CYTOPLASMIC INCLUSIONS IN XENOPUS SPERMATOGENIC CELLS. ULTRASTRUCTURAL AND CYTOCHEMICAL ANALYSIS OF THE ACTION OF ANTIMITOTIC AGENTS ON SUBCELLULAR ELEMENTS

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SUMMARY

The effects of vinblastine sulphate, alone and in combination with other antimitotic agents, were tested in vitro on testicular tissue from the anuran, Xenopus laevis. Treatment with vinblastine alone resulted in dissolution of spindle and intercellular bridge microtubules, but centrioles and sperm tail axonemes remained intact. In addition, no paracrystalloid inclusions typical of those reported in other cell types treated with vinblastine alone were observed in Xenopus spermatogenic cells. Such inclusions were formed, however, when vinblastine was administered in combination with colchicine, colcemid, or puromycin. Under optimal conditions, paracrystalloids were formed in all cells from primary spermatogonia to late spermatids. The maximum size paracrystalloid formed differed in different spermatogenic cell stages, with late primary spermatocytes containing the largest such inclusions, often averaging over 34 μm³ each. Autoradiographic analysis of spermatogenic cells labelled with [3H]leucine prior to drug treatment indicated that some radioactive material was incorporated into paracrystalloids in all cell stages. Treatment of Xenopus spermatogenic cells with vinblastine and puromycin was also employed to investigate the possible relationship between paracrystalloid material and a naturally occurring spermatogenic cell inclusion, the chromatoid body, since previous experiments indicated that microtubule protein might contribute to the mass of this structure. It was found that under certain conditions, both paracrystalloids and giant chromatoid bodies can occur together in the same cell, each inclusion reaching a characteristic size independent of the other. This suggests that the two inclusions are aggregates of materials drawn from different subcellular precursor pools.

In addition, autoradiographic analysis of isotope incorporation indicated that giant chromatoid bodies could be clearly labelled with tritiated amino acids, but not with [3H]uridine. The pattern of isotope incorporation observed in giant chromatoid bodies, coupled with their histochemical staining characteristics, rules out the possibility that these structures function as cytoplasmic nucleoli.

INTRODUCTION

Many spermatogenic cells contain large numbers of microtubules. These organelles have been implicated in the performance of several critical functions during the course of spermatogenesis, including mitosis, intercellular bridge formation, meiosis, postmeiotic nuclear shaping, and formation of the sperm tail axoneme (e.g. Fawcett, Anderson & Phillips, 1971; Kalt, 1973; Phillips, 1974; Wilkinson, Stanley & Bowman, 1975). Several studies on invertebrates have indicated that sperm tail axoneme micro-
tubules, unlike many other microtubules, are resistant to a potent antimitotic drug, vinblastine sulphate (Behnke & Forer, 1972; Wilkinson et al. 1975). While the reason for such drug resistance is not known, it has been demonstrated in invertebrate oocytes that the effects of vinblastine on microtubules may be potentiated by concomitant treatment with the protein synthesis inhibitor, puromycin, or with antimitotic agents which do not compete with vinblastine for binding sites on microtubule protein (Strahs & Sato, 1973; Starling, 1976a, b). Such treatments have been demonstrated to result in an aggregation of free tubulin subunits into large paracrystallloid structures, similar to those observed in mammalian cells treated with vinblastine alone (Bensch & Malawista, 1969).

Since no information exists on either the vinblastine sensitivity of vertebrate spermatogenic cell microtubules, or on the ability of other antimitotic agents to potentiate vinblastine in any spermatogenic cells, the present study has examined these two questions in the vertebrate anuran, *Xenopus laevis*. Attention was also given to assessing the effects of such treatments on other cell inclusions, since puromycin by itself has been shown to induce a 30-fold increase in the volume of a naturally occurring spermatogenic cell inclusion in *Xenopus*, the chromatoid body, and indirect evidence suggests that CBs may be composed in part of microtubules (Kalt, Pinney & Graves, 1975). In addition, autoradiographic analysis of isotope incorporation was employed to determine: (1) whether chromatoid bodies and paracrystalloids contained newly synthesized proteins and/or RNA; (2) whether labelled material would continue to accumulate in the presence of inhibitors of protein synthesis; and (3) whether the kinetics of incorporation of precursors into induced cytoplasmic inclusions differed from those of incorporation into other constituents of the cell.

