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Effects of colchicine on spermiogenesis in the mouse

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SUMMARY

The effects of colchicine on spermiogenesis in the mouse were examined. Mice were injected intratesticularly with colchicine in concentrations of 10^{-4}M to 10^{-6}M for various periods of time. The ultrastructure of the testes was examined and abnormal sperm in the epididymis were scored. Colchicine degraded microtubules in the testis and the treatment caused abnormalities of the head and acrosome of testicular spermatids and also resulted in an increase on the number of epididymal sperm showing abnormal head structure by 5 days after treatment of the testes. These results suggest that structurally intact microtubules are essential for the normal morphogenetic processes of spermiogenesis.

INTRODUCTION

The cellular processes controlling the shaping of sperm cells are not well understood. In the case of the sperm head, where dramatic shaping in the form of elongation and curvature occurs during spermiogenesis, the mechanistic forces have long been the subject of investigation and controversy. While it has been suggested that microtubules cause shaping, it has also been shown that in some species nuclear shaping can occur in the absence of microtubules (Phillips, 1974; van Deurs, 1975). In an informative and provocative review of this subject (Fawcett, Anderson & Phillips, 1971), the hypothesis was advanced that extrinsic factors may not be responsible for head shaping in spermatids, but rather that intrinsic, species-specific patterns of chromatin condensation may determine the final shape of the sperm head. However, in mammals the problem of sperm morphogenesis is particularly complex. Not only are there elaborate arrays of microtubules, the manchette, surrounding the nucleus, but there are also intricate and specialized associations between the germ cells and the supporting Sertoli cells, which themselves contain numerous microtubules. The role of these microtubular structures, and of other structures, such as microfilaments, possibly associated with the microtubules, in controlling sperm morphogenesis are far from clear. Experimental application of agents which degrade microtubules can be of aid in clarifying the role of microtubules.

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The work described in this report was undertaken to analyze the effects of colchicine, a compound which binds to and degrades microtubules (Olmsted & Borisy, 1973; Weisenberg, Borisy & Taylor, 1968), on spermiogenesis in the mouse. The results demonstrate that colchicine treatment results in sperm structural abnormalities and therefore suggest that colchicine, by interacting with microtubules, interferes with normal morphogenetic processes of spermiogenesis.

MATERIALS AND METHODS

Sexually mature male ICR mice (Cumberland View Farm, Clinton, Tenn.) were lightly anaesthetized with ether and a small incision was made in the skin of the scrotal region. The testes were exposed through this incision and 20 μl of colchicine solution was injected into one or both testes. Colchicine (Sigma, St Louis, Mo.) was dissolved in phosphate-buffered saline, pH 7·3 (PBS) in concentrations ranging from 10^{-4} M to 10^{-6} M. At least six mice were injected with each concentration of colchicine. Controls were either uninjected or injected with 20 μl of PBS. (No ultrastructural differences were observed between the two groups of controls.) The testicular route of injection was chosen to minimize possible systemic effects of colchicine. Animals maintained longer than 3 days (for studies of epididymal sperm) were re-injected on the third day. Animals recovered rapidly from this minor surgery and were housed in groups of two to six, with food and water *ad libitum* and with a 12 h light–dark schedule.

Animals were killed by cervical dislocation and the testes and epididymes removed. The testes were stripped of the tunica albuginea and the tubules were placed immediately into 2·5% glutaraldehyde in 0·1 M sodium cacodylate buffer, 0·003 M-$CaCl_2$, pH 7·0, cut into small pieces and fixed for 1 h. The tissue was rinsed in the same buffer, post-fixed for 1 h in 1% OsO_4 in the same buffer, dehydrated in ethanol, and embedded in Epon (Luft, 1961). Thick sections for light microscopy examination and orientation were cut with glass knives and stained (Jeon, 1965). Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate (Venable & Coggeshall, 1965), and examined in a Zeiss EM 9 electron microscope.

