

Spermatozoa-like cell invaders (nuclear vlimata) in human neoplasia

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Summary. Spermatozoa-like cells (nuclear vlimata) have been identified in malignant cell cultures and embryonic cells, also common in the cytology and histology of all types of human neoplasia even after chemotherapy. A new mechanism of invasion of malignant cells has been described, according to which neoplastic cells behave and function as parasites using host-cells to divide, survive and eventually produce nuclear vlimata (bullets). Nuclear vlimata are the end cell products of incomplete, unequal, asymmetrical division of neoplastic cells. The nuclear vlima exhibits similar morphology to spermatozoa and virus (head with, or without, tail) and invades the cytoplasm and/or nucleus of surrounding host-cells by a similar mechanism to sperm-oocyte interaction (fertilization) or viral cell infection, in the events of nuclear vlima → tumor → nuclear vlima → tumor.

The nuclear vlima head contains and transfers DNA, and when incorporated into the host-nucleus is indistinguishable from nucleoli and when in the cytoplasm is similar to sperm pronucleus, observed after sperm penetration of the oocyte. Function of nuclear vlimata is directly dependent on the specific extracellular matrix produced by malignant cells, consisting of glycosaminoglycans-protease-membranes. This mechanism of invasion constitutes the link of all scientific information concerning human neoplasia.

Key words: Nuclear vlimata, Neoplasia, Extracellular matrix, Histology, Cytology

Introduction

Neoplasia development, a genetic disease, is characterized by initiation, promotion and progression

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(Pitot and Dragan, 1991). The stage of initiation reflects a permanent and irreversible change in the initiated cell (Boutwell, 1964) caused by carcinogens (chemical, physical, biological or genetic) which alter the structure of DNA, resulting in carcinogenesis (Pitot, 1987).

Irreversible alterations in the structure of the genome of the neoplastic cell are expressed with increased growth rate, invasiveness, metastatic capability and biochemical changes in the malignant cell (Nowell, 1986).

The most life-threatening characteristics of malignant cells are their ability to detach from the primary tumor, invade surrounding normal tissues, and form metastatic growths at distant sites. The process of metastasis involves invasion and penetration of tumor cells into blood vessels and/or lymphatics, with their subsequent dissemination to distant organs in the blood or lymph (Poste and Fidler, 1980).

Malignant cell deformation (Zeidman, 1961; Sato and Suzuki, 1976), release of hydrolytic enzymes (Strauch, 1972; Bosmann et al., 1973), interactions with clotting components (Wood, 1964) and host immunological defences (Stevenson, 1991), are the determinant factors of invasion.

However, a generalization, with respect to invasion, has not yet been accomplished and how malignant cell invasion into host tissues occurs is not exactly known (Poste and Fidler, 1980).

In the present study spermatozoa-like cell-invaders (nuclear vlimata) are reported as a uniform property to all metastatic human cells.

Materials and methods

Cell culture establishment

Stationary cell cultures were established from human solid tumor tissue specimens by enzymatic digestion (Logothetou-Rella et al., 1988a).

Malignant lung cell lines from metastatic

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lung carcinoma, M-cells, P-cells and B-cells have recently been characterized (Logothetou-Rella et al., 1992a).

Malignant urothelial cell cultures were established from tissue specimen from patients with invasive transitional cell carcinoma. The five established urothelial malignant cell lines were designated as Pa-cells, R-cells, S-cells, Br-cells and IG-cells (Logothetou-Rella et al., 1988b). Only the patient where Pa-cells derived, had received bladder intravesical infusions of anticancer drugs.

Melanoma cell culture (Ha-cells) was originated from a male patient who suffered from primary rectal melanoma, metastasized at the lymph nodes of the right arm where tissue specimen was obtained.

Walker tumor rat cells were donated by Dr. S. Kotaridis.

Normal human liver cells (L-cells) were isolated from liver tissue specimen from a male patient who underwent surgery for the removal of his gallbladder.

All cell cultures were grown in complete medium, RPMI-1640 (Seromed) supplemented with 10% foetal bovine serum (Seromed), glutamine and antibiotics (Seromed), incubated at 37 °C in a CO₂-humidified incubator. Stock cells were stored frozen in liquid nitrogen.

Cytogenetic analysis

Chromosomal analysis of M-cells, P-cells and B-cells has recently been reported (Logothetou-Rella et al., 1992a).

Urothelial malignant Pa-cells consisted of malignant cell clones only, with polyploidies, up to 147 chromosomes and complex structural abnormalities. S-cells consisted of malignant cell clone with regular tetraploidies, up to 20% of the cell population and 80% normal cell clone. Br-cells consisted of normal and malignant cell clones but detailed chromosomal analysis was unsuccessful. Melanoma Ha-cells revealed only double minutes. Liver L-cells were cytogenetically normal.

Cytology of malignant, normal and embryonic cell cultures

Mice embryos harvested at the 2-cell stage were cultivated in complete Earle's balanced salt solution (EBSS supplemented with 10% foetal bovine serum and antibiotics) to the stage of hatched blastocysts. When the embryonic cells had all developed, the culture was fixed in 50% ethanol and stained with Papanicolaou.

Human spermatozoa, 100% motile (after swim up test) were inseminated in human oocytes in EBSS with 10% human serum. The gametes were incubated at 37.5 °C for two hours and then fixed in formalin, embedded in paraffin and sections were stained with haematoxylin-eosin.

All primary cell cultures grown in glass petri dishes (10 cm diameter) after one, two, three and four weeks of continuous growth were fixed in 50% ethanol and stained with Papanicolaou, PAS and PAS-diastrase. Cell cultures and human sperm fixed in 4% formaldehyde in phosphate-buffered saline (PBS) were stained by the feulgen method.

Electron microscopy

Mechanically-dispersed bladder tumor cells, M-cells and P-lung cells were ultrastructurally examined (Logothetou-Rella et al., 1992a,b).

Microscopical examination of tissue sections from human solid tumors and cytology of haematologic tumors

Tissue sections, stained with haematoxylin-eosin (H-E), were donated from the archives of the Pathology Dept., Hellenic Airforce 251 General Hospital. Tissue sections were examined for spermatozoa-like cells from the following human organ solid tumors: lung; intestine; stomach; kidney; pancreas; thyroid; parathyroid; prostate; ovary; mammary gland; and lymphoma.

Bone marrow smears, stained with Giemsa and Papanicolaou, from patients with multiple myeloma or leukemia, were donated from the archives of the First Clinic, Laikon General Hospital.

Results

Cytology of malignant cell cultures

The cytology of all cell cultures showed invasion of large intact cells by small cells, which functioned as parasites (Fig. 1). These parasitic cells consisted of a small hyperchromatic, pycnotic nucleus of a dark blue colour, and very little mucinous cytoplasm of a light blue-green colour. The parasitic cell divided in the host cell cytoplasm and shot its daughter's small nucleus through the extracellular space in the cytoplasm of neighbouring cells indicating functional cells. During shooting the parasitic mother and daughter-cell were connected to each other by an extracellular bridge (dark blue colour) which eventually broke apart (Figs. 2, 3). These parasitic cells kept dividing using a host-cell and kept getting smaller after every division until their nucleus reached the size of a nucleolus (Fig. 4). At this size the parasitic cell exhibited a consistent distinct morphology of a nuclear head and attached tail resembling a small upright immotile spermatozoon. The head size reminded us of fungal spores. These spermatozoa-like cells were observed upright with the head in the space and the tail end attached to the stationary mother-cells. The nuclear heads were round, of different size, within the same cell population, with concrete, well-defined morphology. The distribution and intensity

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of the dark blue colour of haematoxylin stain in the heads, appeared uniform and homogeneous. The nuclear heads were observed intra- and extracellularly detached from the mother-cell, attached to their tails in the extracellular matrix, in the cytoplasm and/or the nucleus of neighbouring cells (Figs. 4-9). When the heads were observed in the cytoplasm, they resembled pronuclei of spermatozoa in the oocyte cytoplasm after fertilization; when in the nucleus, they resembled a nucleolus, which was distinguished from

others by the clear halo around it which was formed during its entrance and which disappeared upon implantation (Figs. 10-14). The production of nuclear heads, the shooting and implantation in other cells occurred mainly in culture areas where extracellular glycosaminoglycans (GSG) and cell membranes were located.

This nuclear head with or without tail is named from now on as nuclear vlima (NV) (Vlima-bullet, Vlimata-bullets).

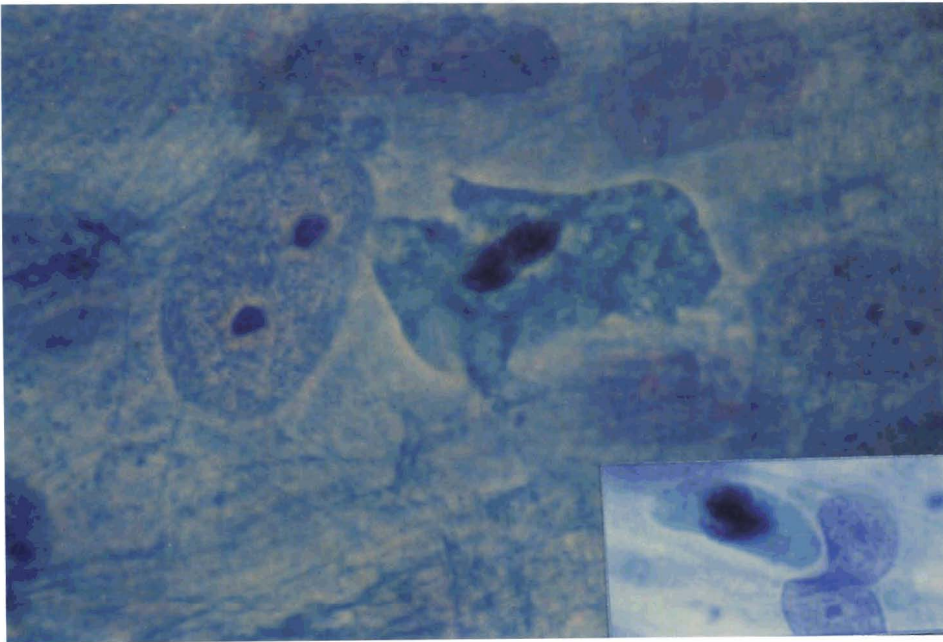


Fig. 1. Parasitic lung cell in a host-cell, in a non-dividing stage. The parasitic cell consists of green mucinous cytoplasm and hyperchromatic, pycnotic, haematoxylinophilic, small nucleus. The halo around the parasitic cells is evidence of its intracellular location. Papanicolaou, phase contrast, x 1,000. Inset: parasitic cell in binucleated cell. Papanicolaou. x 500

