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Cytogenetic analysis and morphology of malignant nuclear vlimata. The life cycle of malignant cells

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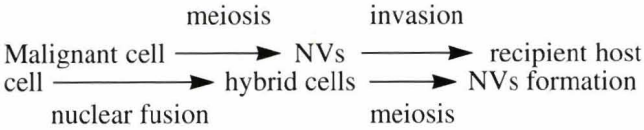
Summary. The karyotype of malignant nuclear vlimata (NVs) was investigated with the in situ and chromosomal spreading techniques. NV metaphases were recognised by the head with tail morphology, kept in situ and evaluated by the chromosomal spreading technique. It was shown that malignant NVs were produced by random, uncontrolled meiosis. NVs contain and carry single, hypodiploid, haploid, hypohaploid, hyperdiploid and atypical sets of chromosomes. NVs are unstable parasitic cellular elements, invading the cytoplasm or the nucleus of host cells, extruding and implanting their chromosomes in the host cell upon contact. Within a malignant cell culture, NVs play the role of chromosomal donors and host cells of chromosomal recipients. NVs were identified as episomatic on host cell nuclei or incorporated into host cell metaphases. The observations are discusssed in terms of fertilization, viral infection and apoptosis. Comparison of malignant NVs with phytohaemagglutinin (PHA)-activated lymphocytic NVs is provided, as well as the life cycle of the malignant cells as follows:



Key words: Cytogenetics, Nuclear vlimata, Malignant cells, Cellular invasion, Meiosis, Life Cycle, Aneuploidy

Introduction

Nuclear vlimata (NVs) are spermatozoa-like cells produced by malignant cells in vitro and in vivo. Malignant NVs, products of unequal, asymmetrical division of neoplastic cells, function as parasites invading the cytoplasm or nucleus of surrounding host-cells (Logothetou-Rella, 1993).

Phytohaemagglutinin (PHA)-activated lymphocytes also produced NVs by meiosis, carrying hypodiploid, haploid and hypohaploid sets of chromosomes (Logothetou-Rella, 1994a).

In this study the cytogenetic analysis and morphology of malignant NVs are investigated, using a) the *in situ* cytogenetic technique, and b) the chromosomal spreading technique.

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Materials and methods

Cell cultures

Three cell lines were used for cytogenetic analysis and morphology of malignant NVs. Walker rat tumor cells, were isolated from transplanted rat tumor and kept frozen for in vitro studies. Human urothelial monolayer Pa-cells (Logothetou-Rella, 1993) and human lung monolayer M-cells (mixed malignant and normal cells) were previously characterized (Logothetou-Rella et al., 1992).

All cell lines were grown in complete medium RPMI-1640 (Gibco) supplemented with 10% foetal bovine serum (Seromed), penicillin (Seromed, 100 U/ml), and streptomycin (Seromed, 100 µg/ml), and incubated at 37 °C in a CO_2-humidified incubator.

Cytogenetic analysis and morphology

Cytogenetic analysis was performed by the chromosomal spreading technique, standard Giemsa, RhG banding of Dutrillaux and Lejeune (1971) and in situ by standard Giemsa. One to two hundred consecutive metaphases were analyzed as previously evaluated (Logothetou-Rella, 1994a).

Chromosomal preparations, besides karyotyping, were also evaluated for cytogenetic morphology, such as chromosomal morphology (condensed vs regular chromosomes) within the same metaphase and cell population, intensity of Giemsa chromosomal staining (hyperchromatic), NV and nuclear morphology, nuclear fusion and fragmentation.

Results

Cytogenetic analysis and morphology

In situ chromosomal analysis showed that the three malignant cell lines exhibited nuclei extruding massive, condensed chromosomes. The number of extruded chromosomes varied from hypohaploid to polyploid (Fig. 1, 1a). Nuclei extruding chromosomes showed hyperchromatic intranuclear texture at the site of extrusion, corresponding to the intranuclear chromosomal content (Fig. 1). Chromosome «0» was present in many metaphases consisting of condensed hyperchromatic chromosomes and hence these metaphases were evaluated as meiotic (Fig. 2). Meiotic metaphases were identified as extracellular, cytoplasmic (Fig. 2) or within the cytoplasm of host cells. There was a characteristic, distinguished, small, hyperchromatic meiotic anaphase, in the cytoplasm of host cells, identified as common in all three cell lines (Fig. 3).