Results indicate that differential sensitivity of separate functional populations of spermatogenic cell microtubules to antimitotic agents exists in *Xenopus*. Furthermore, the maximum size of the paracrystallloid structures formed with appropriate drug treatment differs in different spermatogenic stages. In addition, under certain conditions, both paracrystalloids and giant chromatoid bodies can occur together in the same cell, each reaching a characteristic maximum size independent of the other. This suggests that the 2 inclusions are aggregates of materials drawn from different precursor pools within the same cell. Autoradiographic analysis of isotope incorporation has also ruled out the possibility that the giant chromatoid body in *Xenopus* functions as a cytoplasmic nucleolus (Al-Mukhtar & Webb, 1971).

**MATERIALS AND METHODS**

All animals were obtained from the South African Snake Farm, Fish Hoek, Cape Province, South Africa, and were maintained on a diet of beef heart and liver at 18–19 °C (Kalt, 1976a) for periods of from 1 day to over a year prior to experimentation. Except where indicated, drug and radioactive-labelling experiments were performed using minced chunks of individual testes in the *in vitro* culture system described by Kalt et al. (1975). In some cases, chunks were cultured in serum-free defined medium (Eppig & Dumont, 1976). For each experiment, one control specimen per testis was fixed upon dissection, and a second control specimen was fixed at the termination of the experiment after having been maintained in complete medium without drugs. In all procedures, cell stages were identified using the nuclear criteria outlined by Kalt (1973, 1976a).
Experimental procedures

Effects of antimitotic agents on microtubules. For each experiment, 2 basic drug administration procedures were employed. The 'warm' procedure simply consisted of adding tissue chunks to drug-containing medium at 25 °C and maintaining the same temperature for the duration of the experiment. The 'cold' procedure consisted of adding tissue chunks to medium containing drugs for 30 min at 25 °C, cooling the tissue on ice for 30 min, and finally rewarming to 25 °C for the duration of the experiment.

Vinblastine sulphate (Velban, Eli Lilly) was used alone at concentrations of $10^{-4}$ M and in combination with each of the following: (1) $10^{-4}$ M colchicine (Sigma Chemical Co., DeKalb, Ill., U.S.A.); (2) $10^{-4}$ M colcemid (Sigma); or (3) 50 or 100 μg/ml puromycin (Sigma). In addition, $10^{-3}$ M vinblastine sulphate was tested with a combination of $10^{-4}$ M colchicine plus 100 μg/ml puromycin.

The sensitivity of Xenopus spermatogenic cells to various antimitotic treatments was determined in 2 ways. First, polarized light and phase-contrast light-microscopic observations were made on single, live, wet-mount cells teased out of drug-treated tissue by mechanical dissection. Second, ultrastructural analysis of microtubule integrity was performed on corresponding fixed specimens. For each experiment, chunks from a single testis were fixed 4, 8 and 16 h after addition of the drugs.

The average volumes of induced paracrystalloids were determined by optical measurement of photographs of 1-μm thick Epon-embedded serial sections. Specimens chosen were cold-treated 16 h with $10^{-4}$ M vinblastine plus $10^{-3}$ M colchicine. The cross-sectional area of single paracrystalloids was determined for each section and total volume calculated by addition of the total number of sections in which a single paracrystalloid appeared. Twenty representative cells were chosen for each spermatogenic stage examined.