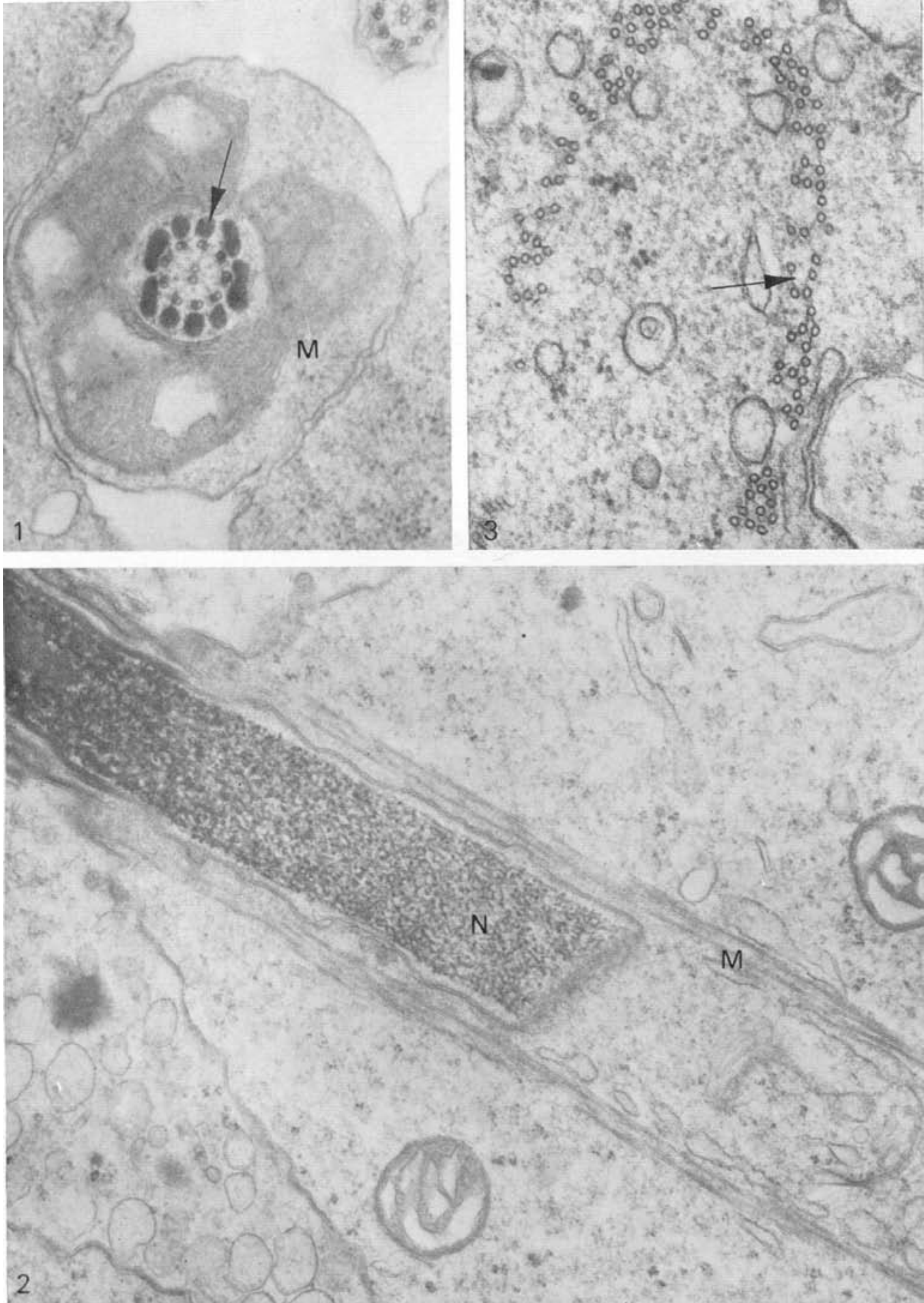
The epididymes of mice treated for 3, 5, and 9 days were bisected to form caput and caudal halves. These were minced in PBS, left undisturbed for 15 min, and then filtered through 80 μm nylon mesh. Eosin Y was added to the sperm suspensions, and air-dried slides were prepared 30 min later. Slides were examined and abnormal sperm scored. The slides were scored blindly, and only after tabulation of the results were the data correlated with the treatment.

RESULTS

The effects of colchicine on spermiogenesis and the structure of the seminiferous epithelium were observed by ultrastructure analysis of thin sections. As detailed below, colchicine treatment resulted in abnormal sperm. Technical limitations preclude quantitative data from thin-section analysis. However, the observed abnormalities increased with increasing colchicine concentration and increasing time of treatment. There were very few abnormalities (comparable to the controls) in mice treated with 10^{-6} M colchicine for 24 h, while abnormalities were increasingly more prevalent in mice treated with 10^{-5} M and 10^{-4} M colchicine and for longer periods of time. This dose-response relationship suggests not only that the range of colchicine concentrations is appropriate but also that the observed effects are, in fact, due to the colchicine treatment.