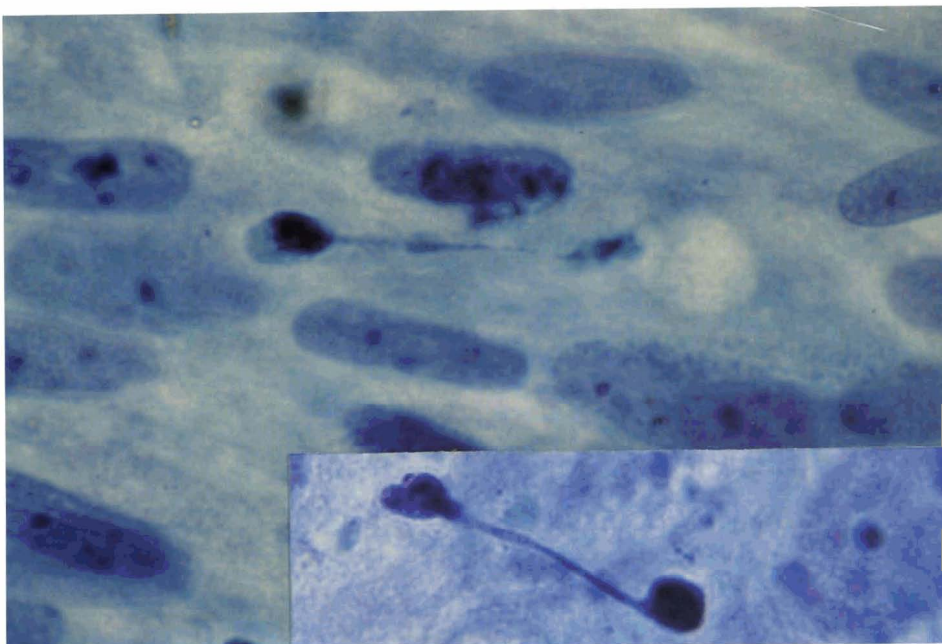


Fig. 2. The parasitic cell during its division in a host-cell, shoots its daughter-cell into another host-cell. The extracellular blue-coloured bridge connects the parasitic mother to its daughter-cell which has smaller nucleus and less cytoplasm than its mother. Inset: Another dividing parasitic cell. Papanicolaou method. x 1,000

Nuclear Vlimata (NVs) were observed vertical, horizontal, or side-ways, in a different focusing level than the stationary mother-cell, indicating their possible motility. NVs differed between each other in the size of the head and the tail length within the cell culture of the same tissue and different tissue origin (Figs. 4-7).

Mother-cells were found supported in the cytoplasm of host cells or in the extracellular GSG fibrillar matrix. During mother-cell abnormal division, its nucleus raised

from the cytoplasm and shot the daughter nucleus (Fig. 15). The green translucent GSG-sacs and cell membranes, PAS and PAS-diastrase positive, constituted a common characteristic of the intra- and extracellular matrix of all the malignant cell cultures examined in this study.

The overall picture of NVs resembled that of human sperm (Fig. 16) in the sense that spermatozoa-like cells were embedded in mucin similar to that of sperm and penetrated other cells by mechanical and enzymatic

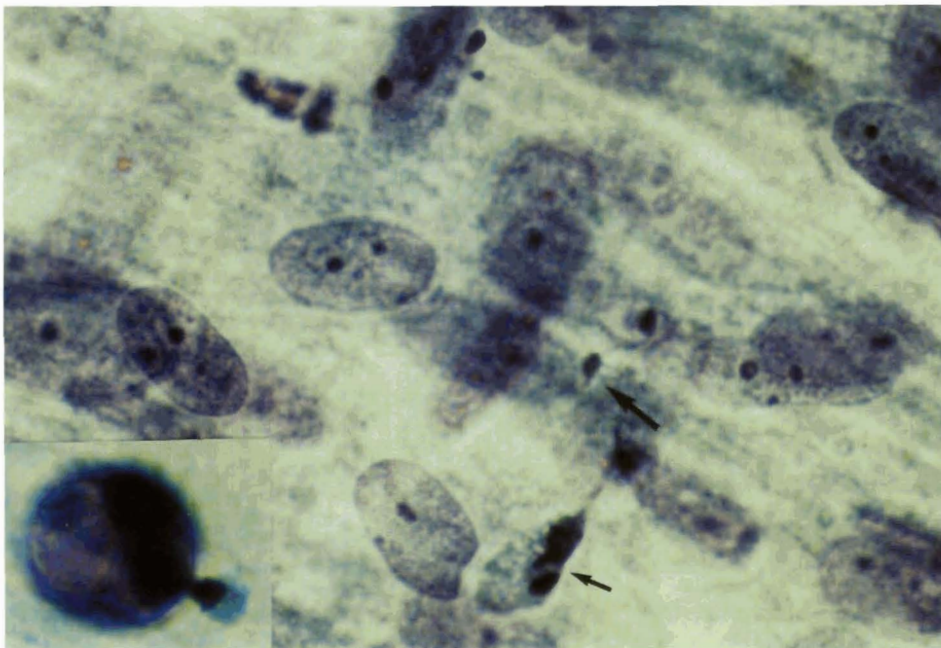


Fig. 3. Two types of parasitic cells, during division. One with cytoplasm (thin arrow) and one without cytoplasm (thick arrow). Papanicolaou. x 500. Inset: A parasitic cell dividing in urine cytology. Papanicolaou. x 1,000

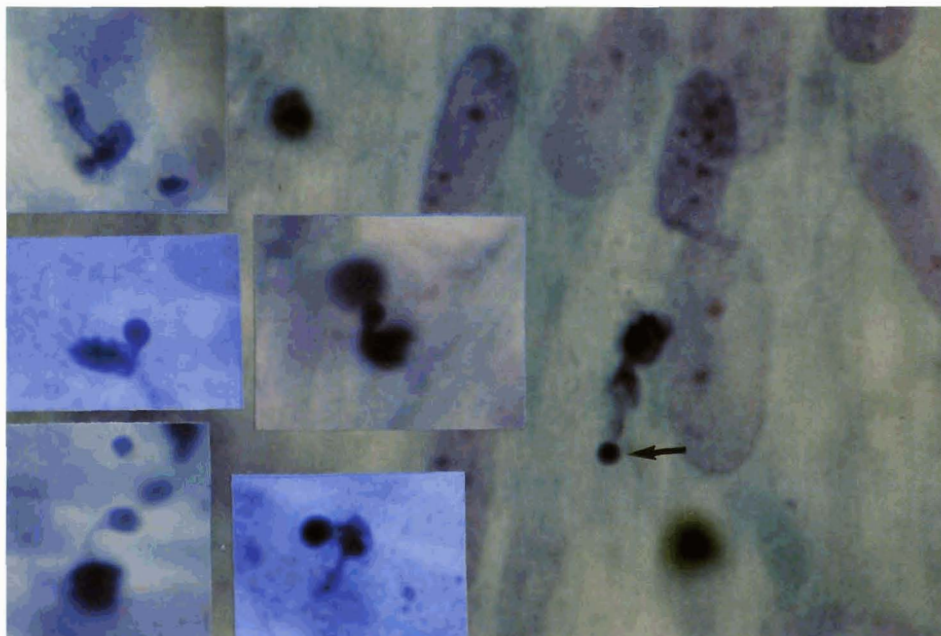


Fig. 4. Final NV (arrow) of M-cells, with round pycnotic nucleus, of homogeneous texture and uniform dark blue colour, almost released from its mother-cell. Inset: Different NVs in lung neoplastic cell cultures with different head size, common morphology (head with tail), attached to the mother-cell focused at the head. Papanicolaou. x 1,000

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means.

Localization of nuclear vlimata in human solid and haematologic tumors

In all the tissue sections NVs were commonly observed to invade the cytoplasm and/or nucleus of cells, forming a halo around their heads at the site of entrance, the same as described in cell cultures (Figs. 17-19). Furthermore NVs were present in tumor sections

and cells of patients who had received chemotherapy. NVs were also present in bone marrow smears from patients with multiple myeloma or leukemia (Figs. 20-23). In haematologic tumor cell smears the cell clumps consisted of plasma cells embedded in green-translucent-fibrillar-GSG-membranous extracellular matrix (Fig. 20 inset). NVs were observed free or during production still attached to the mother-cell. The tail of the free NV was wavy indicating movement. NVs in leukemias have very long tails

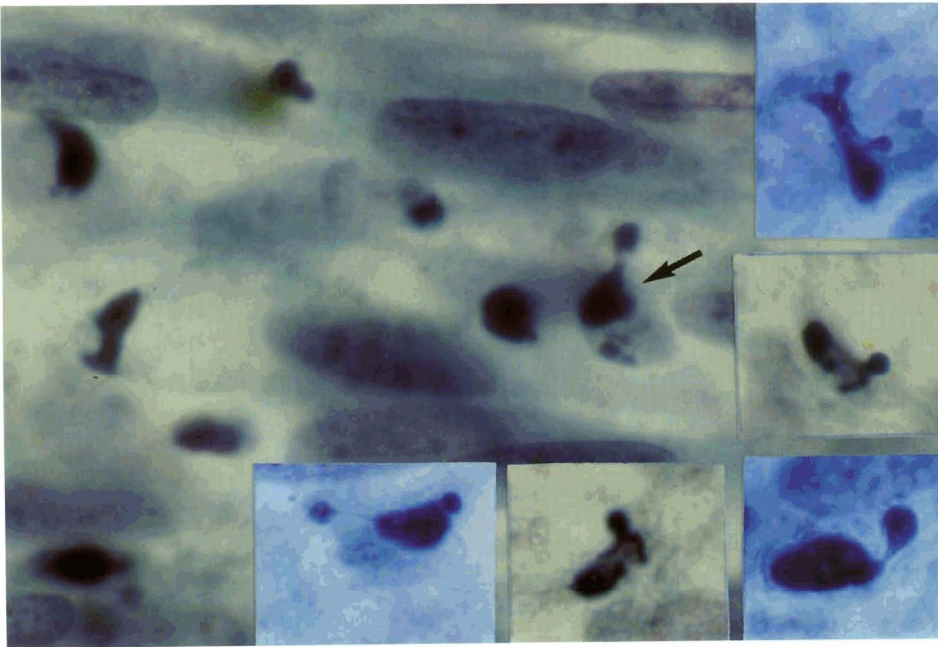


Fig. 5. Lung parasitic cells supported in the green fibrillar extracellular matrix. The nucleus of mother-cell (arrow) raises and buds. Papanicolaou. x 1,000. Insets: Nuclear vlimata with one or two tails just detached from mother-cell. Also mother-cells with one or two daughter-heads (buds). Papanicolaou. x 1,000