Radioactive labelling of cells containing paracrystalloids induced by antimitotic drug treatments was also examined by incubating tissue in leucine-free Defined Nutrient Oocyte Medium (Eppig & Dumont, 1976) containing 10% heat-inactivated foetal calf serum (Gibco, Grand Island, New York, U.S.A.) to which was added 50 μCi/ml [3H]leucine (New England Nuclear Co., Boston Mass., U.S.A.) sp. act. 5 Ci/mm. $5 \times 10^{-4}$ M vinblastine sulphate plus $5 \times 10^{-4}$ M colchicine was added 2, 4 or 8 h after isotope addition, and the tissue was incubated at 25°C for an additional 12 h. Upon termination of the experiment, and prior to fixation, specimens were rinsed in 3 10-min changes of complete medium with drugs but without isotope.

Chromatoid body experiments. Several cytochemical procedures were used to analyse chromatoid bodies observed during the course of the present investigation. Ultrastructural localization of nucleic acids was performed on trihaldehyde-fixed Xenopus testes (Kalt & Tandler, 1971) which had been reacted with indium trichloride according to the procedure of Watson & Aldridge (1961). After staining, tissues were dehydrated in acetone and embedded in Vestopal resin (Electron Microscopy Sciences, Inc. data sheet, Fort Washington, Pa., U.S.A.). In some instances thin sections were post-stained with lead citrate (Reynolds, 1963), a procedure found to enhance contrast non-specifically.

In separate experiments, cells were labelled with each of the following isotopes at a final concentration of 50 μCi/ml: [3H]uridine, sp. act. 50 Ci/mm; [3H]lysine, sp. act. 70 Ci/mm; [3H]leucine, sp. act. 50 Ci/mm; [3H]phenylalanine, sp. act. 50 Ci/mm. (All isotopes were obtained from the New England Nuclear Corp.) For each procedure, 2 drugs dissolved in DNOM medium containing 10% heat inactivated foetal calf serum were used in separate experiments: puromycin (Sigma) at a concentration of 100 μg/ml; and chloramphenicol (Sigma) at a concentration of 250 μg/ml. Three basic incorporation studies were performed with each isotope to determine the labelling kinetics of giant chromatoid bodies: (1) the isotope was added 8 h prior to the addition of drugs, so normal incorporation would occur; (2) the isotope was added with drugs, so that incorporation during the onset of drug effect could be assessed; (3) the isotope was added 4 h after the addition of drugs, so that induced chromatoid body enlargement and any inhibitory effect of the drug on cellular metabolism should have already been well underway. In all experiments, incorporation was terminated after 16 h of labelling by transferring specimens through 3 changes of complete medium with drugs but without isotope. Control experiments were run using the same labelling protocol without drugs to establish the normal in vitro pattern of incorporation into testicular tissue.
Xenopus spermatogenic cell inclusions

In all autoradiographic experiments, tissues were fixed and processed for autoradiography as described previously (Kalt, 1976a). For all electron-microscopic observations, preparation and examination of specimens was performed as described by Kalt et al. (1975).

Figures 2, 7, 11–13, 15 and 16 are light micrographs. All others are electron micrographs.

RESULTS

Morphological effects of vinblastine alone and with other antimitotic agents

Vinblastine alone at concentrations of \(10^{-4}\) M or greater caused mitotic and meiotic arrest, as measured by increases in metaphase cells over controls; partial loss of spindle birefringence, as measured on teased-out wet-mount cell preparations; and ultra-structural disruption of both spindle and intercellular bridge microtubules in all stages where such structures were normally present. In contrast, vinblastine at concentrations up to \(10^{-3}\) M in medium with or without serum was ineffective in producing any morphological disruption of centrioles or sperm tails, even when the 'cold' procedure was used. In no case did vinblastine-treated cells develop the paracrystallloid structures commonly associated with the use of this agent, both in mammalian cells (Bensch & Malawista, 1969) and in oocytes, including those of Xenopus (e.g. Dumont & Wallace, 1972).

