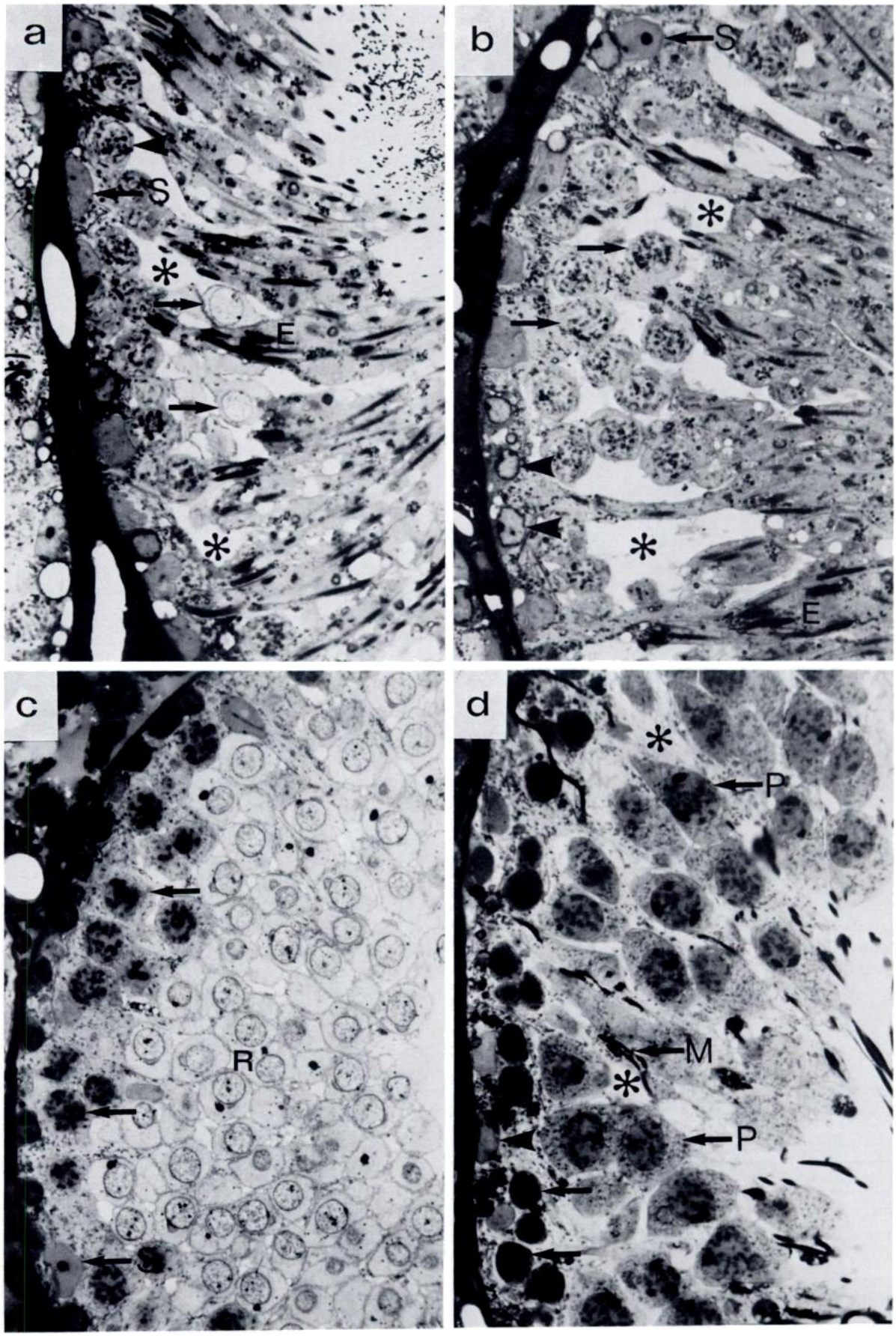


Fig. 1c-d. Three days after treatment with methoxy acetic acid illustrating seminiferous epithelium at stage XII. Note the presence of a ring of extracellular spaces (*) in place of pachytene spermatocytes but an otherwise normal epithelium comprising A-type spermatogonia (arrowheads), zygotene primary spermatocytes (arrows) and elongating step 12 spermatids (E); S = Sertoli cell nuclei (× 800). **d:** Seven days after treatment with methoxy acetic acid illustrating seminiferous epithelium at stages VII-VIII. Note the complete absence of pachytene primary spermatocytes but an otherwise normal epithelium containing A-type spermatogonia (arrowhead), preleptotene primary spermatocytes (arrow) and round (R) and elongate (E) spermatids (× 800).