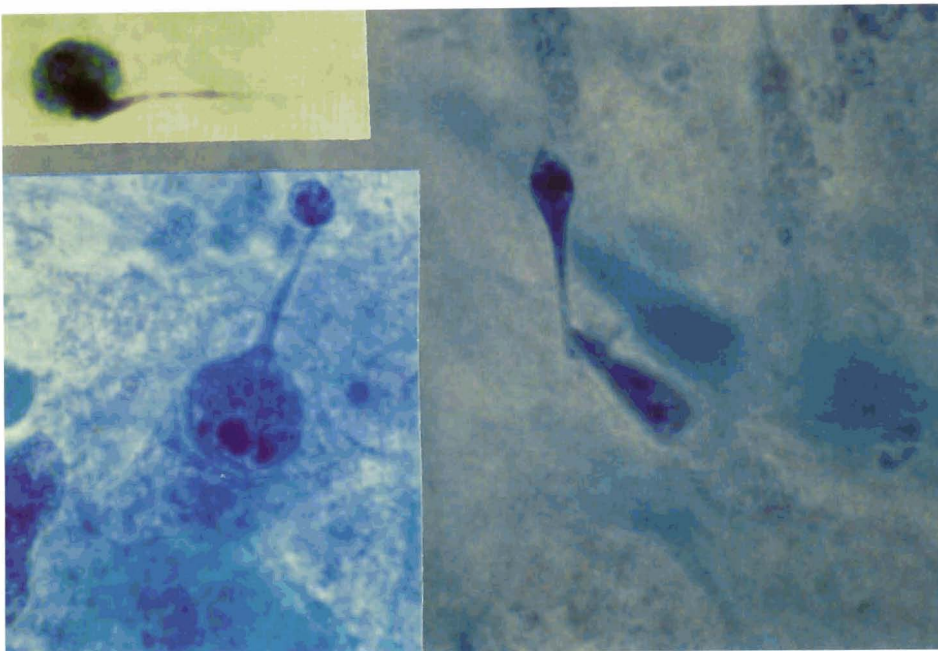


Fig. 6. S-cell culture (bladder carcinoma). The NV pops out of the stationary mother-cell, perpendicularly, with very close resemblance to spermatozoa. Insets: bottom, smaller size of daughter nucleus; top, a free NV in urine cytology.

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and haematoxylinophilic and/or eosinophilic heads or heads with both haematoxylinophilic and eosinophilic areas (Fig. 23).

Nuclear vlimata in the cytology of embryonic and normal cell cultures. Description of karyolysis

NV production with round head and short tail was observed by some embryonic cells. One NV was caught fixed, while injected from the nucleus into the cytoplasm

of the same cell forming a halo around it. In embryonic cells nuclear vlimata were produced by the nucleoli (Fig. 24).

The established normal liver cell line consisted of endothelial, epithelial and fibroblastic cells. The cytological study of L-cells exhibited several cells undergoing karyolysis which was defined by the amorphous, non-homogeneous, non-uniform nucleus which was stained by haematoxylin, in some areas dark blue and in other areas light blue (Fig. 25). Cell cultures

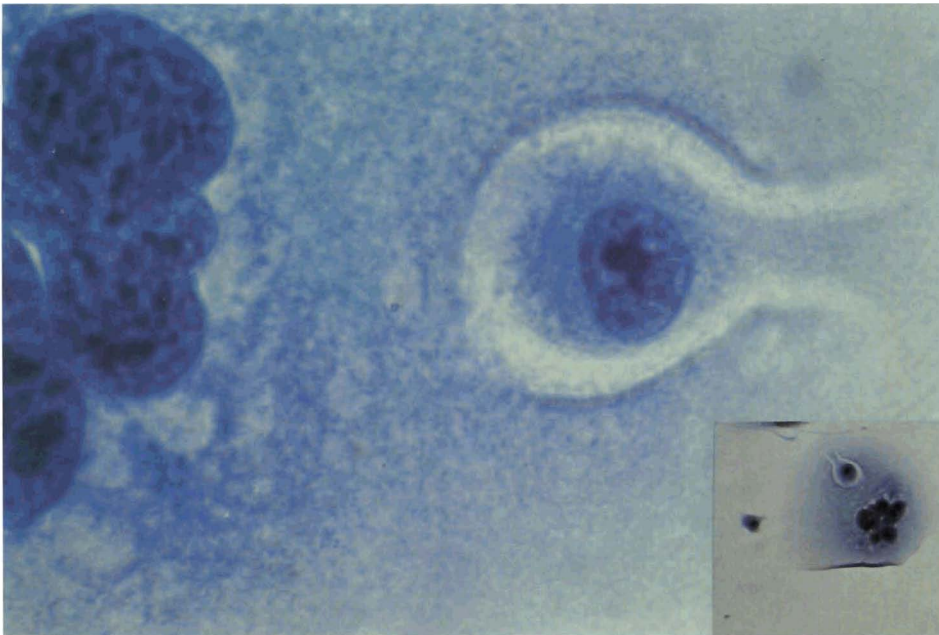


Fig. 7. Pa-cell culture (bladder carcinoma). A small cell invading a multinucleated host-cell. The invading cell has taken the morphology of a head (large nucleus, little cytoplasm) and tail. Papanicolaou. x 1,000. Inset: The overall picture. x 100

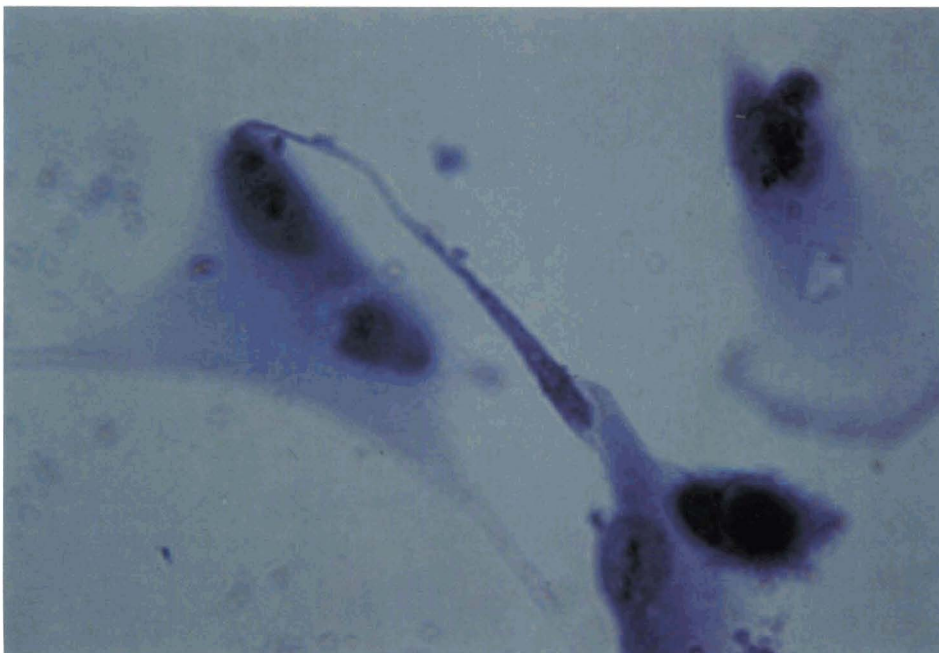


Fig. 8. Production of daughter NV by stationary parent melanoma cell. The field is focused on the daughter NV, the tail of which is bent, almost detached from its parent-cell, and the head contacts a neighbouring cell. Papanicolaou. x 400

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of normal granulosa, urothelial, fibroblasts and endothelial cells were also cytologically examined. NVs were not found in any of the normal cell cultures, and they were not related to karyolysis.

Spermatozoa, in sperm-oocyte paraffin section, were similar to NVs observed in malignant tissue sections (Fig. 26).

The DNA content of nuclear vlimata (feulgen method)

The invasive NVs and human spermatozoa were

positive to the DNA-specific stain (Feulgen), staining bright purple. NVs contained condensed or decondensed chromosomes (Figs. 27, 27a,c). Spermatozoa contained only condensed chromosomes (Fig. 27b). NVs were embedded in PAS-diestase-positive extracellular matrix (Fig. 27d).