NV metaphases were identified by the head with tail morphology and consisted of intact condensed nuclear head (Fig. 4a,b,h) or condensed nuclear head extruding regular and «0» chromosomes (Fig. 4f) or fibrous chromatin with or without regular chromosomes (Fig. 4c-e,g,i). During meiotic division the mother cells is connected with the daughter cell by a nuclear bridge (Fig. 4i); upon separation the nuclear bridge breaks and constitutes the tail of NVs. The head size and tail length of NVs were variable. NVs consisted of hypodiploid, hypohaploid, hyperdiploid and polyploid sets of chromosomes even in the same cell population. NV aneuploid metaphases were extracellular, cytoplasmic (Fig. 5) or in the cytoplasm of host cells (Figs. 5f, 6).

The nucleus of malignant cells formed thin or thick hyperchromatic segments frequently with attached nuclear buds (Fig. 7). Thin nuclear segments were identified in the heads of NVs (Fig. 7g) and thick ones attached on host cell nuclei (Fig. 7c,e). These segments contained chromosomes, as the in situ chromosomal

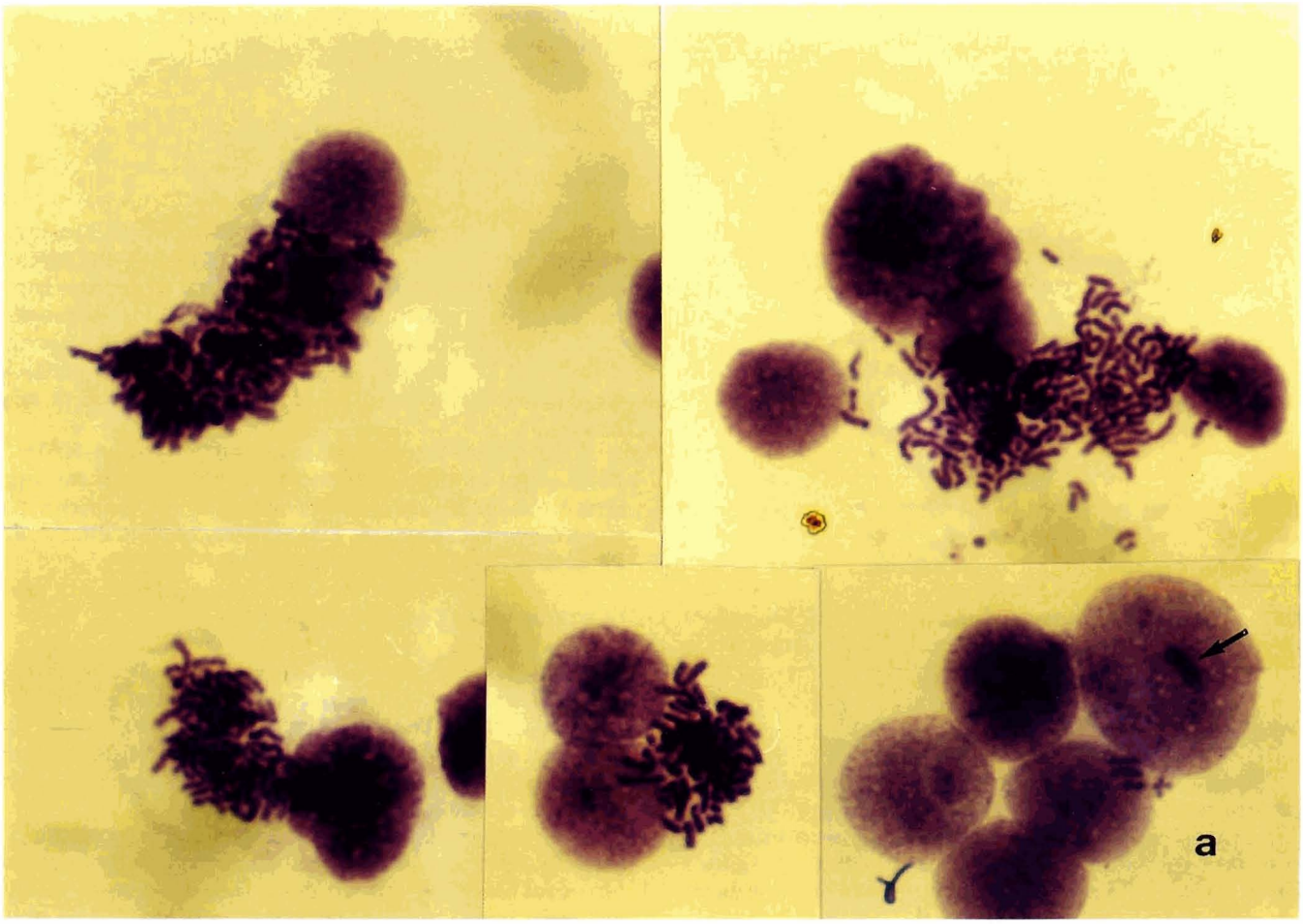


Fig. 1. In situ meiosis of Pa-cells. Extrusion of hyperchromatic condensed chromosomes of variable number. Intranuclear condensed hyperchromatic regions are associated with massive chromosomal extrusion. **Inset a:** Extrusion of 4 chromosomes and intranuclear chromosome is visible. Giemsa. x 1,000

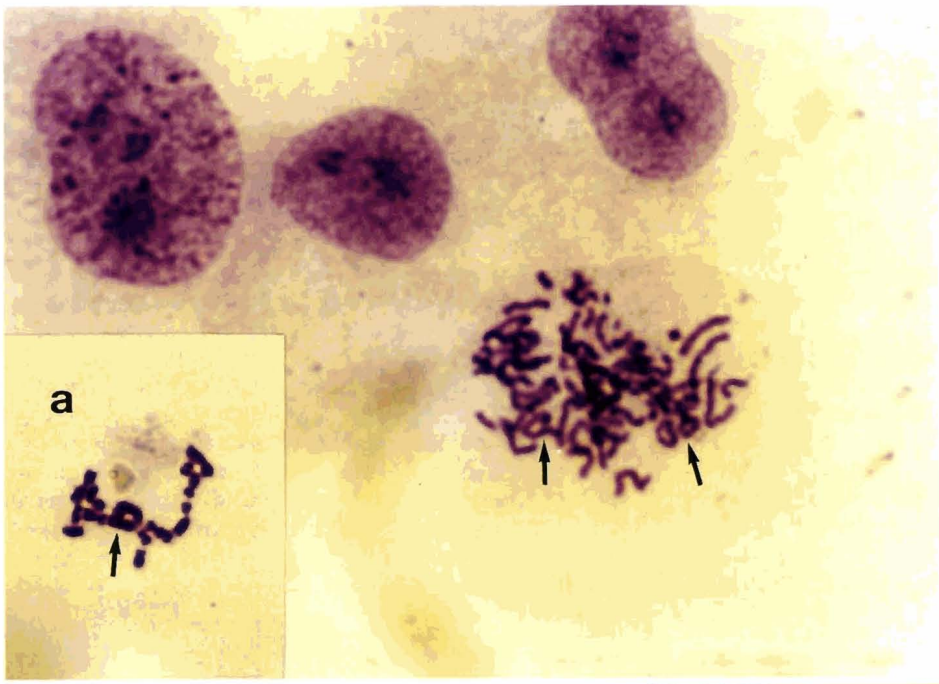


Fig. 2. Meiotic cytoplasmic Pa-cell metaphase exhibiting «0» and broken chromosomes. **Inset a:** A hypohaploid meiotic Pa-cell metaphase with «0» (arrows) and hyperchromatic condensed chromosomes. Giemsa. x 1,000