Microtubules of the cells of the seminiferous epithelium showed differential sensitivity to colchicine. Regardless of colchicine concentration and independent of length of treatment, no evidence was found for a degradative effect of colchicine on the axonemal complex (Fig. 1). There was also no evidence for an effect of colchicine on the initiation and elongation of the axonemal complex since spermatids exposed to colchicine for the entire period of axonemal elongation possessed apparently normal axonemes. Another prominent microtubular complex of the spermatid is the manchette. Microtubules of the manchette of mouse spermatids are initially associated with the post acrosomal portion of the head and are later seen as a sheath surrounding and extending beyond the caudal portion of the elongating nucleus (Fig. 2). Observed in cross-section, these microtubules are clustered and frequently cross-linked (Fig. 3). The microtubules of the manchette were found to be relatively resistant to colchicine treatment in that at least some microtubules were observed at all colchicine concentrations (Fig. 4). However, structured and cross-linked arrays were not observed as frequently in colchicine-treated animals as in the control animals. Microtubules of the Sertoli cells were quite sensitive to colchicine treatment, and were rarely observed in the treated animals.

In addition to these specific effects of colchicine treatment on the microtubular arrays of the seminiferous epithelium, general effects were observed on sperm morphology and on the structural organization of the seminiferous epithelium. Abnormal spermatid head profiles were frequently observed in the testes of animals treated with higher concentrations of colchicine (10^{-5} M and 10^{-4} M) and for longer periods of time (48–72 h). One of the most frequently observed abnormalities was indentations of the nucleus containing pockets of spermatid cytoplasm (Fig. 4). Sertoli cells were also observed to be susceptible to morphological effects of colchicine treatment. Though these cells frequently showed apical bulges and intracellular clefts (particularly at the time of spermiation) in the controls, these effects were exaggerated in the colchicine-treated animals,



Figs. 1–3. For legends see facing page.

where the cells showed a lack of normal associations, protruded into and nearly obscured the lumen (Fig. 5).

By the time spermatids exposed to colchicine in the testis would be expected to reach the epididymis, there was an increase in the number of sperm in the epididymis with abnormal head structure. Normal and abnormal head shapes scored are shown in Fig. 6 and the percent abnormalities with various treatments is shown in Table 1. The caput and caudal regions of the epididymis were scored after 3, 5, and 9 days of treatment. (By 5 days following the initial colchicine injection, significant numbers of spermatids exposed to colchicine in the testis during the elongation phase of spermiogenesis should have reached the epididymis.) For tests of significance, abnormalities resulting from different concentrations of colchicine were grouped, as there appeared to be no direct correlation between colchicine concentration and percent abnormalities (see below). At 3 days after treatment, there were no significant differences in the number of abnormal sperm in the colchicine-treated animals when compared to the saline-injected controls. By 5 days, the number of abnormal sperm in the caput region (closest to the testis) was significantly greater than in the controls; by 9 days after initial treatment, there was a significant increase in caput sperm abnormalities over the 5-day values (and over the control value). In the cauda epididymis (further from the testis and therefore receiving sperm subjected to colchicine several days later than the caput epididymis), there was a significant increase over controls in the number of abnormal sperm by 9 days after initial treatment. (All significant differences were at the level of $P < 0{\cdot}05$, using t tests.) It is particularly interesting to note that there is a general decrease in the number of sperm in standard dilutions with increasing concentration of colchicine and time after treatment, and that no sperm were observed in the caput epididymis after 9 days of exposure to colchicine at the highest concentration. Spermatids which were exposed to colchicine in the testis from the acrosomic phase of early nuclear elongation (stages 8–10, Oakberg, 1956) through spermiation, would be expected to have reached the epididymis by this time. It can be suggested that colchicine interferes with normal spermiation and/or transport or that colchicine results in sperm degeneration, and that these effects result in