elongating spermatids and many stages exhibited extensive extracellular spaces in the epithelium (Fig. 2b); stages VII and VIII appeared qualitatively normal. By 21 days, the latter stages would have progressed to stages II to III and, accordingly, tubules at these stages appeared normal at this time. Tubules at stages IV to VIII were totally lacking in elongated spermatids at 21 days after methoxy acetic acid (Fig. 2c) and the majority of tubules were in this category. Tubules at stages IX to XI lacked early spermatids and exhibited abnormal retention of step 18 to 19 spermatids (Fig. 2d). The latter tubules also appeared to have excessive numbers of pachytene spermatocytes, but morphometry will be necessary to deter-

Fig. 2a-d. Seven days after treatment with methoxy acetic acid illustrating seminiferous epithelium at stage IV. Note the almost complete absence of round spermatids (arrow) and the presence of a ring of extracellular spaces (*), but the normal presence of pachytene primary spermatocytes (arrowheads), elongate spermatids (E) and Sertoli cell nuclei (S) (× 800). **b.** Fourteen days after treatment with methoxy acetic acid illustrating seminiferous epithelium at about stage II. Note the extensive extracellular spaces (*) in place of round spermatids but an otherwise normal-looking epithelium containing Sertoli cell nuclei (S), A-type spermatogonia (arrowheads), pachytene primary spermatocytes (arrows) and elongate spermatids (E) (× 800). **c.** Twenty-one days after treatment with methoxy acetic acid illustrating seminiferous epithelium at stage VI. Note the complete absence of elongate spermatids but the normal presence of pachytene primary spermatocytes (arrows) and round spermatids (R) (× 800). **d.** Twenty-one days after treatment with methoxy acetic acid illustrating seminiferous epithelium at around stages IX to XI. Note the abnormal retention of mature spermatids (M) but the absence of elongating step IX to XI spermatids and the presence of extracellular spaces (*). Spermatogonia (arrowhead) and leptotene primary spermatocytes (arrows) are evident while pachytene primary spermatocytes (P) appear to be present in abnormally high numbers (× 800).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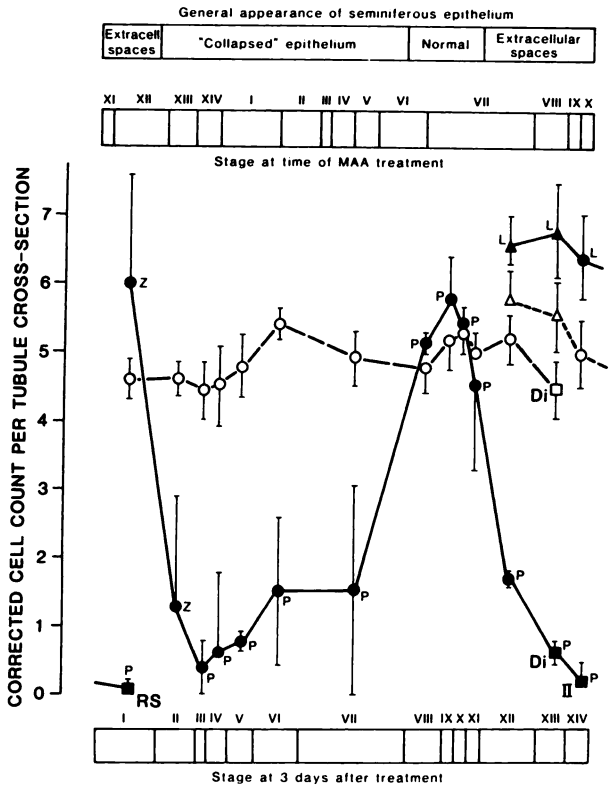

Fig. 3. Stage-dependent changes in spermatocyte numbers at 3 days after treatment with methoxy acetic acid (MAA). Results are shown as the mean and range for two controls (open symbols) and two rats treated with methoxy acetic acid (solid symbols). Data for zygotene (triangles), pachytene (circles), diplotene (Di) and secondary (II) spermatocytes are shown together with the number of round spermatids (RS) at stage I. Note that in controls the RS count (18.9 ± 1.3) is not illustrated and secondary spermatocytes (Stage XIV) were not counted. The stages of the spermatogenic cycle at the time of sampling (day 3) and those deduced for the time of initial exposure to methoxy acetic acid (day 0) are illustrated in the bottom and top panels, respectively, with the width of each stage corresponding to its relative duration (Clermont, 1972). The deduced stage of spermatocyte development on the day of treatment with methoxy acetic acid (day 0) is shown alongside each cell count (L = leptotene, Z = zygotene, P = pachytene). Stage-dependent differences in the general appearance of the seminiferous epithelium are also indicated in the uppermost panel (see text for explanation). Analysis of spermatocyte counts at stages I to XIII by 2-factor ANOVA indicated a signficant ($P < 0.001$) effect of methoxy acetic acid and a significant ($P < 0.001$) interaction with the stage of the spermatogenic cycle.