Fig. 1. Late secondary spermatogonial or premeiotic S cell cold-treated with \(10^{-4}\) M vinblastine plus \(10^{-3}\) M colchicine 16 h. A large drug-induced paracrystallloid (p) occupies most of the cytoplasmic area of the section. N, nucleus. \(\times 12500\).

Fig. 2. Early primary spermatocytes from the same specimen as Fig. 1. Large juxta-nuclear paracrystalloids (arrows) are visible in several cells. \(\times 900\).

Fig. 3. Pachytene primary spermatocyte from same specimen as Figs. 1 and 2. A large paracrystallloid (p) exhibiting a regular hexagonal packing of subunits occupies the centre of the micrograph. A portion of the nucleus (n) is visible at the lower right. \(\times 20000\).

Fig. 4. Chromatoid body region (cb) of an early spermatid warm-treated 16 h with \(10^{-4}\) M vinblastine plus \(10^{-3}\) M colchicine. A typical vesiculated chromatoid body (see figs. 26–27, Kalt, 1973) is juxtaposed with a vinblastine-induced paracrystallloid (p). \(\times 27000\).

Fig. 5. Cross-section of the neck region of a late spermatid warm-treated 16 h with \(10^{-4}\) M vinblastine plus \(10^{-3}\) M colchicine. A large vinblastine-induced paracrystallloid (p) fills the protruding collar region of the neck, surrounding the descending sperm tail axoneme (a) which still contains an intact '9 + 2' arrangement of microtubules. Other intact axonemes are evident in the surrounding cells. \(\times 20000\).

Fig. 6. Cytoplasmic region of a primary spermatogonium, warm-treated 16 h with 100 \(\mu\)g/ml puromycin plus \(10^{-4}\) M vinblastine from an animal which was 'puromycin insensitive' with respect to chromatoid body enlargement. A 'normal' sized spermatogonial chromatoid body (cb) and a portion of a loosely organized aggregate (ag) are present. Contrast this aggregation induced in the presence of puromycin to that induced in the presence of colchicine (Fig. 3). \(\times 29000\).

Fig. 7. Pachytene spermatocytes warm-treated 16 h with 100 \(\mu\)g/ml puromycin plus \(10^{-4}\) M vinblastine from an animal which was puromycin sensitive with respect to chromatoid body enlargement. Both a giant chromatoid body (arrow) and vinblastine-induced aggregations (ag) are present. Note the size of the paracrystalloids relative to the nucleus and the fact that one paracrystallloid is contained in each cell. \(\times 1600\).
Further experiments, therefore, were undertaken to test the effects of vinblastine potentiating drugs (see Introduction) on *Xenopus* spermatogenic cells. Initial results were dramatic. When $10^{-4}$ M vinblastine was administered with $10^{-4}$ M or more colchicine or colcemid, within 4–16 h virtually all spermatogonia, primary and secondary spermatocytes, and early spermatids formed typical vinblastine paracrystalloids (Figs. 1–5). The largest paracrystalloids characteristically appeared in meiotic prophase cells, where a single structure averaging approximately $34 \mu m^3$ was observed within all cells of a spermatocyst (Figs. 2, 3 and Table 1). This response occurred throughout the tissue, but longer culture times were required to produce a uniform response all the way to the centre of the specimen. In postmeiotic spermatid stages, a morphologically intact axoneme remained evident in the tail, while large paracrystalloids formed in the neck region of the same drug-treated cells (Fig. 5). Mature spermatozoa, in contrast to other cell stages, remained motile and ultrastructurally unchanged with all combinations of antimitotic drugs tested. Live spermatozoa examined by phase-contrast light microscopy after extended treatment with vinblastine plus colchicine still exhibited motility. In addition, ultrastructural examination of such spermatozoa, regardless of drug treatment, revealed the continued presence of intact axonemes and centrioles, and an absence of paracrystalloids.