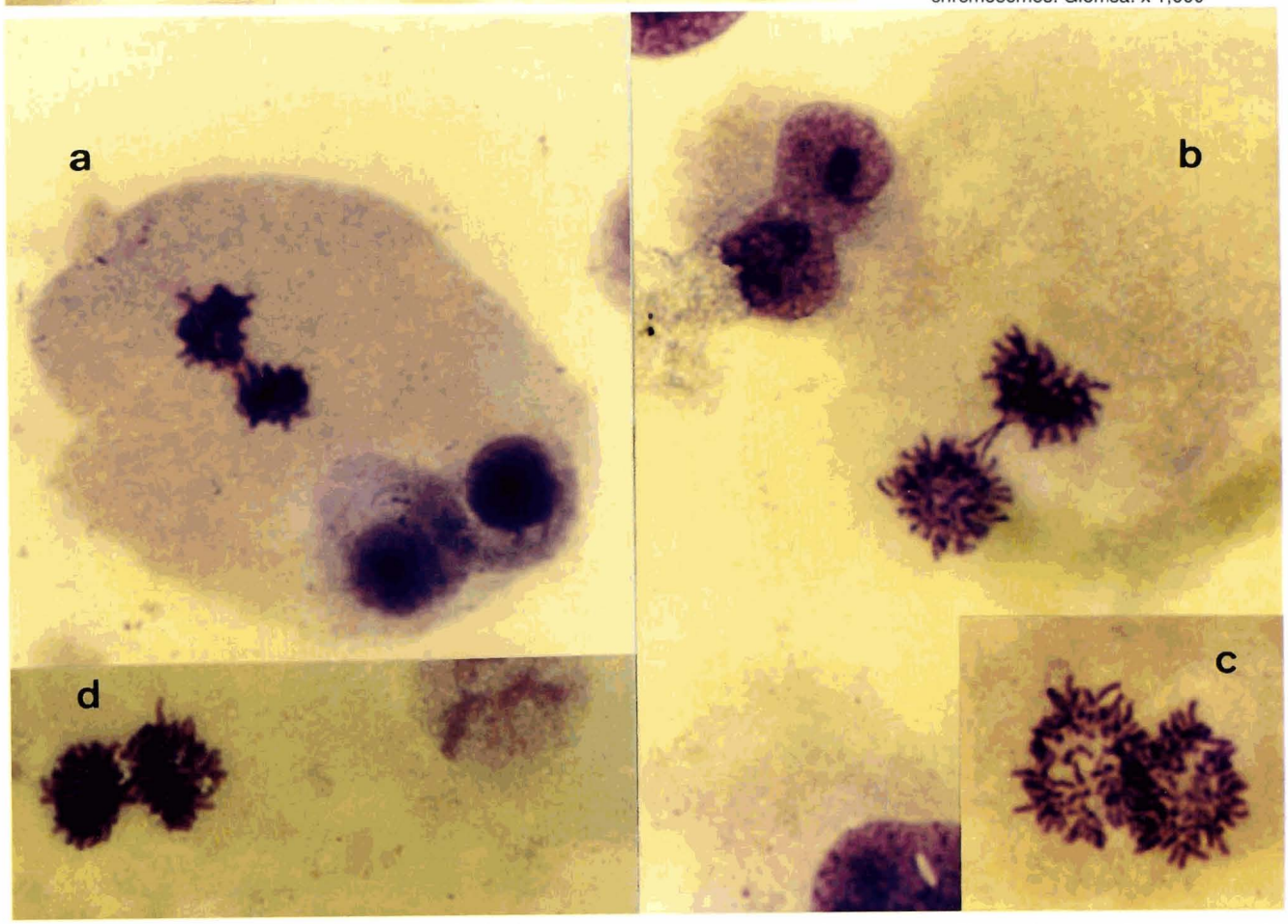


Fig. 3. A distinguished, meiotic anaphase in host cell cytoplasm, common in all cell lines examined. **Inset a**: Walker rat tumor cells. **Insets b, c:** Pa-cells. **Inset d:** M-cells. Giemsa. x 1,000

analysis showed metaphases of segment morphology. Nuclear segments consisted of regular chromosomes or fibrous chromatin with condensed chromosomes (Fig. 8).

The morphology of the thin nuclear segments correlated well with that of NV tail and nuclear bridge. Thin nuclear segments were identified in the cytoplasm and/or the nucleus, passing from nucleus to cytoplasm and from nucleus into another nucleus (Fig. 9). Nuclei containing thin nuclear segments extruded chromosomes arranged in line (Fig. 9a-c). These nuclei did not exhibit intranuclear, condensed, hyperchromatic regions of massive chromosomal extrusion. In addition, the thin nuclear segments gave rise to chromosomes upon decondensation (Fig. 9e,f). Fine fibrous chromatin was also extruded by malignant cell nuclei into the cytoplasm (Fig. 9d).

NVs, of condensed, variably-sized nuclear head, were observed inside or passing through or attached on host cell nuclei (Fig. 10) and also directed at a host cell metaphase (Fig. 10g,h,i). NVs, during their meiotic production, implanted their chromosomes in the cytoplasm of host cells undergoing meiosis (Fig. 11).