mine whether this increase is real or is due to compression of the epithelium.

Testicular morphology was not studied at 28 or 42 days after treatment. At 70 days, tubules at all stages of the cycle appeared qualitatively normal. At the light microscope level, all other aspects of testicular morphology (vasculature and interstitium) appeared unaffected by treatment with methoxy acetic acid.

Spermatocyte Numbers

To confirm that treatment with methoxy acetic acid had selectively depleted pachytene spermatocytes, the numbers of all zygotene and later spermacytes were counted at 3 days after treatment at each stage of the spermatogenic cycle. From the known duration of each stage (Clermont, 1972), the initial cell type(s) affected by methoxy acetic acid could thus be deduced (Fig. 3). This analysis indicated that late zygotene (stage XIII) and early (stages XIV to IV) and late (late stage VII to stage XII) pachytene spermatocytes were highly susceptible to methoxy acetic acid and were reduced in numbers by 60 to 90% when compared with control values. In contrast, the number of earlier zygotene spermatocytes (stage XII) and pachytene spermatocytes in early- to mid-stage VII were not affected by treatment. As judged by the numbers of zygotene (stages XII to XIII) and early pachytene (stage XIV) spermatocytes at 3 days post-treatment, leptotene spermatocytes also were resistant to the initial effects of methoxy acetic acid, although the apparent increase in the number of the latter cells compared with the controls may be due to compression of the epithelium. Finally, diplotene and secondary spermatocytes were either unaffected or were only marginally affected by treatment. The number of round spermatids at stages II to IV appeared normal (e.g. Fig. 1b), which contrasted with the virtual elimination of round spermatids at stage I (Fig. 3). These cells would have derived from late pachytene spermatocytes at stages XI to XII when exposed to methoxy acetic acid.

Although the degree of initial loss of pachytene spermatocytes induced by methoxy acetic acid was generally similar at all stages other than VII of the spermatogenic cycle, this resulted either in the appearance of extracellular spaces in the vicinity of the missing germ cells or collapse of the epithelium, leaving no such spaces (Figs. 1 to 3). These two types of lesions were clearly distinguishable up to 21 days after treatment and their stage-specificity may offer a possible clue to their origin (Fig. 3). Extracellular spaces therefore appeared only in tubules that were at late stage VII to stage XII at the time of treatment, which coincides with the period when translocation of early spermatocytes from the basal to the adluminal compartment takes place (Russell, 1977). It should be emphasized, however, that these early spermatocytes were clearly unaffected by treatment with methoxy acetic acid (Fig. 3).