**Effects of puromycin**

Puromycin has been demonstrated to potentiate the effects of vinblastine in invertebrate oocytes and, by itself, has been shown to cause marked increases in the size of chromatoid bodies in *Xenopus* spermatogenic cells (see Introduction). Therefore, in the hope of demonstrating a possible interrelationship between chromatoid bodies and microtubules, experiments were conducted using puromycin in combination with vinblastine and colchicine.

Puromycin at a dosage of 100 $\mu g/ml$ was able to potentiate the antimitotic effects of vinblastine with the same stage specificity as described in the previous section. In this instance however, the result often was not a fully formed paracrystalloid, but rather a loosely organized aggregate of material with little regular structure evident.
in sections observed with the electron microscope (compare Fig. 6 to Fig. 3). Addition of colchicine to mixtures of puromycin and vinblastine resulted in more ordering of substructure in paracrystalloids (Fig. 10), but did not increase their size or frequency of occurrence.

Initial treatments of cells with vinblastine and puromycin elicited erratic results with respect to chromatoid body enlargement. While induction of the typical paracrystalloid arrangement described above was reproducible, the identical drug dosage in some instances resulted in marked enlargement of the chromatoid body, and in others produced no change at all in this structure (Figs. 6-10) as compared to control specimens from the same testis (see Table 2 for normal size values). Careful scrutiny of experimental parameters including photoperiod, temperature, feeding, source and handling of drugs, and shipment lot of animals ruled out all identifiable variables except seasonal fluctuations or source of animals as possible factors influencing puromycin sensitivity of chromatoid bodies. Ultimately, testicular biopsy of specimens from several different shipments indicated that there appeared to be a subpopulation of *Xenopus* which was sensitive to drug treatment, and one which was not. In addition, biopsy revealed a small number of frogs from different shipments which had been in the laboratory for periods of time ranging from 1 week to over a year that contained germ cells with naturally occurring giant chromatoid bodies. Ultrastructural analysis showed these naturally occurring structures to be identical to those induced by puromycin. Re-examination of animals with naturally occurring giant chromatoid bodies was then carried out periodically for up to 6 months after initial identification. Results indicated the continued presence of giant chromatoid bodies. Conversely, no animals which biopsy revealed to contain 'normal' sized chromatoid bodies developed the 'giant' chromatoid body syndrome within the same period of time. Prior to performing subsequent experiments, therefore, it became necessary to biopsy all frogs used to establish first, whether they contained small or giant chromatoid bodies; and second, whether their germ cells were sensitive to puromycin or chloramphenicol. Further results described below are from animals preselected in this manner.

When animals containing puromycin-sensitive chromatoid bodies were treated with vinblastine and puromycin, or vinblastine, puromycin and colchicine, both giant chromatoid bodies and paracrystalloids always formed in the appropriate spermatogenic stages (Figs. 7-9). Likewise, when animals with naturally occurring giant chromatoid bodies were treated with similar drug combinations, an identical pattern of giant chromatoid bodies and vinblastine paracrystalloids was observed (Fig. 10). To summarize, in all cases where both giant chromatoid bodies of any origin and paracrystalloids occurred in a single cell, no diminution in the typical size or structure of any inclusion was detected, and no obvious continuity between the 2 types of inclusions was observed in hundreds of cells examined. Finally, when puromycin was administered to animals which already contained natural giant chromatoid bodies, no further increase in size was detected, and the only change in composition appeared to be a slight increase in the number of peripheral ribosomes associated with the structure.
Xenopus spermatogenic cell inclusions

Cytochemical analysis of spermatogenic cell inclusions

Autoradiographic studies demonstrated that the amount and pattern of grain localization in spermatogenic cells was dependent on the length of incorporation, the presence or absence of drugs, and the cell stage. Under optimal conditions, both naturally occurring and drug-induced giant chromatoid bodies in all cell stages were definitely labelled with all amino acids tested (see Materials and methods). The degree of grain localization over giant chromatoid bodies, however, did not appear to differ significantly from the grain densities observed over other labelled structures within the cell (Figs. 11–13). Administration of puromycin prior to, or at the same time as