Within the same cell population, there were chromosomes of different morphology and Giemsa staining intensity such as: regular chromosomes with two arms and light Giemsa staining intensity; condensed chromosomes with light Giemsa staining intensity; condensed chromosomes with strong Giemsa intensity (hyperchromatic) (Fig. 12, 12a). Some metaphases consisted of regular and condensed hyperchromatic chromosomes (hybrids), resulting from the fusion of two different NVs (Fig. 12b,c). Condensed head NVs (Fig. 12d,e) or thick nuclear segments (Fig. 12f-i) were located among chromosomes of a metaphase. Very fine, short, hyperchromatic, condensed nuclear tail segments were attached (inserted) on regular chromosomes or located among metaphase chromosomes (episomatic)

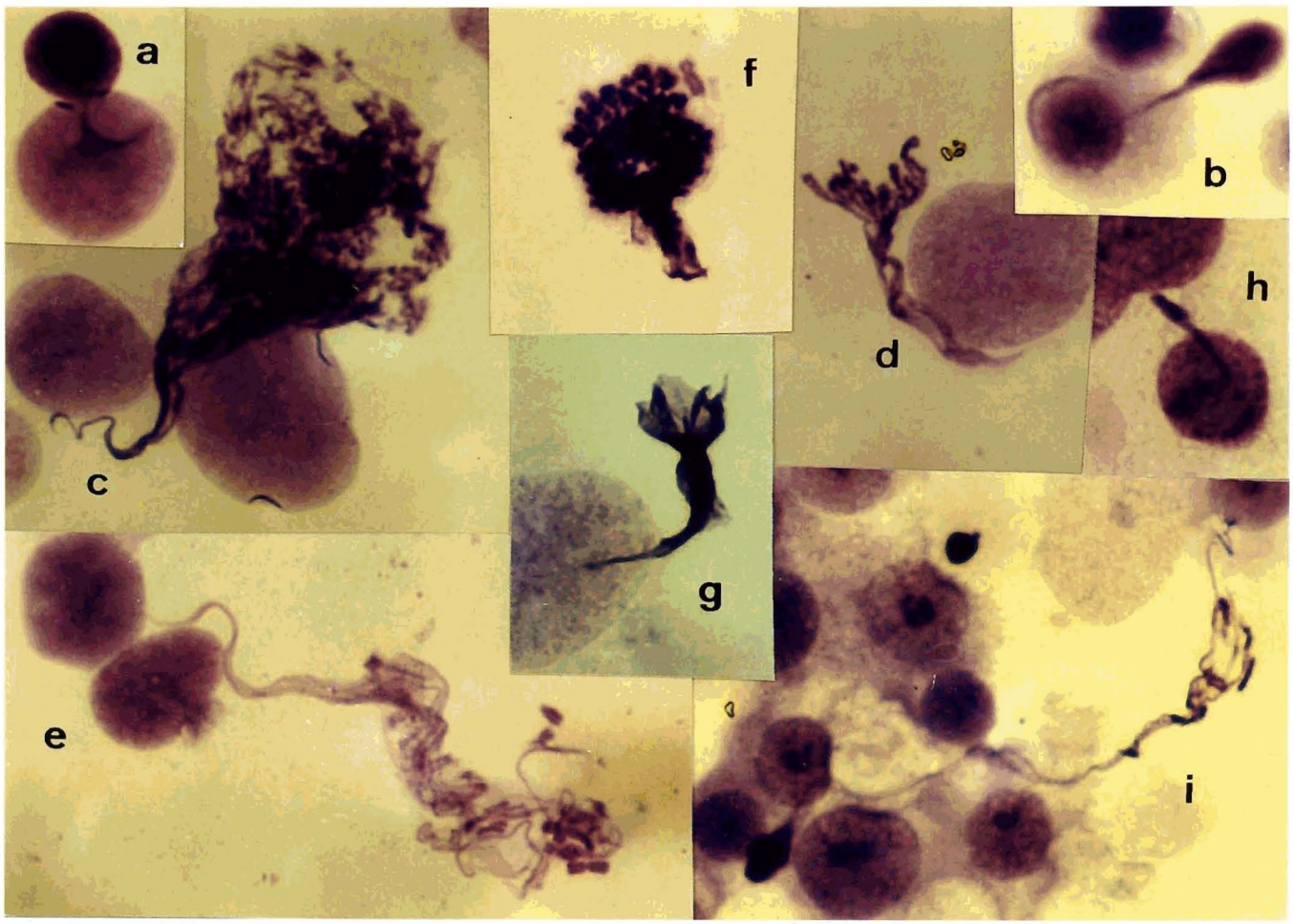


Fig. 4. In situ cytogenetic morphology of NVs. **Insets a,b:** Pyknotic nuclear head Pa-NV during its production. **Insets c, d, e:** Polyploid and hypodiploid Pa-NVs of fibrous chromatin and condensed or regular chromosomes. **Inset f:** A Pa-NV extruding «0» and regular chromosomes. **Inset g:** An unusual morphology of a Pa-NV. **Inset h:** A small condensed-head Walker rat tumor NV. **Inset i:** Walker rat tumor mother and daughter NVs connected by a nuclear bridge. One is in meiotic metaphase. Giemsa. x 1,000

(Fig. 13).

