

and seed set. Seed lots from A₂ steriles originally obtained from post-rainy season of 1990–1991 as well as those from A₂ steriles which have set seed in summer and rainy seasons (1991) behaved exactly similar and were all sterile. The breakdown of sterility is perhaps due to high temperatures of summer and rainy seasons. The sterility will perhaps be maintained only if the temperatures are low as in the case of post-rainy season. In addition, it is possible that the shorter photoperiod of the post-rainy season might also be affecting developmental changes to result in male sterility. Temperature and/or photoperiod effects can be distinguished if studies are made in growth chambers with controlled photoperiod and temperature.

These observations are of special significance in commercial seed production programmes. Normally, male sterile lines in sorghum and also other crops like maize and pearl millet are maintained by growing the male sterile lines and their maintainers side by side in isolated seed production plots. Seeds from only the sterile plants which are crossed by the maintainer are harvested. This results in reduced yields of A line. In addition to this reduction in seed quantity, production

of male sterile seed is associated with several problems like synchronization of A and B lines, pollen dispersal, etc. These problems can be eliminated if the cytoplasmic system reported in the present study is utilized. The sterile parents using the cytoplasm can be simply maintained by growing them in the summer. Even if the seed set is less, still it will be economical since the other problems are eliminated.

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Giant spermatogonial cells generated by vincristine and their uses

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Vincristine treatment to albino rats has earlier been shown to cause formation of giant cells in the seminiferous tubules, by probably affecting spermatogenic mitosis. The present paper reports on the fate of the giant cells thus formed. These cells, highly intact, reach the caput epididymis, where also they maintain their identity, and then the cauda epididymis where they undergo fragmentation, cytolysis and phagocytosis. The study, in addition, demonstrates that vincristine can cause azoospermia and possibly sterility. It is also proposed that spermatogenic giant cells can thus be generated in animal models and conveniently collected from the caput epididymal tubule for use as a tool in cell biology.

In the reported background that combination cancer chemotherapeutic regimens containing the cytotoxic spindle poison vincristine as one of the drugs^{1–7} can cause several side-effects like nausea, vomiting, leukopenia, alopecia, stomatitis, peripheral neuropathy, cardiopathy, hepatocellular damage, pulmonary fibrosis, etc⁸, including male gonadal dysfunction like azoospermia, oligospermia, gynaecomastia and germinal aplasia^{3,9–13}, the specific gonadal toxicity of vincristine has not been

studied while administration of total alkaloids of *Vinca rosea* (*Catharanthus roseus*; Apocynaceae), the plant from which vincristine is obtained, to adult male rats and mice brings about arrest of spermatogenesis, regression of Leydig cells and derangements in sperm^{14–18}, we reported that vincristine, when administered to rats, can cause thorough disorganization of the seminiferous tubules, narrowing down of the layers of cells in the latter, absence of meiotic elements and, more importantly, the occurrence of giant cells in the seminiferous tubules¹⁹. Giant cell formation in the testis has been reported under several experimental conditions^{20–28}, but invariably the cells undergo cytolysis and/or phagocytosis by macrophages²⁰. Therefore the intact nature of the giant cells in the seminiferous tubules caused by vincristine is intriguing. Therefore the investigation was extended further to trace the fate of the giant cells at the epididymis.

The experimental protocol has already been reported¹⁹. It consisted essentially of administration of vincristine sulphate to Wistar strain adult male albino rats through intraperitoneal route (group I, 10 µg and group II, 20 µg/day/animal) with the control rats receiving the vehicle (group III). Rats were sacrificed on the day 16 by cervical dislocation and the testis and epididymis were dissected out. Slices of testis, caput epididymis and cauda epididymis were fixed in Bouin–Hollande fixative. Serial paraffin sections, 8 µm thick, were stained in Delafield haematoxylin and eosin²⁹ (Figure 1).