Table 2. Chromatoid body size in Xenopus spermatogenic cells

<table>
<thead>
<tr>
<th>Population</th>
<th>Normal diameter, μm/μm³</th>
<th>Puromycin induced</th>
<th>Natural giant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1° spermatogonia</td>
<td>2.5/0.2</td>
<td>8.0/0.268</td>
<td>8.0/0.268</td>
</tr>
<tr>
<td>2° spermatogonia</td>
<td>2.0/0.2</td>
<td>4.0/0.335</td>
<td>3.5/0.324</td>
</tr>
<tr>
<td>Meiotic prophase</td>
<td>0.5/0.07</td>
<td>2.5/0.2</td>
<td>2.5/0.2</td>
</tr>
<tr>
<td>Early spermatid</td>
<td>0.2/0.004</td>
<td>1.5/0.1</td>
<td>1.0/0.5</td>
</tr>
</tbody>
</table>

* Data taken from Kalt et al. (1975).
† Measurements made from normal animals treated with 100 μg/ml puromycin for 8 h in vitro as described by Kalt et al. (1975).
‡ Data taken from untreated biopsy specimens studied in the present investigation.

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Fig. 8. Pachytene spermatocyte warm-treated 16 h with 100 μg/ml puromycin plus 10⁻⁴ M vinblastine from an animal which was puromycin insensitive with respect to chromatoid body enlargement. A vinblastine-induced aggregation (ag) is present at right, while 2 normal sized chromatoid bodies (cb) and 2 intact centrioles (c) are present to the left of the nucleus. × 11,500.

Fig. 9. Pachytene spermatocytes from the same specimen as Fig. 7 illustrating similar features at an ultrastructural level. Note the vinblastine-induced aggregate (ag), the enlarged chromatoid body (cb) (compare to Fig. 8) and the still-intact centrioles (c). n, nucleus. × 15,000.

Fig. 10. Portions of 2 secondary spermatogonia-premeiotic S cells from a specimen with naturally occurring giant chromatoid bodies cold-treated for 8 h with 100 μg/ml puromycin, 10⁻⁴ M vinblastine and 10⁻⁴ M colchicine. The giant chromatoid body (cb) is flanked by, but is separate from, vinblastine-induced paracrystalloids (p). n, nucleus. × 10,000.

Fig. 11. Puromycin-induced giant chromatoid bodies (arrows) in two 2 spermatogonia labelled with [³H]leucine 8 h prior to the addition of drugs. Note that the labelling of the chromatoid body is not significantly enhanced with respect to the rest of the cytoplasm. × 1000.

Fig. 12. Naturally occurring giant chromatoid body (arrow) in a primary spermatogonium labelled 16 h with [³H]leucine. Again, the chromatoid body is labelled, but not to a greater extent than other cellular structures. × 1000.

Fig. 13. Puromycin-induced giant chromatoid bodies (arrows) in a cluster of primary spermatocytes labelled with [³H]lysine for 8 h prior to the addition of drugs. × 1000.
isotope, while decreasing grain counts in both giant chromatoid bodies and the cell as a whole, did not change the relative grain distribution between structures within treated spermatogenic cells, and did not change the ultimate size of the giant chromatoid bodies in any cell stage. Administration of vinblastine and puromycin produced similar results, and in addition, produced paracrystalloids which were labelled when isotope was administered prior to drug treatment in all cell stages from primary spermatogonia up to early spermatids (data not shown).

In contrast to amino acids, in no case was substantial labelling of chromatoid bodies or paracrystalloids observed with tritiated uridine. Instead, at best only a scattered, largely peripheral grain localization was observed in some giant chromatoid bodies. More often, most chromatoid bodies in a cell cluster were not labelled at all, and even
those structures which were labelled had grain concentrations no heavier than that of 
the cytoplasm as a whole (Figs. 14, 15).