A variable number of chromosomes transferred by NVs were extruded and implanted in the cytoplasm of host cells (Fig. 14, 14a). Buds of nuclear segments also extruded chromosomes (Fig. 14b).

Single chromosomes and aneuploid metaphases of NV morphology observed by the in situ chromosomal technique document that aneuploid metaphases belonged to NVs and were not artefactual. Detailed karyotyping was performed using the chromosomal spreading technique, for better chromosomal spreading, evaluating single chromosomes and aneuploid metaphases as content of NVs. There was no repetitiveness of a particular aneuploid metaphase within the same cell line (Table 1). Repetitiveness was only numerical. M-cells exhibited 36% diploid, 4% tetraploid and 60% hypodiploid metaphases. Aneuploid chromosomal sets of M-cells are shown in Fig. 15. Pa-cells showed 20% hyperdiploid, 13% polyploid and 67% hypodiploid metaphases (Table 1). Walker rat tumor cells showed 28% diploid (2n= 42) and 72% hypodiploid metaphases (Table 1). A large percentage of the hypohaploid metaphases are in sets of 1-10 chromosomes in the three cell lines. Repetitiveness of each chromosome in 1-10 or

Table 1. Numerical analysis of malignant cell metaphases.

CELL LINE	%DIPLOIDY	% HYPERDIPLOIDY	% TETRAPLOIDY	% POLYPLOIDY	% HYPODIPLOIDY	HYPODIPLOID AND HYPOHAPLOID SETS				
						1-10	11-20	21-30	31-45	41-45
M	36	0	4	0	60	44	7	4	4	1
Pa	0	20	0	13	67	59	6	2	0	0
	2n= 42					1-10	11-20	21-30	31-41	
Walker	28	0	0	0	72	39	5	1	27	