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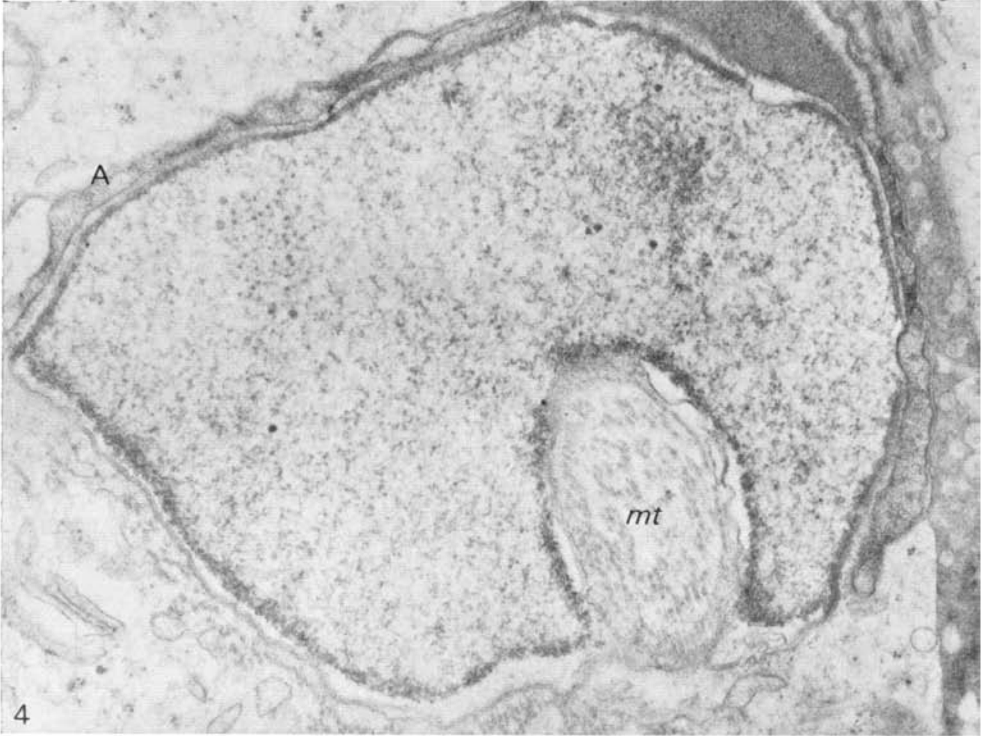
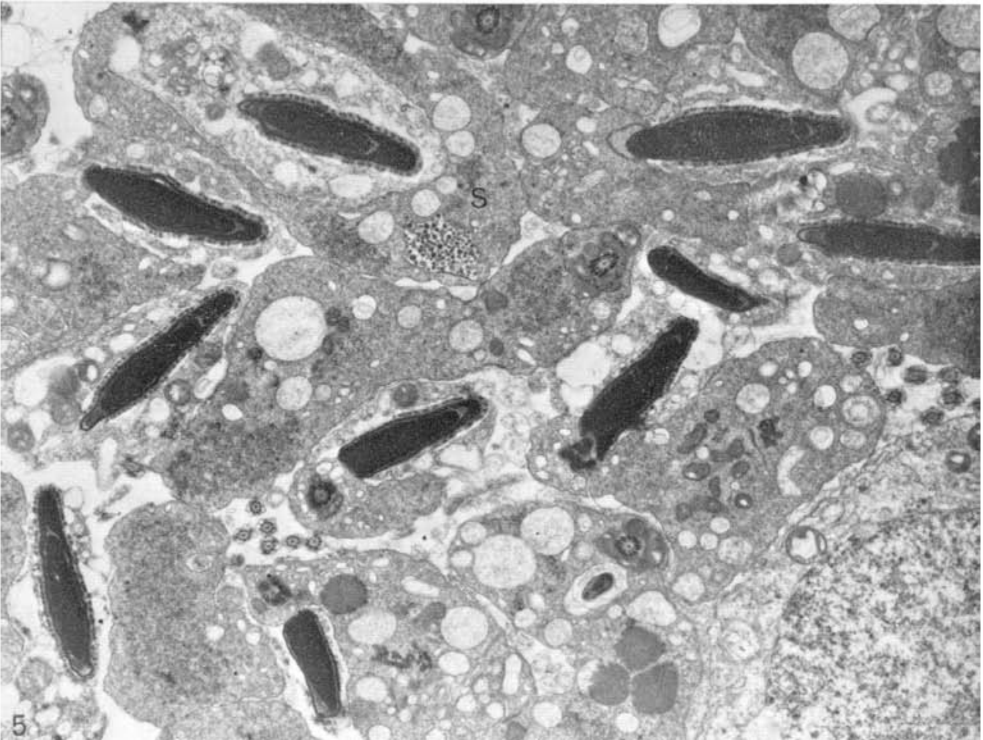
FIGURES 1–3

Fig. 1. This photograph is of a section through a testis tubule which was treated for 48 h with 10^{-5} M colchicine. The cross-section of the middle piece shows, in addition to the central axonemal complex, mitochondria (M) and the outer dense fibers (arrow).