As might be expected, nuclei and nucleoli were labelled with tritiated uridine. All 
spermatogenic cell stages up to and including postacrosomal stage spermatids exhibited 
diffuse labelling over nuclei. However, the relative amount of nuclear labelling in a 
single specimen was different for each stage, with spermatids exhibiting relatively little 
incorporation, spermatogonia and early meiotic prophase cells moderate incorporation, 
and late meiotic prophase cells the heaviest incorporation. With respect to nucleoli, 
primary and secondary spermatogonia consistently were the most heavily labelled cell 
types (Fig. 15). In contrast, possible nucleolar labelling in meiotic prophase and post-
meiotic spermatogenic cells was not discernible due to high nuclear background, even 
though these cell stages have been demonstrated in vitro to synthesize ribosomal RNA 
(Kalt, 1976b). Another notable feature about uridine incorporation into Xenopus 
spermatogenic cells was the low degree of cytoplasmic label obtainable relative to the 
nucleus, even with extended incubation periods and chase times (Figs. 14, 15). Again, 
this phenomenon was most apparent in pachytene cells, where grain counts indicated 
that slightly over half of the radioactivity never left the nucleus (Fig. 14).

The distribution of nucleic acids in the cytoplasm of spermatogenic cells, as 
visualized by indium staining, was found to be consistent with the patterns of uridine 
incorporation observed above. In all cases, virtually no indium-stained material or 
ribosome-like particles were found within the mass of giant chromatoid bodies. In-
stead, indium-stained particles were confined to the periphery of these inclusions, 
and to the remainder of the cytoplasm (Fig. 16). Lead enhancement of contrast in 
indium-treated specimens also indicated a similar morphological disparity between 
the structure of the giant chromatoid body and that of both ribosomes and chromatin 
(data not shown).

**DISCUSSION**

Data from the present investigation demonstrate that 2 classes of microtubules exist 
in Xenopus spermatogenic cells. One class, encompassing spindle and intercellular 
bridge microtubules, is sensitive to treatment with appropriate antimitotic agents. A 
second class including centrioles and sperm tail axonemes, appears to be both structur-
ally and functionally resistant to similar drug treatments. The drug resistance of 
centrioles and axonemes cannot simply be due to lack of penetration of drugs, since 
vinblastine-colchicine induced paracrystalloids occur in other areas of the same cell 
(Figs. 5, 8, 9), and in parts of the tissue further removed from a free surface. While 
current data in Xenopus are not sufficient to establish the exact cause of differential 
drug sensitivity, one or more of several possible mechanisms have been demonstrated 
to exist in other cells. First, there may be a complete lack of tubulin turnover in 
resistant microtubules (e.g. Margulis, 1973; Olmsted & Borisy, 1973). Second, 
chemical differences may exist between drug-sensitive and drug-resistant structures, 
either within the tubulin subunits or within the microtubule-associated proteins 
(Stephens, 1970; Sloboda, Rudolph, Rosenbaum & Greengard, 1975; Bibring,
Baxendall, Denslow & Walker, 1976). Finally, there may be interactions of tubulin or drug-binding sites with other molecules within the cell that preclude disassembly by the agents employed.

Whatever mechanisms underly the resistance of *Xenopus* sperm tail axonemes and centrioles to drugs, such findings are in general accord with observations made on sperm in several invertebrate species (Behnke & Forer, 1972; Wilkinson *et al.* 1975). Likewise, the potentiation of the action of vinblastine by other antimitotic agents also has precedents in the literature (Strahs & Sato, 1973; Starling, 1976a, b). *Xenopus* spermatogenic cells appear to be somewhat more resistant to vinblastine than other organisms, however, in that no paracrystalloid formation at all occurred when vinblastine was administered by itself.