Electron microscopy

Ultrastructural detection of NVs was not successful. In bladder tumor cells there were nuclear projections

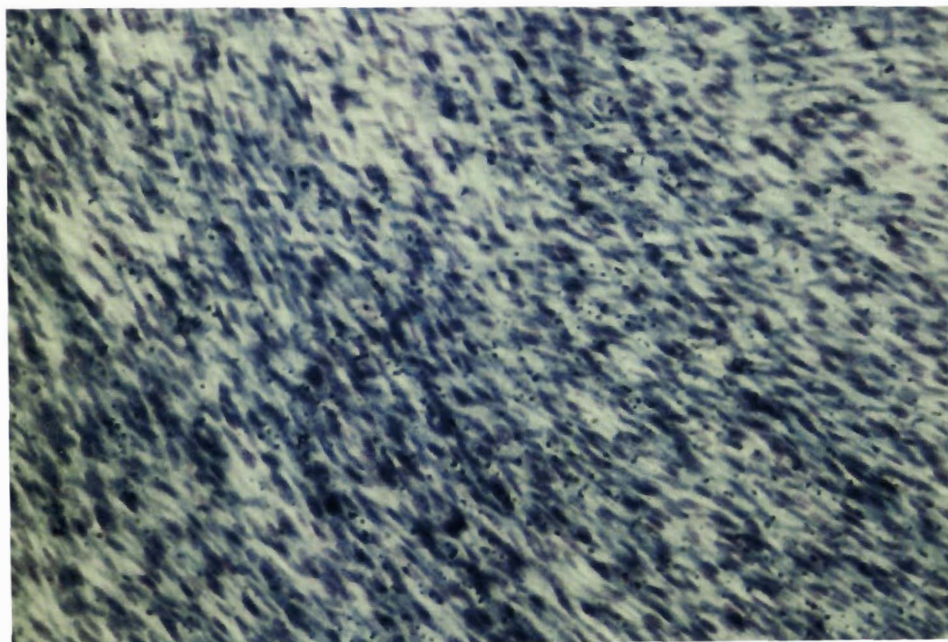


Fig. 9. Confluent cell culture of M-cells (lung): The green extracellular fibrillar GSG on top and between the cells is obvious. The small dark blue dots are the NVs (arrow). Papanicolaou. x 40

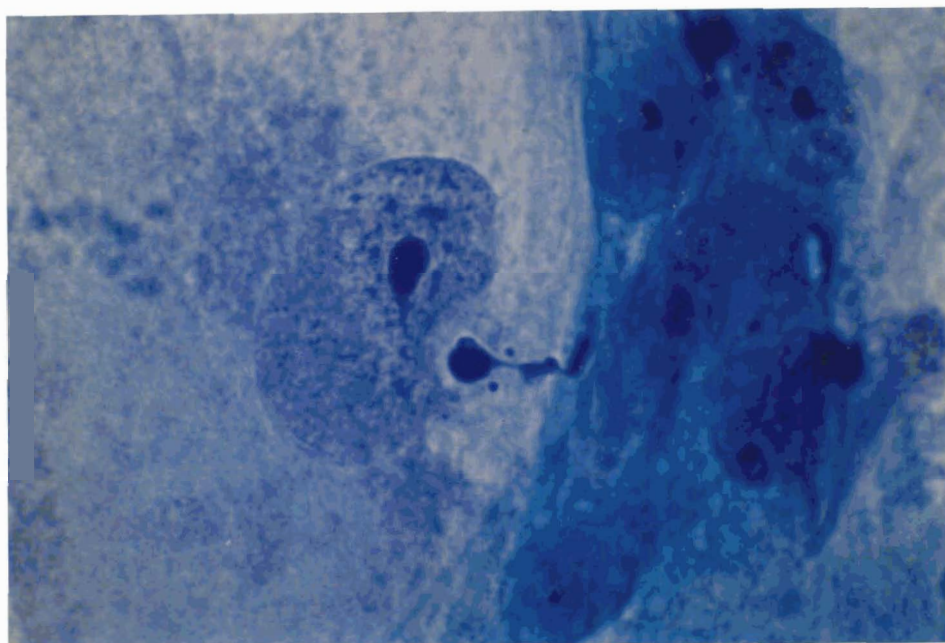


Fig. 10. NV invades a host-cell nucleus. The halo around its head indicates that the head is inside the host-cell and the tail outside (no halo). The host-cell nucleus contains a nucleolus which has the same morphology, size, and colour but no halo and shorter tail than the invading NV. This one has been incorporated into the host-cell nucleus. Bladder carcinoma cell culture. Papanicolaou. x 1,000

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in the form of head and tail resembling NV, or micronuclei (Fig. 28). A host-cell nucleus was observed, at a semi-circular, engulfment position around a micronucleus probably for synkaryosis (Fig. 28 inset).

Discussion

The pathogenesis of neoplasia is commonly viewed as the evolution of increasingly malignant cell

populations that arise via a series of mutations, selection and epigenetic mechanisms in the descendants of a single initially transformed cell (Nowell, 1976). This view is further strengthened by the presence of nuclear vlimata in all types of human neoplasia.

NVs are end cell products of incomplete, unequal, asymmetrical division of malignant cells. Morphologically they consist of a nuclear head and tail. Upon their production they eventually detach

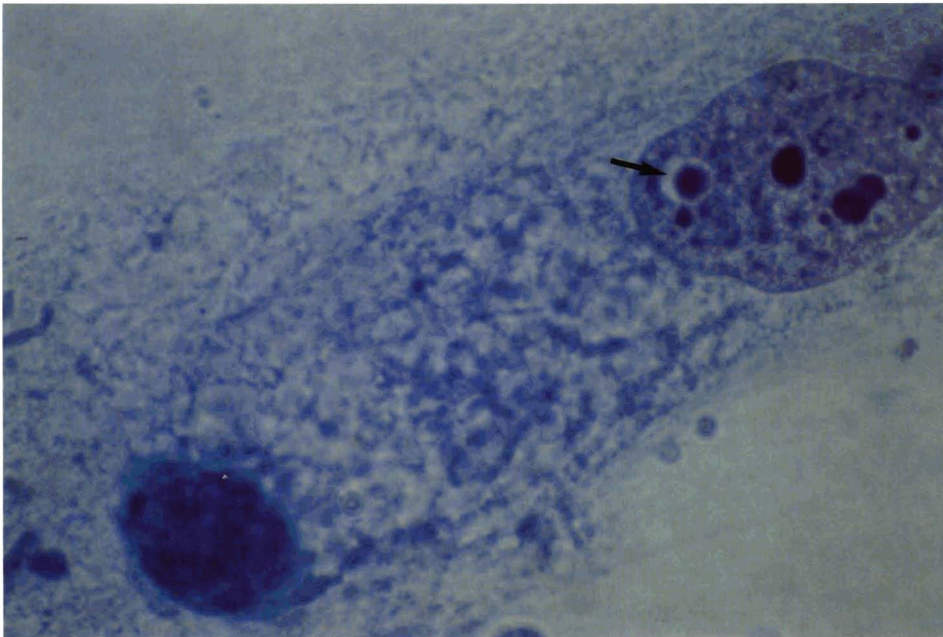


Fig. 11. Host-cell nucleus with two incorporated NVs (dark blue coloured) and a third one with halo, during its entrance (arrow). The incorporated vlimata can be mistaken for nucleoli. Bladder carcinoma cell cultures. Papanicolaou. x 1,000

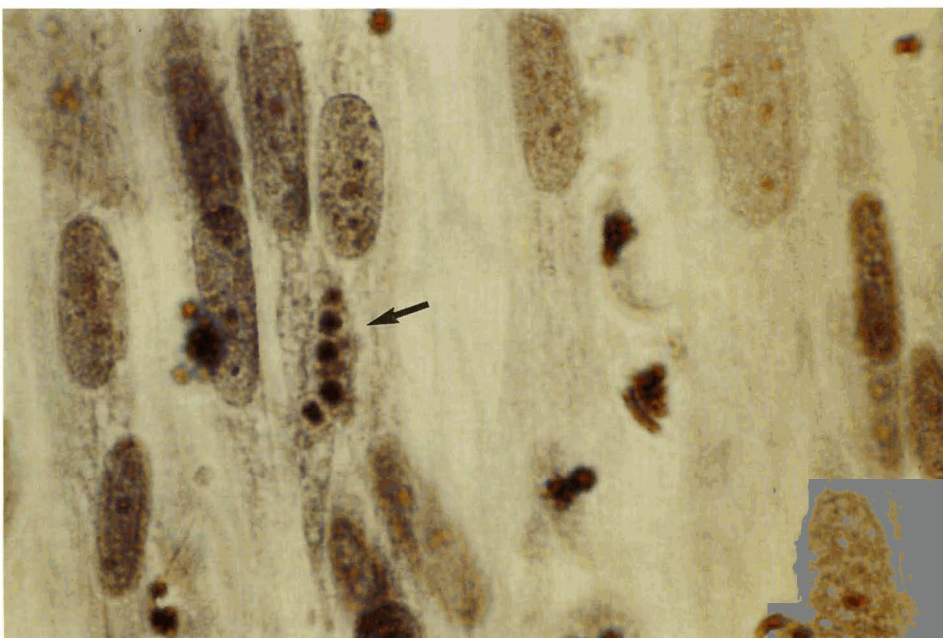


Fig. 12. Four nuclear heads of nucleolar size entering the nucleus (arrow) of a host-cell and a fifth one entering the cytoplasm of the same host-cell. Lung cell cultures. Papanicolaou. x 1,000

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from their mother-cell and seek a host cell at random. According to this study, when a NV is implanted and incorporated into the nucleus of a normal host-cell, it can be considered as a process similar to fertilization or viral infection. As a result, the host-cell genotype and phenotype is altered and behaves like a transformed cell (or embryo) exhibiting the main properties of the uncontrolled cell division, invasiveness, metastatic capability and biochemical changes. An infected or fertilized host-cell, by NV,

after many divisions, loses its cytoplasm (Logothetou-Rella et al., 1992b), and cannot divide by itself; it needs support from a host-cell or extracellular matrix, it is thus forced to become a parasite and produce NVs. Once, at the stage of NV, the life cycle of the malignant parasite host-cell repeats itself. There is no doubt that malignant cells metastasize, in order to divide, propagate and survive in the events of nuclear vlima → tumor → nuclear vlima.