Fig. 2. This photograph shows the microtubules of the manchette (M) associated with the most terminal portion of the nucleus (N) and extending posteriorly back through the spermatid cytoplasm. × 23660.

Fig. 3. Illustrated in this micrograph is a cross-section of the manchette posterior to the nucleus. Note the clustering of the microtubules and the occasional cross-bridges (arrow) linking adjacent microtubules. × 39000.





Figs. 4 and 5. For legends see facing page.

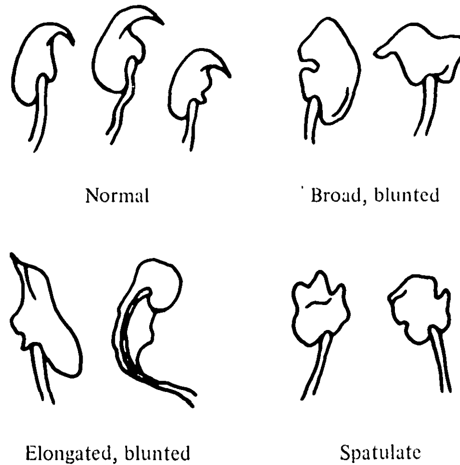


Fig. 6. Drawings of normal and abnormal sperm heads scored in the epididymes of control and treated mice.

Table 1. *Percentages of abnormal sperm in caput and cauda epididymes after colchicine treatment**

Colchicine concentration	Caput			Cauda		
	3 days	5 days	9 days	3 days	5 days	9 days
1×10^{-5} M	4·6 (0·9)	6·2† (1·0)	10·5 (1·1)	3·8 (0·7)	8·2 (1·0)	11·3 (1·1)
5×10^{-5} M	3·3 (0·7)	6·0 (1·2)	8·3 (1·2)	2·9 (0·7)	6·4 (0·9)	6·6 (1·1)
1×10^{-4} M	9·6 (1·1)	6·4 (0·8)	—‡	4·4 (0·8)	5·3 (0·9)	7·1† (1·2)
Control	4·2 (0·9)	4·4 (0·8)	5·7 (0·9)	2·4 (0·6)	4·8 (1·0)	6·2 (0·7)

* Between 500 and 900 sperm (with pooling of data from different mice) were scored except where noted. Standard errors are indicated in parentheses.

† Low numbers of sperm were present; between 350 and 450 sperm were scored.

‡ No sperm were present.

a decrease of sperm numbers. This effect may well account for the apparent lack of correlation between colchicine concentration and sperm abnormalities, since large numbers of abnormal sperm produced by treatment with high concentrations of colchicine may simply not be reaching the epididymis.

FIGURES 4 AND 5

Fig. 4. This figure illustrates a spermatid head from a testis treated for 48h with 10^{-4} M colchicine. Note the nuclear indentation containing microtubule-filled (*mt*) cytoplasm. Acrosomal cap (A) ×22750.

Fig. 5. This figure illustrates the luminal region of a testis tubule treated for 72 h with 10^{-5} M colchicine. The Sertoli cells (S) are highly vacuolated and contain multivesicular bodies. ×6750.

DISCUSSION

The work reported here demonstrates that colchicine, a microtubule degradative agent, causes sperm abnormalities and disruption of the seminiferous epithelium. Abnormalities of spermatids in the testis and of sperm in the epididymis include distortion of the shape of the head and acrosome. It is improbable that colchicine has caused abnormal head structure by interfering with chromatin condensation; in fact spermatid heads with normally condensed chromatin were seen in colchicine-treated animals. The principal mode of action of colchicine is degradation of microtubules by binding to tubulin subunits, and it is therefore not unreasonable to suggest that colchicine has disrupted normal morphogenetic processes in the testis by interaction with microtubules. The critical morphogenetic phases affected include the acrosomic phase (stages 8–11, Oakberg, 1956) during which head elongation is initiated and occurs to nearly the fullest extent (Dooher & Bennett, 1973), and the maturation phase (stages 12–16, Oakberg, 1956) during which chromatin condenses and rostral curvature of the spermatid head occurs. Judging by the abnormalities in head structure observed in the testis at these stages and those observed in the epididymis, it appears that these stages are particularly vulnerable to effects of colchicine treatment. It is interesting to note that these stages are apparently quite sensitive, for similar structural abnormalities are found in sperm of wild-type mice (the percentage abnormalities being strain specific), and furthermore, a number of mutations affecting male fertility in the mouse also give rise to similar abnormalities (see Hillman & Nadijcka, 1978*a*, *b* for further data and a review of the pertinent literature). In a sense, therefore, colchicine acts to produce a phenocopy not only of mutational effects but also of 'mistakes' which apparently occur naturally. These combined observations suggest that these stages of spermiogenesis are particularly sensitive to alterations in the microenvironment, whether genetically induced or chemically induced. The induction of similar effects by colchicine, a chemical with a known mode of action, can provide insight into some of the morphogenetic processes occurring during these critical stages of spermiogenesis.