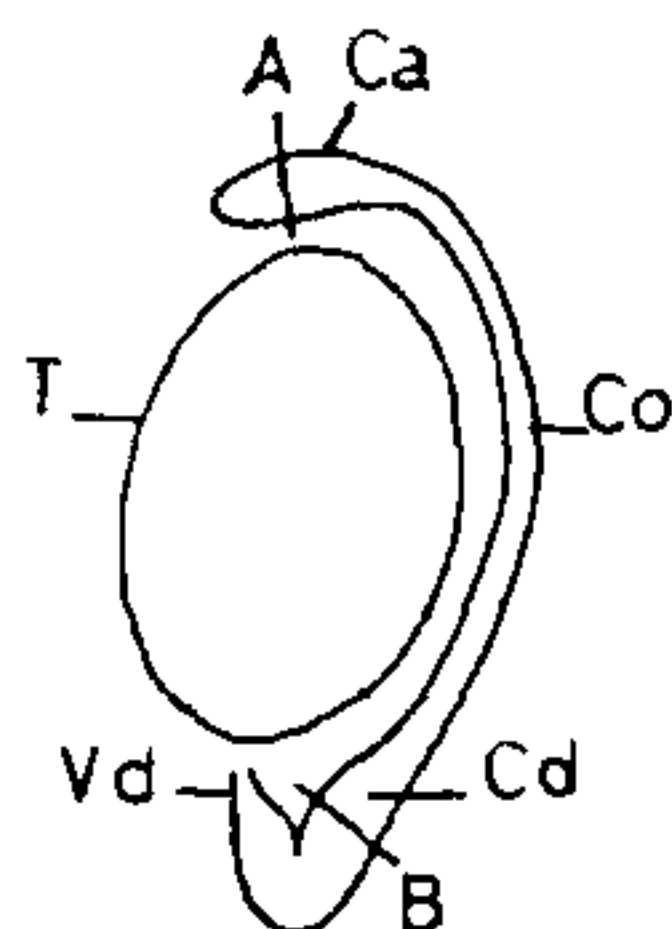


Figure 1. Schematic diagram of the epididymis of rat showing the levels at which sections were obtained in the present study (A, caput; B, cauda). Ca, caput; Cd, cauda; Co, corpus; T, testis, Vd, vas deferens.

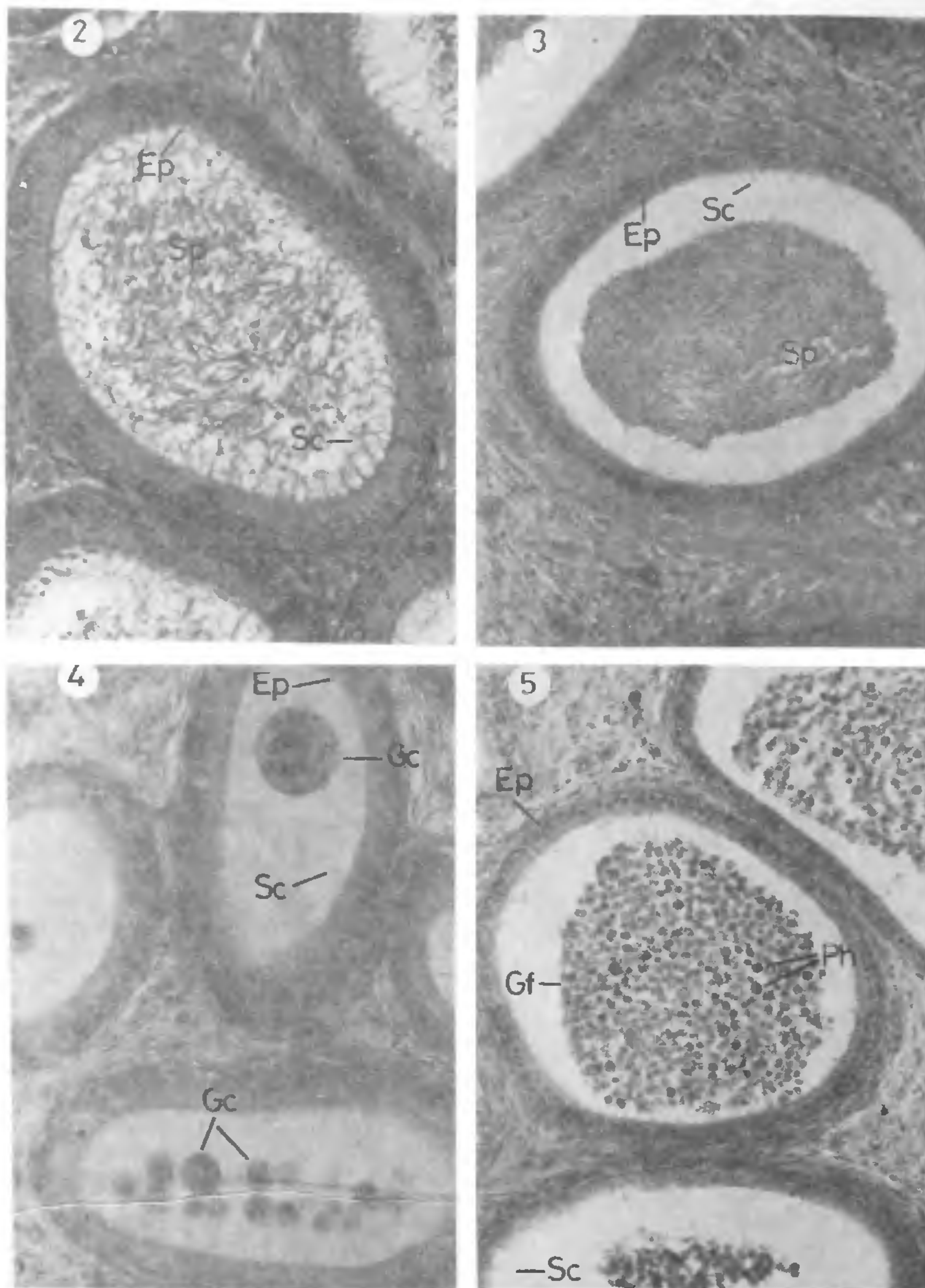
Observation of the testis of the treated rats confirmed the formation of giant cells in the seminiferous tubules. The caput (Figure 2) and cauda (Figure 3) epididymis of the control rats revealed the typical histoarchitecture, the former containing tall columnar cells with abundant and long stereocilia and dense but dispersed sperm in the lumen and the latter containing short columnar cells with short stereocilia and a dense compact mass of sperm in the lumen. On the other hand sperm were totally absent in the lumen of caput epididymis of the treated rats, which however contained giant cells in various numbers (Figure 4). The cells were spherical and ranged in diameter from 10 to 40 μm . Each cell contained a single nucleus, the size of which differed among the cells. The chromatin appeared as clumps, dispersed inside the nucleus. The profile of the cell was highly intact; there was no trace of cytolysis or phagocytosis. There was no indication of the giant cells having been produced from the caput epididymal epithelium. The cauda epididymis of the treated rats (Figure 5) also lacked sperm. Its lumen was densely packed with the giant cells, but the cells appeared to have been fragmented and cytolytic; phagocytic activity was also evident.