NVs within the same cell population differ among

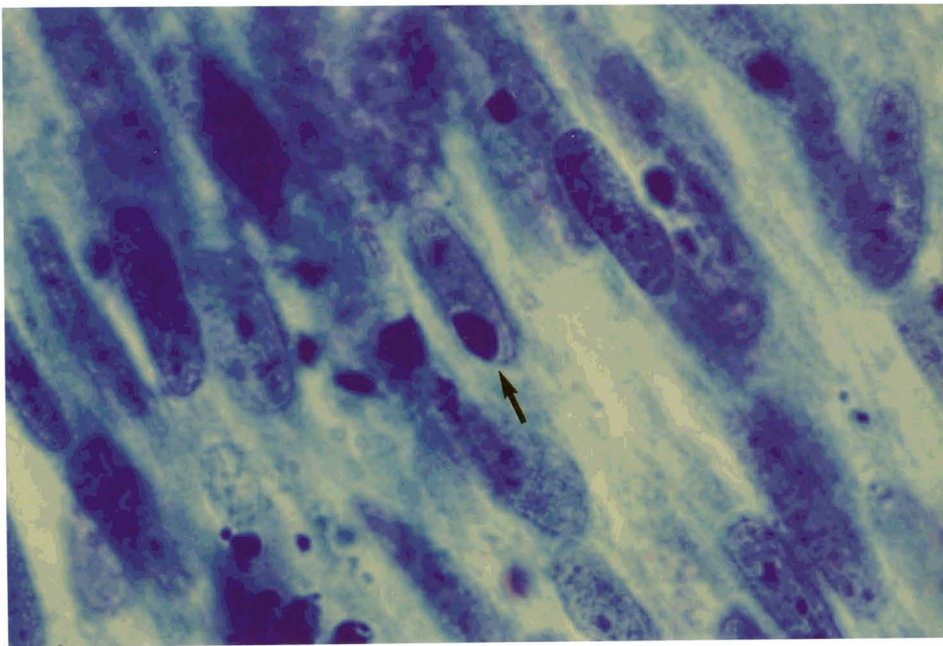


Fig. 13. NVs of dark blue colour at different focusing planes of lung carcinoma cultures. The field is focused on a nuclear head (arrow) entering the nucleus of a host-cell with incomplete halo. Papanicolaou. x 1,000

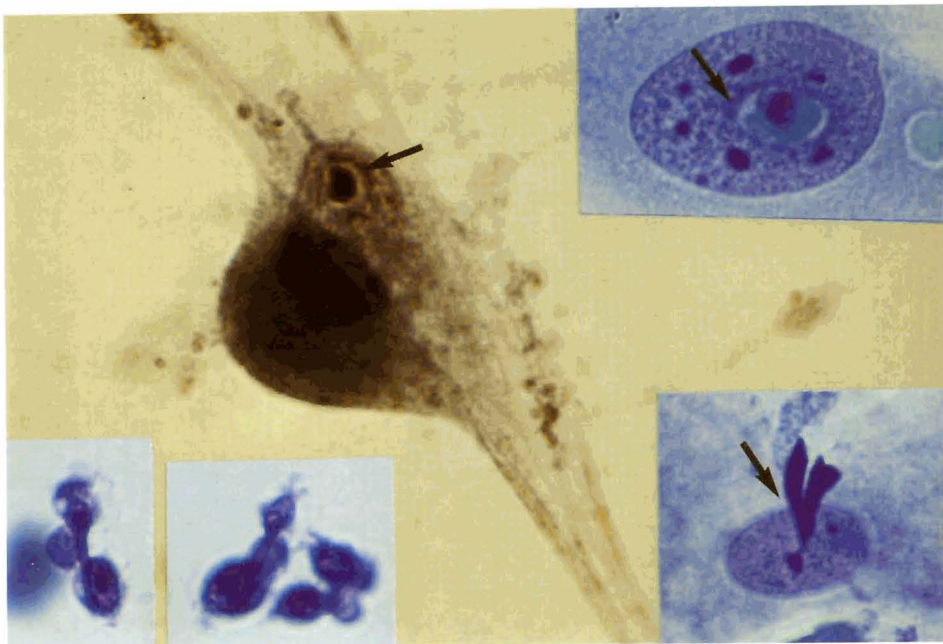


Fig. 14. Walker tumor rat cell culture. NVs entering the nucleus of a stromal rat cell (arrows). Inset: bottom-left, NV production by Walker tumor cells in suspension. Papanicolaou. x 1,000

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each other in the size of the nuclear head and tail length. NVs with an intact nucleus and reduced cytoplasm when invading the cytoplasm of a host-cell result in the formation of multinucleated host-cells (syncytia) which do not usually survive in culture, and exfoliate. Multi-nucleated malignant cells are common in exfoliated cytology and histology of malignant tissues. NVs of small heads, when invading the host-cell nucleus become indistinguishable, for they are cytologically identical to nucleoli. Increased

number of nucleoli in malignant cells, has been observed and evaluated by cytology. Increased number of nucleoli are also found in malignant cells in culture (Logothetou-Rella et al., 1988a).

Micronuclei production by malignant cells treated with anti-cancer drugs (Kliesch and Adler, 1987; Walker et al., 1990) are probably heads of NVs which failed to invade host-cells and are found free extracellularly, due to drug effect.

Scientists have considered the observation of one

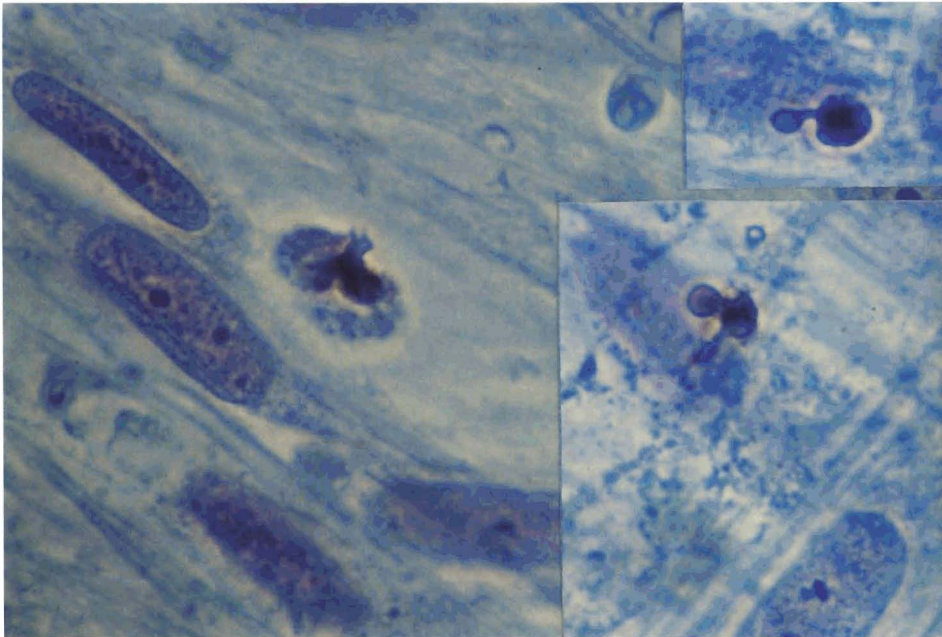


Fig. 15. Parasitic mother-cell nucleus (lung cell culture) raises in order to bud. Insets: Parasitic cell division resembling the asexual process of yeast budding. Papanicolaou, phase-contrast. x 1,000

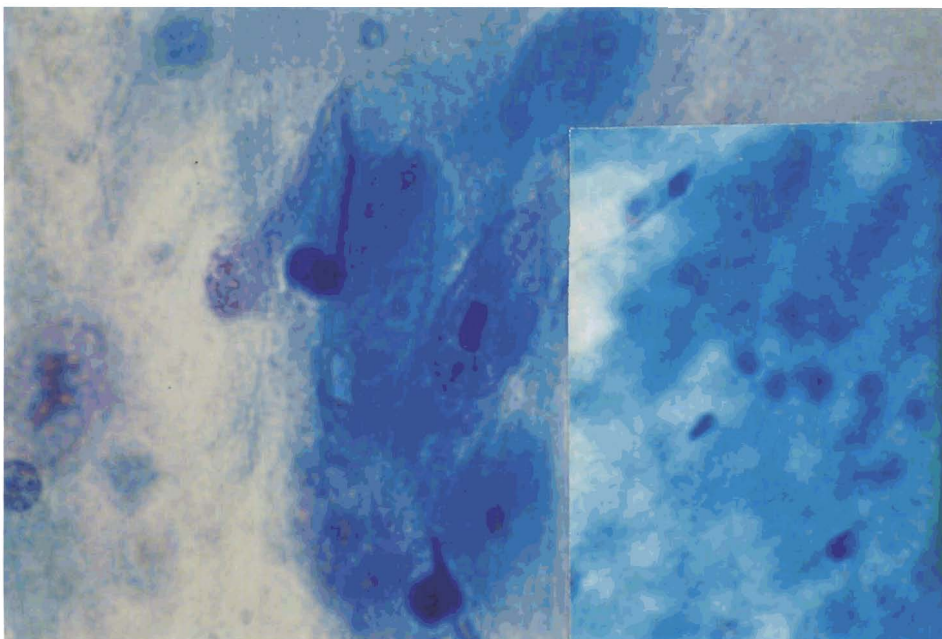


Fig. 16. NVs (bladder cell culture) embedded in green fibrillar-translucent mucin similar to human sperm. Papanicolaou. x 1,000. Inset: Human spermatozoa embedded in mucin are of similar texture to that of malignant cells. Papanicolaou. x 800

cell into another among malignant cell populations, as phagocytosis and cannibalism of malignant cells. The present study shows that the intracellular parasitic cells divide and are therefore functional cells. Consequently, cells within cells are not all involved in the process of phagocytosis but also in the host-parasite process. Among cells, in mixed cell cultures of normal and malignant cells in this study, there were host cells which, upon entrance of NVs produced GSG-sacs, in which NVs were wrapped and destroyed,

resulting in translucent GSG-sac in the host cell. In other host-cells NVs were observed dividing. Unfortunately it has been impossible, so far, to distinguish normal from malignant host-cells in culture, as NVs do not discriminate between the two. It is, though, certain that the extracellular matrix of GSG-protease-membrane production by malignant cells (Logothetou-Rella et al., 1988a, 1992b) is a result of cell host-parasite interaction.