The analysis of the morphogenetic effects can best be evaluated by first examining the site of colchicine-sensitive microtubules. Microtubules are found both in the Sertoli cells and in the spermatids. In Sertoli cells the microtubules are situated predominantly in the apical region, that portion of the cell closely associated structurally (and in all probability, functionally) with the spermatids. Microtubule profiles were not frequently observed in Sertoli cells after colchicine treatment. The degradation of Sertoli cell microtubules by colchicine was also observed by Wolosewick & Bryan (1977). A variety of abnormalities were observed in the Sertoli cells, including disorganization of normal contacts and extreme apical bulging into the lumen. This latter loss of cell shape was so extensive that in some cases the normal structure of the seminiferous epithelium

was disrupted and the lumen was obscured. The alteration of cell shape can reasonably be attributed to degradation of microtubules in the Sertoli cells. These are highly asymmetric cells, extending from the periphery of the tubule to the lumen. On the basis of similar experimental studies, microtubules have previously been implicated in the maintenance of cell shape (Handel & Roth, 1971; Karfunkel, 1971; Downie, 1975). In the Sertoli cells, where there are not specialized intercellular anchoring devices at the luminal end to aid in shape maintenance, the microtubules may be essential in maintaining cell shape.

Microtubules of the spermatid include those of the axonemal complex and of the manchette. The present study demonstrates that colchicine treatment does not disrupt the axonemal complex. Microtubules of the manchette are reduced in number, but not completely degraded by colchicine (also observed by Wolosewick & Bryan, 1977). The relative resistance may be due to the fact that manchette microtubules are associated with terminal densities in the nuclear ring (Rattner & Brinkley, 1972; Rattner & Olson, 1973). It has been observed previously that cytoplasmic microtubules associated with dense (perhaps stabilizing) material are more resistant to degradation (Handel & Roth, 1971; Wolosewick & Bryan, 1977).

A functional role for the microtubules of the manchette in shaping of sperm heads has been suggested on the basis of descriptive ultrastructural studies (Kessel, 1966; McIntosh & Porter, 1967), on the basis of studies of genetic mutation in *Drosophila* (Shoup, 1967; Wilkinson, Stanley & Bowman, 1974; Habliston, Stanley & Bowman, 1977) and in the mouse (Dooher & Bennett, 1974, 1977), and on the basis of experimental studies with vinblastine (Wilkinson, Stanley & Bowman, 1975) and Colcemid (Rattner, 1970). The present study adds to the above experimental evidence for the involvement of microtubules in sperm shaping in that it demonstrates abnormalities of both testicular sperm and of epididymal spermatozoa exposed to colchicine.

The available experimental evidence, therefore, clearly implicates microtubules as important elements in the morphogenesis of sperm cells. What is not clear, however, is whether the microtubules of the manchette are responsible for shaping and what is the magnitude of the contribution of the microtubules of the Sertoli cells. These problems must be resolved by further experimental analysis. Furthermore, contrary to the common assumptions, the morphogenetic effects following degradation of microtubules do not necessarily permit the interpretation that microtubules are directly responsible for morphogenetic forces ('Maxwellian demons', as envisioned by McIntosh & Porter, 1967). Microtubules are part of an intricate cytoskeleton (the structure of which is not well understood) and may well be linked to other structures, such as actin filaments, which may be more directly responsible for the shaping forces. Degradation of microtubules might well result in disorganization of force generating structures. Therefore, colchicine-induced sperm abnormalities may be an indirect result of microtubule degradation. These data suggest that

microtubules are essential, but not necessarily directly responsible, for maintaining the normal processes of spermiogenetic morphogenesis.

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