The study conclusively demonstrates that vincristine treatment to rats can cause azoospermia (no sperm ejaculated). Further, the study provides a direct histological evidence to the effect that the azoospermia or oligozoospermia reported in case studies involving treatment of cancer patients with chemotherapeutic regimens containing vincristine^{3,9-13} is a contribution of the latter drug, probably through its spindle poisonous nature affecting spermatogonial mitosis^{30,31}.

Mammalian epididymis, formed of a single long tubule, highly coiled and convoluted, is divisible into caput, corpus and cauda³². It contributes considerably to functional maturation of the sperm like initiation of motility and fertilizability³³⁻³⁶. Its epithelium is known to secrete several proteins, some of which have been

traced to the sperm surface and the latter are mainly responsible for the functional maturation of the sperm³⁷⁻⁴². Though the proteins are believed to be secreted along the entire length of the epididymis, their association with the sperm occurs mostly at the caput and proximal corpus³⁹. The cauda is essentially an organ of storage of sperm until ejaculation; this region is also endowed with the property of disintegrating and absorbing the dead sperm as well as the multivesicular bodies^{41,43}. The transit to and accumulation in the epididymis of the giant cells is interesting. The cells are neither disintegrated nor phagocytosed by macrophages when in the testis, as it usually occurs when induced by other causative factors^{18,20}, but reach the caput epididymis intact, in a manner similar to sperm. Further, the cells which are of varied shapes when in the testis, acquire spherical shape during transit to the caput epididymis. The same cells on reaching the cauda epididymis are disintegrated into fragments and are acted upon by phagocytes as it happens in the case of dead sperm⁴³. The intact nature of the giant cells in the caput epididymis can be attributed to the physiological peculiarity of this region in processing the sperm arriving from the testis towards functional maturation. The disintegration of the giant cells at the cauda epididymis can be attributed to the fact that only differentiated sperm leave this region for ejaculation.

In addition to throwing light on the negative impact of vincristine treatment in causing azoospermia, and probably sterility, the present study opens up a new avenue of application of vincristine on the positive side whereby vincristine can be used to induce the formation of giant spermatogenic cells in animal models. The cells thus produced, since they reach the caput epididymis intact, can be isolated free from contamination by flushing of the caput epididymal tubule, and studied further in terms of the nucleocytoplasmic ratio, ploidy, DNA content, tubulin, etc. In addition, the possibility of culturing these cells *in vitro* exists and such cultured giant cells can be of paramount value as a tool in cell biology in nuclear transplantation experiments involving polyploid nuclei⁴⁴, in elucidating nucleocytoplasmic interactions⁴⁵ in spermatogenic cells, etc. For nuclear transplantation involving polyploid nuclei, untreated renal adenocarcinoma cells are used which are rather small; further, to achieve successful transplantation, several nuclei need to be transplanted into the enucleated host egg⁴⁴. The giant spermatogonial cells would offer two advantages in this regard, namely (i) the large size, overcoming the technical difficulty of smallness of the nuclei of renal adenocarcinoma cells, and (ii) the giant polyploid cells are germinal rather than somatic. Giant HeLa cells used in elucidating nucleocytoplasmic interactions are produced *in vitro* by subjecting HeLa cells to X-irradiation, which treatment invariably results in the formation of nuclear frag-



Figures 2-5. 2, Transverse section of the caput epididymis of control rat ($\times 400$). 3, Transverse section of the cauda epididymis of control rat ($\times 200$). 4, Transverse section of the caput epididymis of treated rat ($\times 400$). 5, Transverse section of the cauda epididymis of treated rat ($\times 200$). Ep, epithelium; Gc, giant cells; Gf, giant cell fragments; Ph, phagocyte; Sc, stereocilia; Sp, sperm.

ments⁴⁵. The giant spermatogonial cells produced *in vivo* would overcome this problem.

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