This host-parasitic relationship, in malignant cells,

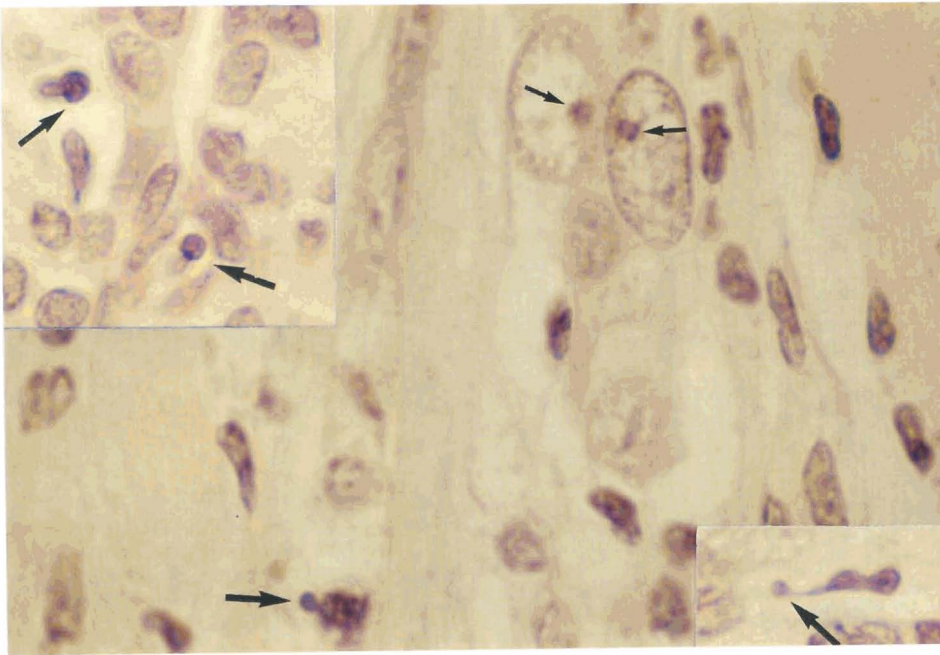


Fig. 17. NVs in tissue section of breast carcinoma (thick arrows). Thin arrows show host-nucleus containing nuclear head (of the same colour and size as NVs) which could be mistaken for nucleolus. H-E. x 1,000. Insets A, B: Free, attached and intracellular NVs in other fields of the breast tissue section. H-E. x 1,000

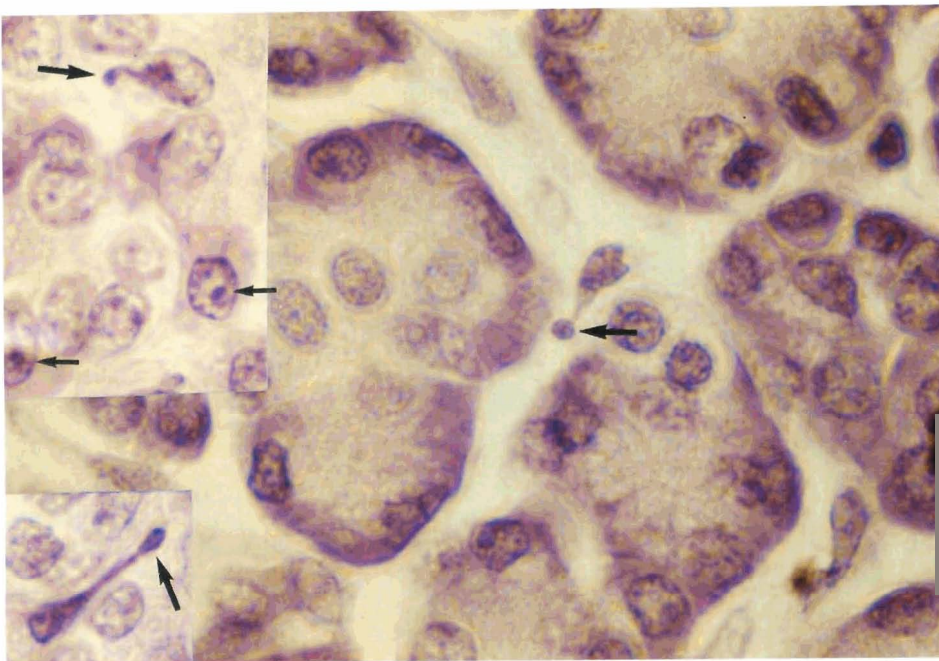


Fig. 18 and insets. Different forms of NVs (thick arrows), within the same tissue section of pancreas carcinoma. Incorporated heads in host-cell nuclei (thin arrows). H-E. x 1,000

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resembles fertilization in the sense that NVs morphologically resemble spermatozoa containing condensed or decondensed DNA and penetrate surrounding host-cells which may play the role of the oocyte, in the events: gametes → embryo → gametes → embryo.

Multiplication of NVs in the extracellular matrix reminds us also, of the asexual process of yeast budding. The malignant mother-cell nucleus raises, leaving behind its cytoplasm attached on the culture vessel and buds.

Daughter-cell (bud) does not always separate immediately but may remain attached, while one or more additional buds form on the mother-cell. At this stage the nuclei remain unseparated like the advanced stage of budding of *Saccharomyces cerevisiae* (Carpenter, 1967).

The heterogeneity of cell clones within a tumor, that is, transitional and stem cells (Mackillop et al., 1983), can be explained in terms of the different head size of NVs, transferring and infecting (or

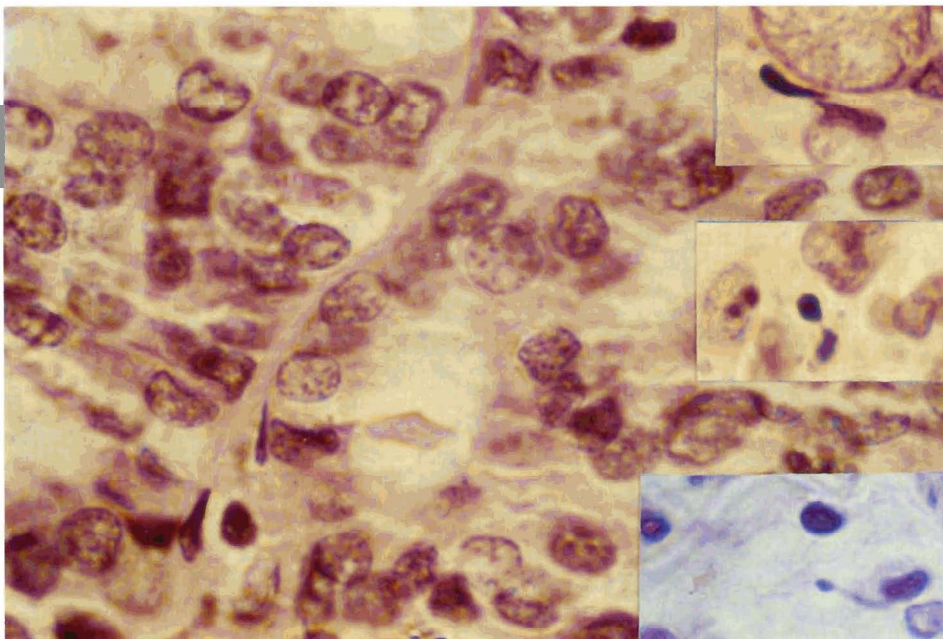


Fig. 19. NVs in tissue section of thyroid carcinoma. H-E, x 1,000. Insets: top right, NV in section of intestinal carcinoma; middle, lymphoma; bottom, lung carcinoma where M-cells were derived. H-E. x 1,000

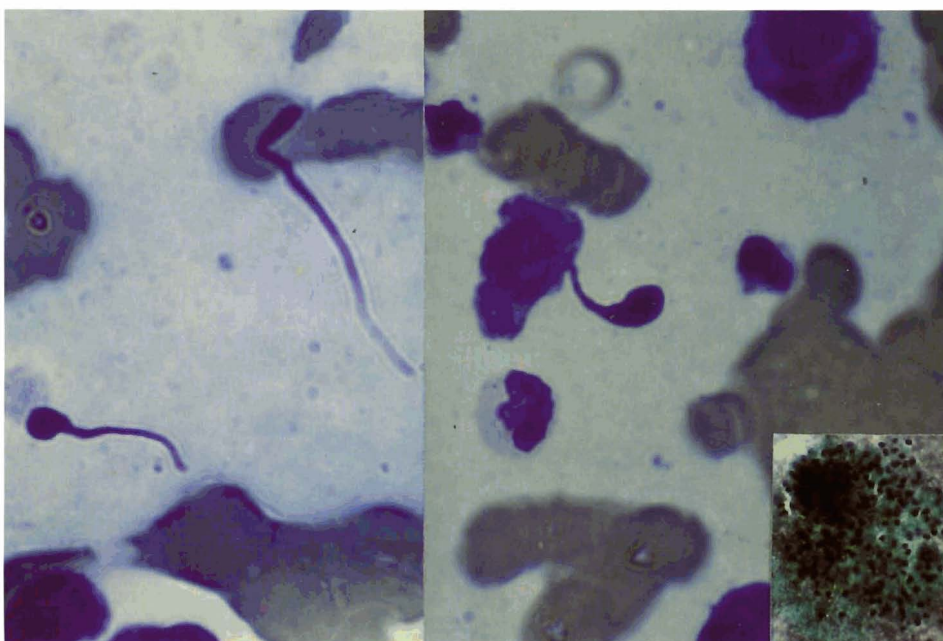


Fig. 20. Bone marrow cell smears (multiple myeloma), a NV during its production. Inset: left, free NVs, tails indicate movement. Giemsa. x 1,000. Inset: right bottom, cell clump of plasma cells embedded in green, fibrillar, translucent mucin. Papanicolaou. x 100

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fertilizing) host-cell nucleus with different amounts or DNA.

Scientists had realized the possibility of viral DNA being involved in human malignancies but the absence of any human cancer, clearly associated with a virus, greatly contributes to the notion that it may occur in some animals (Dagleish, 1991). Transfer of nuclear material, by NVs and its incorporation in other nuclei resembles viral (episomes) infection. However, the implanted nuclear material is not of viral origin,

neither is it constant and could not until now be identified.