The relationship between the disappearance of drug-sensitive microtubules and the appearance of paracrystalloids in *Xenopus* spermatogenic cells suggests that, as in many other cell types, the paracrystalloids consist predominantly of microtubule protein (Wilson, Bryan, Ruby & Mazia, 1970; Bryan, 1971, 1972). If such paracrystalloids are assumed to be of a similar nature in each spermatogenic cell stage, then several additional inferences may be drawn. First, based on size of induced paracrystalloids, it appears as if pachytene spermatocytes contain slightly more total vinblastine-precipitable protein than in any other spermatogenic stage, including mitotically active primary spermatogonia. Second, autoradiographic incorporation observed over vinblastine-induced paracrystalloids suggests that this material is synthesized at least in part both in meiotic prophase cells and in spermatids. Third, the total amount of paracrystalloid material observed per cell suggests that vinblastine-precipitable proteins may be one of the predominant species of cytoplasmic proteins in primary spermatocytes as well as in postmeiotic stages.

While giant chromatoid body material must also constitute a significant amount of cytoplasmic protein, the distribution of putative microtubule protein in different spermatogenic cell stages (as visualized by induced paracrystalloids) is dissimilar to the stage-specific distribution pattern observed with giant chromatoid body material, in which the primary spermatogonial cell contains 30 times more chromatoid body material by volume than chromatoid bodies in meiotic prophase stages (Kalt *et al.* 1975) (Figs. 11-16). Therefore, chromatoid body enlargement cannot totally be explained as aggregation of vinblastine-sensitive microtubule protein in all stages, which was one possible explanation of the phenomenon (Kalt *et al.* 1975).

The fact that paracrystalloids reach the same maximum size in all cells of a given stage, regardless of the size chromatoid body that they contain, also suggests that material in vinblastine-induced paracrystalloids is not drawn from the same pool as chromatoid body material. This does not rule out, however, the possibility that the chromatoid body, like centrioles and axonemes, may contain tubulin in a form insensitive to any of the drug combinations tested.

Several additional conclusions about the composition of the chromatoid body in *Xenopus* spermatogenic cells may be drawn from labelling patterns observed in the present series of experiments. First, since no localized accumulation of newly synthesized material occurred when tritiated amino acids were administered concomitant
with or after puromycin (which severely inhibits protein synthesis in these cells) (Kalt, unpublished), it can be concluded that the formation of drug-induced giant chromatoid bodies probably does not require the synthesis of new proteins. At the same time, however, labelling of naturally occurring giant chromatoid bodies suggests that, in such instances, some synthesis of CB material occurs in all cells from primary spermatogonia to spermatids. With respect to RNA, both tritiated uridine incorporation patterns and indium trichloride staining argue against the presence of large amounts of RNA in Xenopus spermatogenic cell chromatoid bodies. The small amount of labelling observed in the present investigation appears to be associated with the presence of ribosomes at the periphery of the chromatoid body. This pattern is consistent with previous interpretations of Xenopus chromatoid body structure (Kalt, 1973; Kalt et al. 1975), and with the observation that the majority of labelled RNA localized in the cytoplasm of isolated Xenopus spermatogenic cells is transfer and ribosomal, not informational (Kalt, 1976a, 1977). In any case, evidence gathered to date indicates that the chromatoid body in Xenopus, while superficially similar in structure to a nucleolus, does not function as one in terms of the synthesis or accumulation of RNA.

The discovery of enlarged chromatoid bodies in apparently normal animals, along with drug resistance to puromycin and chloramphenicol in many others, requires comment. These observations suggest that the original study describing drug-induced giant chromatoid bodies (Kalt et al. 1975), while valid for the animals used in that investigation, may have to be modified for larger populations of Xenopus. At present, it is not certain whether these different chromatoid body states reflect genuine phenotypic variation in the population, or simply are the result of some environmental factor(s). Breeding experiments and continued biopsies are being carried out in the hope of resolving this question.

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