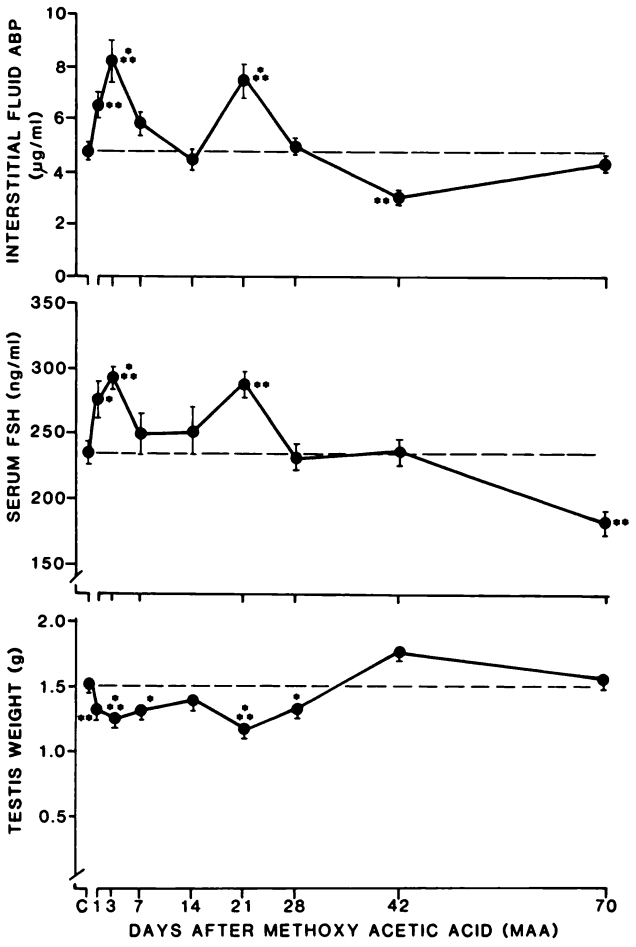

Fig. 4. Temporal changes in (top) the levels of ABP in testicular interstitial fluid and (middle) the serum levels of FSH in relation to (bottom) testicular weight, after a single oral dose of methoxy acetic acid. Values are the mean ± SEM for 10 rats per group except for controls (c), which consisted of 27 rats. The horizontal dashed line shows the control mean. $^{*}P<0.05$, $^{**}P<0.02$, $^{***}P<0.005$, in comparison with respective control value.

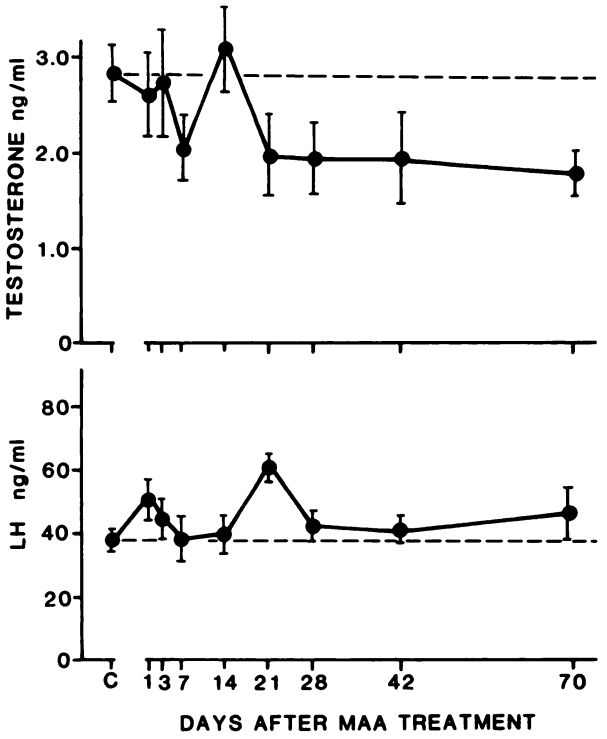

Fig. 5. Serum levels of LH and T at various times after a single oral dose of methoxy acetic acid (MAA). Other details are as in the legend to Fig. 4.

Testicular Weight

Within 24-hours of treatment with methoxy acetic acid, testicular weight decreased significantly when compared with controls and remained significantly decreased at 3 and 7 days (Fig. 4). Testicular weight then recovered slightly at 14 days before falling at 21 days to the lowest values observed during the experiment (Fig. 4). Following this second decline, testicular weight recovered to reach control levels by 42 days.

Serum Hormone Levels

Serum levels of FSH were increased significantly within 24 hours of treatment with methoxy acetic acid and increased still further at 3 days (Fig. 4). At 7 and 14 days after treatment, serum FSH returned to control levels before showing a second significant increase at 21 days. Serum FSH then returned to control levels at 28 to 42 days before decreasing significantly at 70 days (Fig. 4); the latter decline is entirely unexplained as testicular morphology appeared qualitatively normal at this time (see above).

Serum levels of LH and T did not change significantly after treatment with methoxy acetic acid although there was evidence of higher LH levels at 1 and 14 days, times when FSH levels were significantly elevated (Fig. 5).

Interstitial Fluid Levels of ABP

The levels of ABP in testicular interstitial fluid showed a pattern of change that was virtually identical to that of serum levels of FSH at 1 to 28 days after treatment (Fig. 4). Thus, ABP levels in interstitial fluid were increased significantly at 1 and 3 days, returned to control levels at 7 and 14 days before increasing to a second peak at 21 days. Thereafter, ABP levels in interestitial fluid remained at (28 and 70 days) or below (42 days) control levels (Fig. 4).