NVs, also present in mouse embryonic cells, are implanted in the cytoplasm of the same cell. It is attractive to suggest that in normal embryogenesis, NVs, injected within the same cell, are destroyed by cytoplasmic karyolytic enzymes; while in abnormal cases NVs could be injected into the nucleus of surrounding cells instead, resulting, for example, in uniparental origin of chromosomes or genes associated

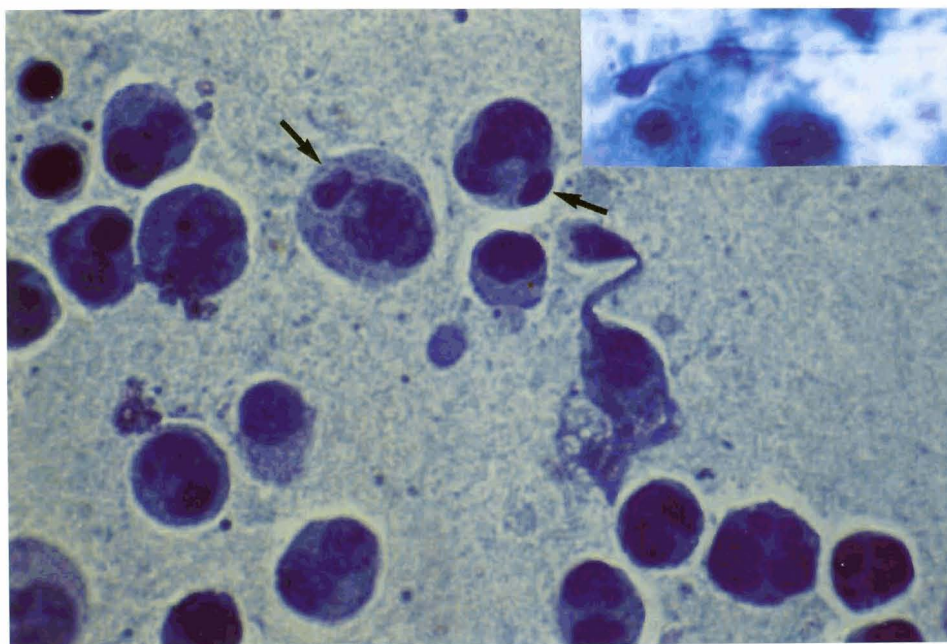


Fig. 21. Chronic myeloid leukemia. A parasitic cell divides while the host nucleus remains inert. Arrows indicate parasitic nuclei in other cells. Inset: free NV. Papanicolaou. x 1,000

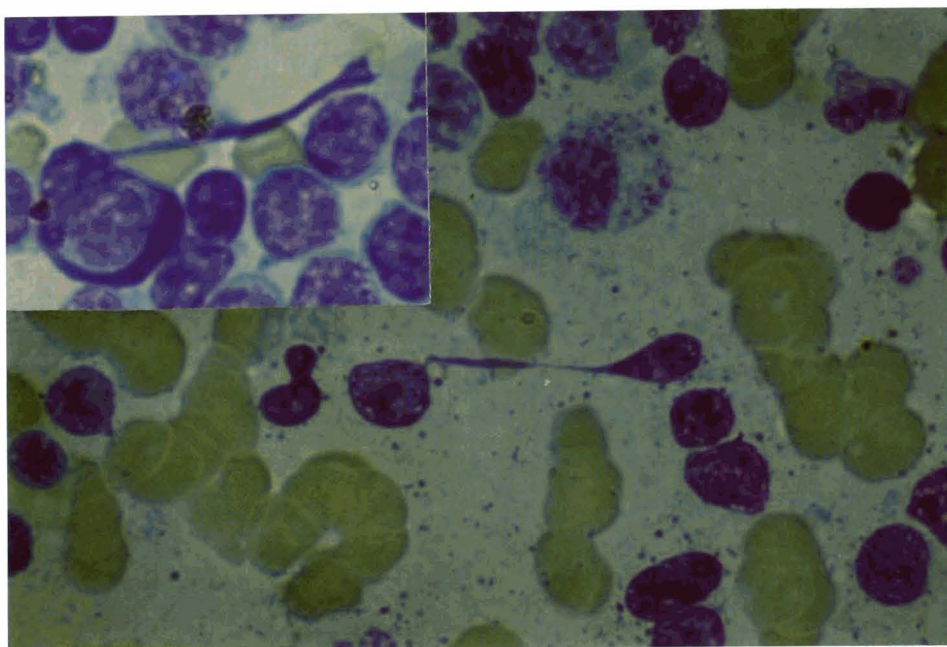


Fig. 22. NV production in chronic myeloid leukemia. Giemsa. x 1,000. Inset: NV in chronic lymphocytic leukemia has surrounded another cell. Giemsa. x 1,000

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with intrauterine, neonatal and childhood disorders (Hall, 1990).

The mother-cell nucleus, after NV production, obviously loses nuclear material equal to the size of the head of the NV. The injected host-cell nucleus gains additional nuclear material. This mechanism of nuclear material transfer among cells may be the explanation for genetic instability and loss of heterozygosity in tumorigenesis (Ponder, 1988; Dryja et al., 1989).

NVs, once in the normal host-cell nucleus, may be integrated into its genome (episomes), and cause mutations of proto-oncogenes into actively transforming oncogenes or may trigger loss of tumor suppressor genes or provoke position effects, all being genetic events associated with human neoplasm development (Cooper, 1990).

There is no doubt that NVs are morphologically identical to spermatozoa, viruses and bacterial phages (head with, or without tail), all vehicles of biological

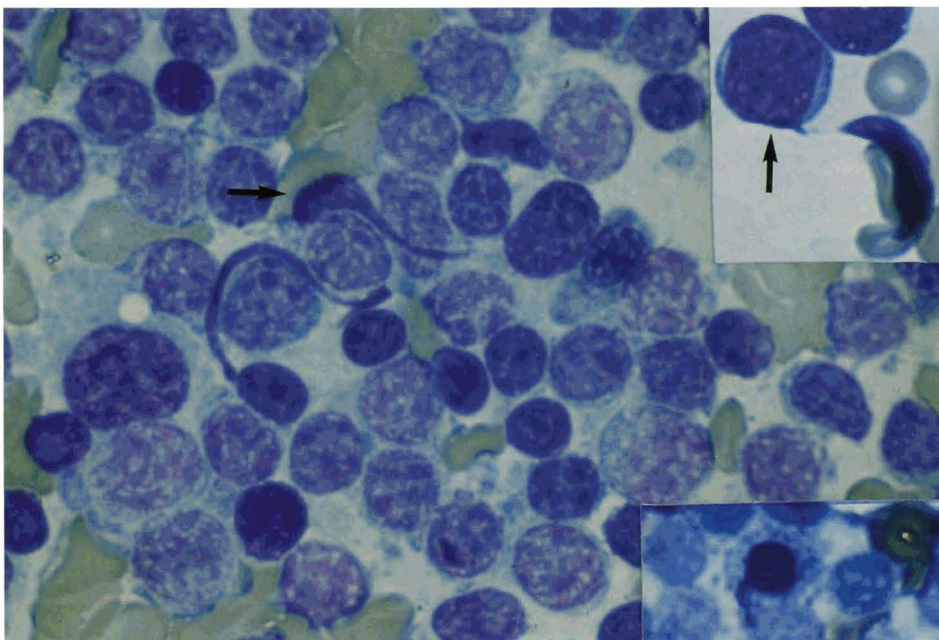


Fig. 23. NVs in chronic lymphocytic leukemia. Arrow points at free NV with haematoxylinophilic and eosinophilic head. Giemsa. x 1,000. Inset: top right, a haematoxylinophilic head (episome) is attached to eosinophilic nucleus (arrow) during its detachment from its mother-cell; bottom right, a hyperchromatic-pycnotic NV infecting another cell. Giemsa. x 1,000

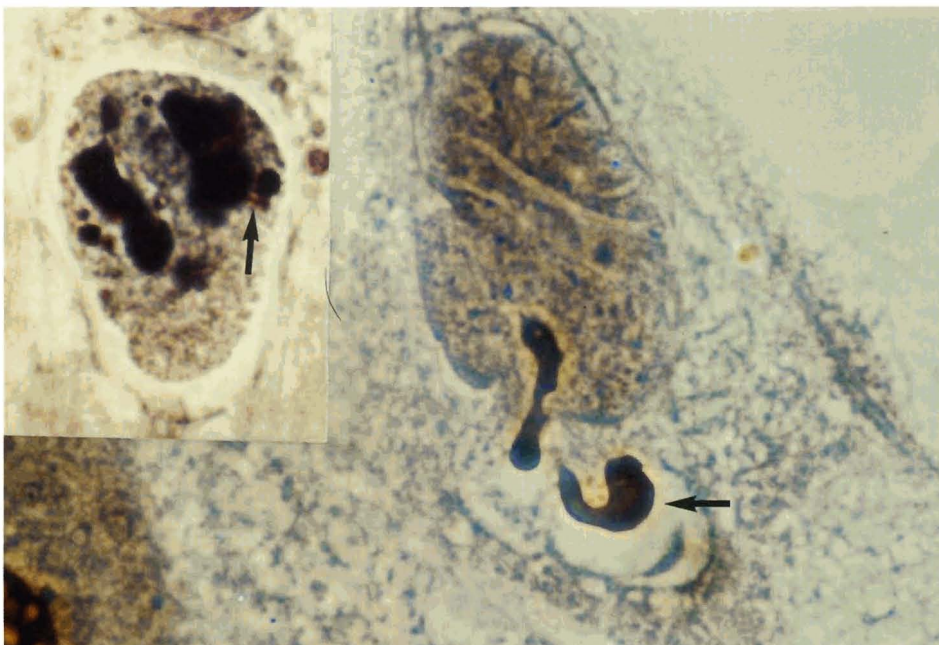


Fig. 24. NV with halo in mouse hatched blastocyst cells (arrows), injected in the cytoplasm of the same cell. Inset: Parasitic nucleus in embryonic host-cell; its nucleolus is producing NVs (arrow). Papanicolaou, phase-contrast, x 1,000

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active DNA inside other cells.

Pathologists have observed NVs in malignant tissue sections but have evaluated them as fragments of cell degeneration. This is justifiable as tissue sections do not provide a three dimensional picture as cell cultures do. Indeed even spermatozoa in paraffin sections appear as nuclear fragments of cell degeneration.

The present study shows that the consistent

morphology of NVs, their implantation and division in other cells are not all events and products of karyolysis but of abnormal cell division involved in cell to cell invasion.

In conclusion, NVs are common in all types of human malignancies and transfer DNA to other cells in a process related to fertilization or viral infection.

Further research is in progress to investigate this new process in human malignant cell populations.

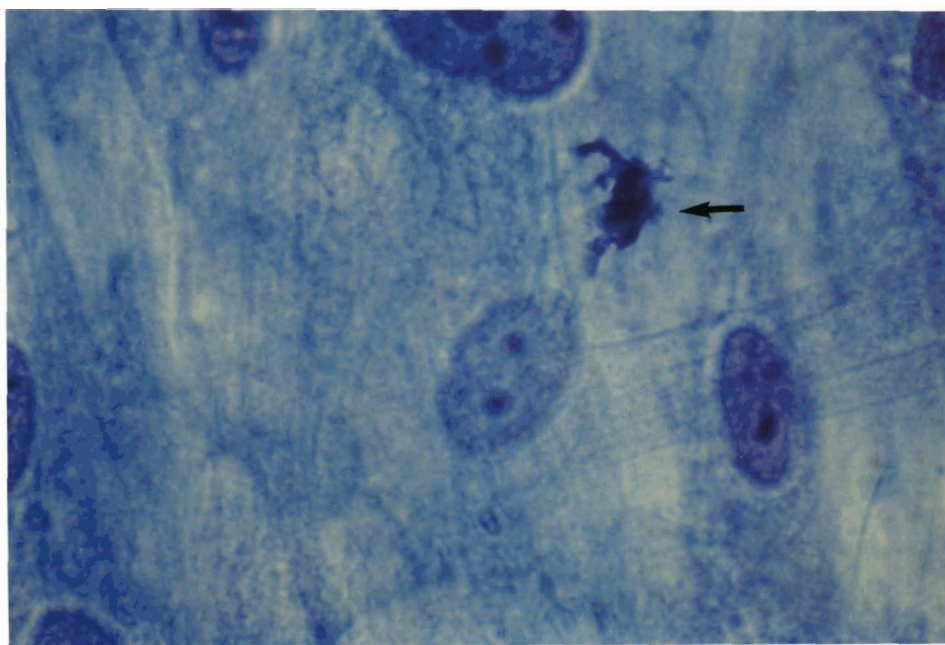


Fig. 25. Karyolysis in normal liver cell culture (arrow). It is characterized by the amorphous nuclear mass, of non-uniform, light- and dark blue-coloured areas. Papanicolaou. x 1,000

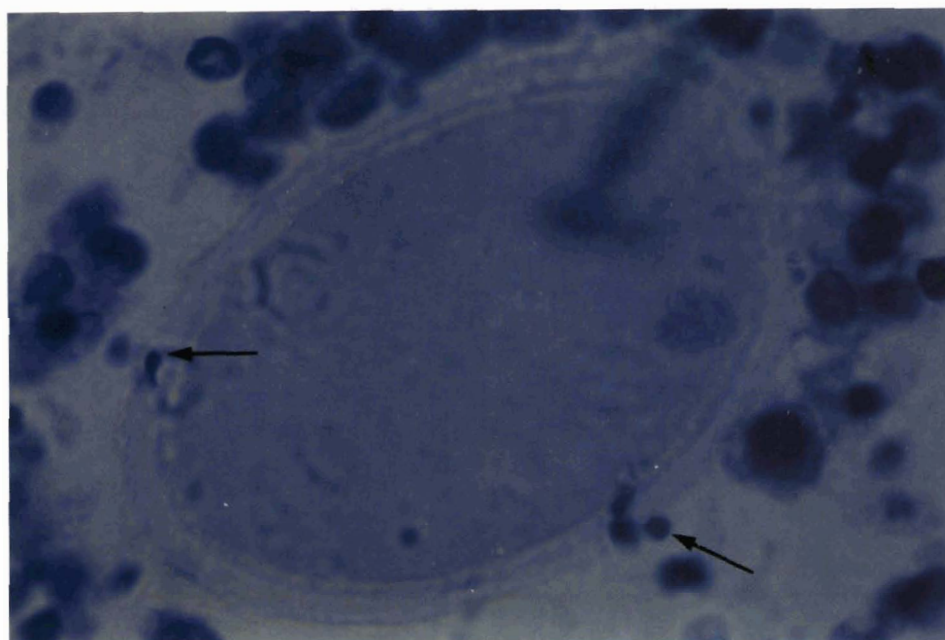


Fig. 26. Paraffin section of spermocytes. Spermatozoa are similar to NVs in malignant tissue sections (arrows). H-E. x 1,000

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Fig. 27. Free NV in melanoma cell culture. Feigen, x 1,000. Insets: a, decondensed chromosomes carried by a free NV in malignant bladder cell culture, Feigen, x 1,000; b, culture, Feigen, x 1,000; c, condensed chromosomes transferred by spermatozoa, Feigen, x 1,000; d, heads of NVs in lung malignant cell cultures, Feigen, x 1,000; e, lung NV in glycosamino-glycan extracellular matrix, PAS-diastase, x 1,000

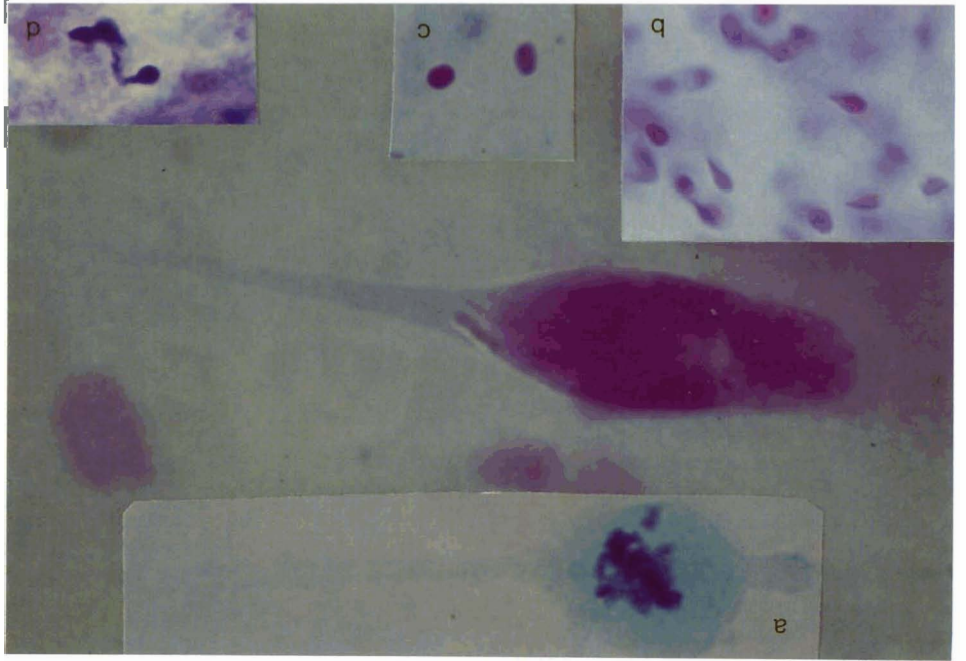
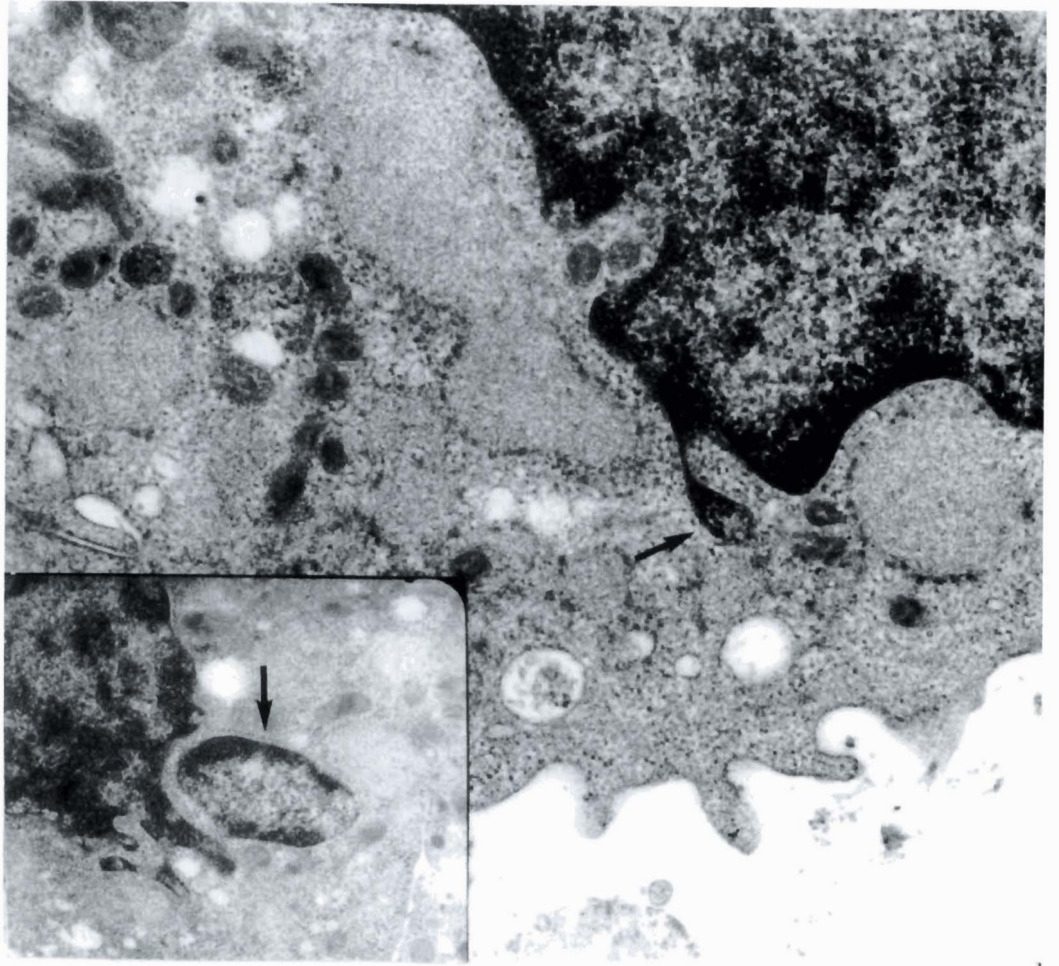


Fig. 28. Ultrastructure of bladder tumor cells. Nuclear projection resembles NV (arrow). x 5,300. Inset: Microtubules and host-cell nucleus in a position of probable synkaryosis. x 2,500



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