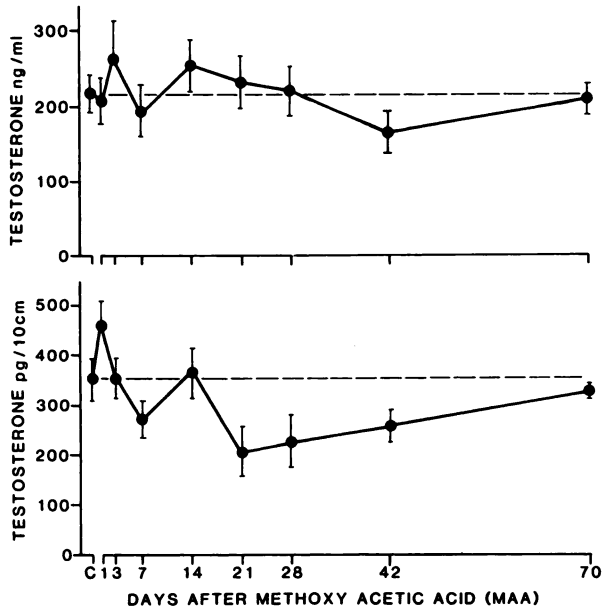

Fig. 6. Testosterone levels in isolated seminiferous tubules (bottom) and in testicular interstitial fluid (top) at various times after a single oral dose of methoxy acetic acid (MAA). Other details are as in the legend to Fig. 4 except that in the bottom panel each point is the mean ± SEM for either 20 (control-C) or five (all other points) rats per group.

Intratesticular Testosterone Levels

Testosterone levels inside and outside (in interstitial fluid) of the seminiferous tubules did not change significantly at any time after treatment with methoxy acetic acid although there was a tendency towards lower intratubular levels at 21 to 42 days post-treatment (Fig. 6).

Discussion

The general aim of the present experiments was to test whether chemicals with toxic effects on specific germ cell types in the testis could be useful tools for the study *in vivo* of testicular paracrine interactions. For this study, we chose methoxy acetic acid, which is the major active metabolite of ethylene glycol monomethyl ether (EGME), a water-miscible organic solvent that is widely used in the paint and printing industries (see Foster et al, 1983; 1987).

Toxicologic studies (Nagano et al, 1979; Foster et al, 1983; 1984) and more recent studies of testicular morphology and *in vitro* effects (Creasy et al, 1985; Foster et al, 1987) have all suggested that methoxy acetic acid and its parent compound (EGME) produce selective and stage-specific damage and loss of pachytene primary spermatocytes. Our time-course study confirms these reports in showing that at 3 to 21 days after treatment with methoxy acetic acid, all germ cell losses can be traced back to an initial effect on late zygotene or pachytene spermatocytes. Other than early- and mid-stage VII, all other stages of the spermatogenic cycle were susceptible to this effect of methoxy acetic acid, again confirming earlier findings (Creasy et al, 1985; Foster et al, 1987). It has also been demonstrated that doses of methoxy acetic acid lower than that (650 mg/kg) used in the present study exert effects over a more restricted range of stages (Foster et al, 1987). The reason for the stage-dependent differences in susceptibility to methoxy acetic acid is not known.

After the loss of pachytene spermatocytes induced by methoxy acetic acid, spermatogenesis progressed apparently normally, both in the unaffected seminiferous tubules (stage VII at the time of treatment) and in those lacking, or with a reduced complement of, one layer of germ cells. In relation to current ideas about the importance of Sertoli-germ cell interactions during spermatogenesis (Sharpe, 1986), this finding suggests that a full complement of germ cells is not necessary for the progressive development and differentiation of unaffected germ cells. This does not necessarily mean that the germ cells that develop under these abnormal conditions will give rise to completely normal spermatozoa since there is evidence for deficiencies in their function (Chapin et al, 1985; Holloway et al, 1986). It remains to be shown whether the latter changes are the result of a more subtle effect of methoxy acetic acid on germ cells other than pachytene spermatocytes or whether the changes are a consequence of germ cell development under abnormal conditions, ie, development in a tubule lacking a complete layer of germ cells. With respect to the latter possibility, the present study has produced two pieces of evidence to suggest that Sertoli cell function was perturbed by the absence of a layer of germ cells.

First, in treated rats, serum levels of FSH were raised significantly at 1 to 3 and again at 21 days after treatment with methoxy acetic acid. Although this increase was small in magnitude, it is a repeatable finding (unpublished data) and may reflect an overall decrease in the secretion of inhibin by the Sertoli cell, since steroid feedback (ie, serum T levels) remained unchanged in the treated rats. However, serum LH also showed some evidence of raised levels at 1 and 21 days, which may indicate that the secretion of both gonadotropins had been altered by treatment. Second, the levels of ABP in testicular interstitial fluid

showed a pattern of change identical to that of serum FSH, at least up to 28 days post-treatment, with levels increased significantly at days 1 to 3 and again at 21 days. In other recent studies we have shown that major experimental disruption of the seminiferous epithelium by treatments such as local heating, cryptorchidism or T withdrawal all result in an increase in the interstitial fluid levels of ABP, indicating a change in the secretory function of the Sertoli cell (Bartlett and Sharpe, 1987; Sharpe and Bartlett, 1987). The physiologic significance of this change is unknown.

The increase in levels of FSH in serum and of ABP in interstitial fluid after treatment with methoxy acetic acid occurred in two peaks (days 3 and 21), with a return to normal levels in the intervening period (days 7 and 14). These findings are of particular interest since they may shed light on the nature of the intratesticular factors/events that control Sertoli cell function. Two possible explanations for the observed changes are that they are (1) related to the type of germ cell missing from the epithelium of the majority of tubules after treatment, or (2) related to the stage of spermatogenesis in the affected or unaffected seminiferous tubules. With respect to the former possibility, there is growing evidence that specific germ cell types may influence the secretion of ABP and/or inhibin by the Sertoli cell, both *in vitro* (Galdieri et al, 1984; Le Magueresse et al, 1986) and *in vivo* (Jegou et al, 1984; Pinon-Lataillade et al, 1986).

In the present study, the first increase in FSH and ABP levels (days 1 and 3) was associated with the absence of pachytene spermatocytes from most tubules, while at 14 days, when FSH and ABP levels had returned to normal, the germ cell type missing was predominantly round spermatids. The secondary increase in FSH and ABP levels at 21 days coincided with the absence of elongated spermatids. It is therefore noteworthy that, in previous studies, pachytene spermatocytes have been clearly implicated in the control of the secretion of both ABP (Galdieri et al, 1984; Le Magueresse et al, 1986) and inhibin (Pinon-Lataillade et al, 1985; Delic et al, 1986), while elongate spermatids have also been implicated in the control of ABP secretion (Pinon-Lataillade et al, 1986). However, it is not possible to compare the present findings in detail to those cited above because of differences in experimental procedures. In other situations of induced seminiferous tubule damage, the interstitial fluid levels of ABP are increased (Bartlett and Sharpe, 1987; Sharpe and Bartlett, 1987) when overall ABP secretion, and particularly secretion into the tubule lumen, is decreased drastically (Hagenas and Ritzen, 1976; Jegou et al, 1983). Therefore, the finding of increased ABP levels in interstitial fluid in the present study may only reflect a proportionate change in the direction of ABP secretion by the Sertoli cell.

Interpretation of the present results is also complicated by the fact that not all seminiferous tubules were affected by methoxy acetic acid, and this may provide a second possible explanation for the temporal pattern of change in ABP and FSH levels. It is well established that the secretory function of the Sertoli cell changes according to the stage of the spermatogenic cycle (Sharpe, 1986) and, *in vitro*, ABP secretion by isolated seminiferous tubules is highest at stages VII to VIII (Ritzen et al, 1982). Therefore, it is perhaps significant that the period of elevated ABP levels in interstitial fluid from treated rats (days 3 and 21) occurred when tubules at stages VII to VIII were damaged. Conversely, at 14 days tubules at these stages appeared normal and are presumed to have been normal at 28 days. At both of these times, ABP levels also were normal. While these results may appear to be the opposite of that expected from the *in vitro* findings (ie, low ABP levels would have been predicted at 7 and 21 days when stages VII to VIII were damaged), ABP levels in interstitial fluid apparently are increased only when ABP secretion into the lumen is reduced, as described above.

In the present studies, treatment with methoxy acetic acid caused no significant change in T levels in serum or within the testis, both inside and outside of the seminiferous tubules. However, since some of the tubules in the testis of treated animals remained apparently normal and since damaged and undamaged tubules were not distinguished during tubule isolation, it remains possible that methoxy acetic acid could have induced a change in intratubular T that was masked by the presence of "normal" tubules.

From this study it is concluded that methoxy acetic acid and other chemicals with selective germ cell toxicity should be useful tools for the study of testicular paracrine interactions *in vivo*. This approach will also permit the elucidation of the relationship between observed intratesticular changes and subsequent fertility, which remains to be established, and may provide insight into possible causes of idiopathic infertility in man and animals.

Acknowledgments

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