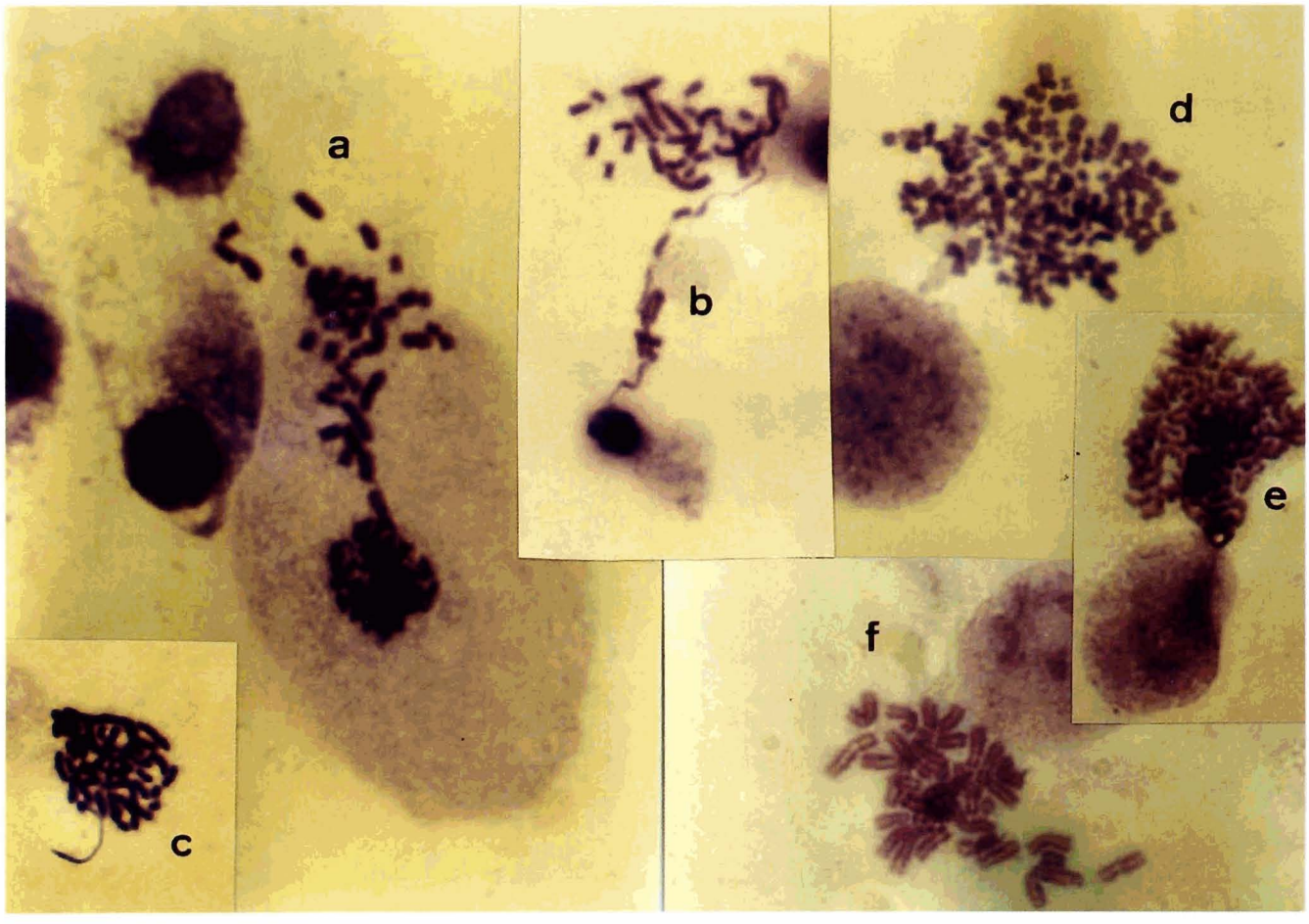


Fig. 5. In situ NV meiotic metaphases keeping the head with tail morphology. **Insets a, b, c:** Walker rat tumor NV metaphases. **Inset d:** A hyperdiploid Pa-NV metaphase. **Inset e:** A hyperdiploid M-NV metaphase. **Inset f:** A hypodiploid M-NV in the cytoplasm of a host cell. Giemsa. x 1,000

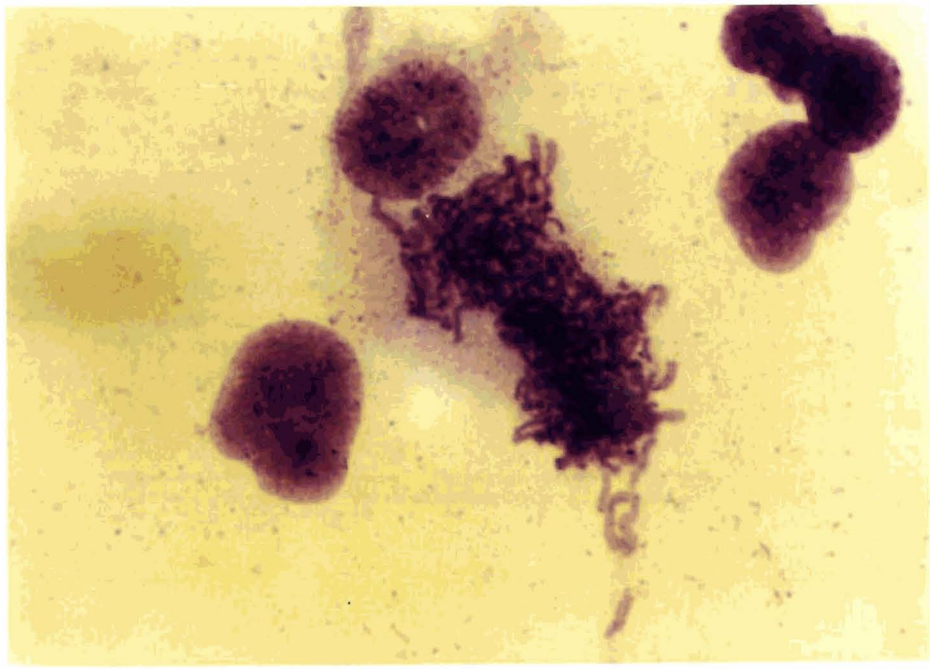


Fig. 6. In situ polyploid Pa-NV metaphase in the cytoplasm of a host cell. Giemsa x 1,000

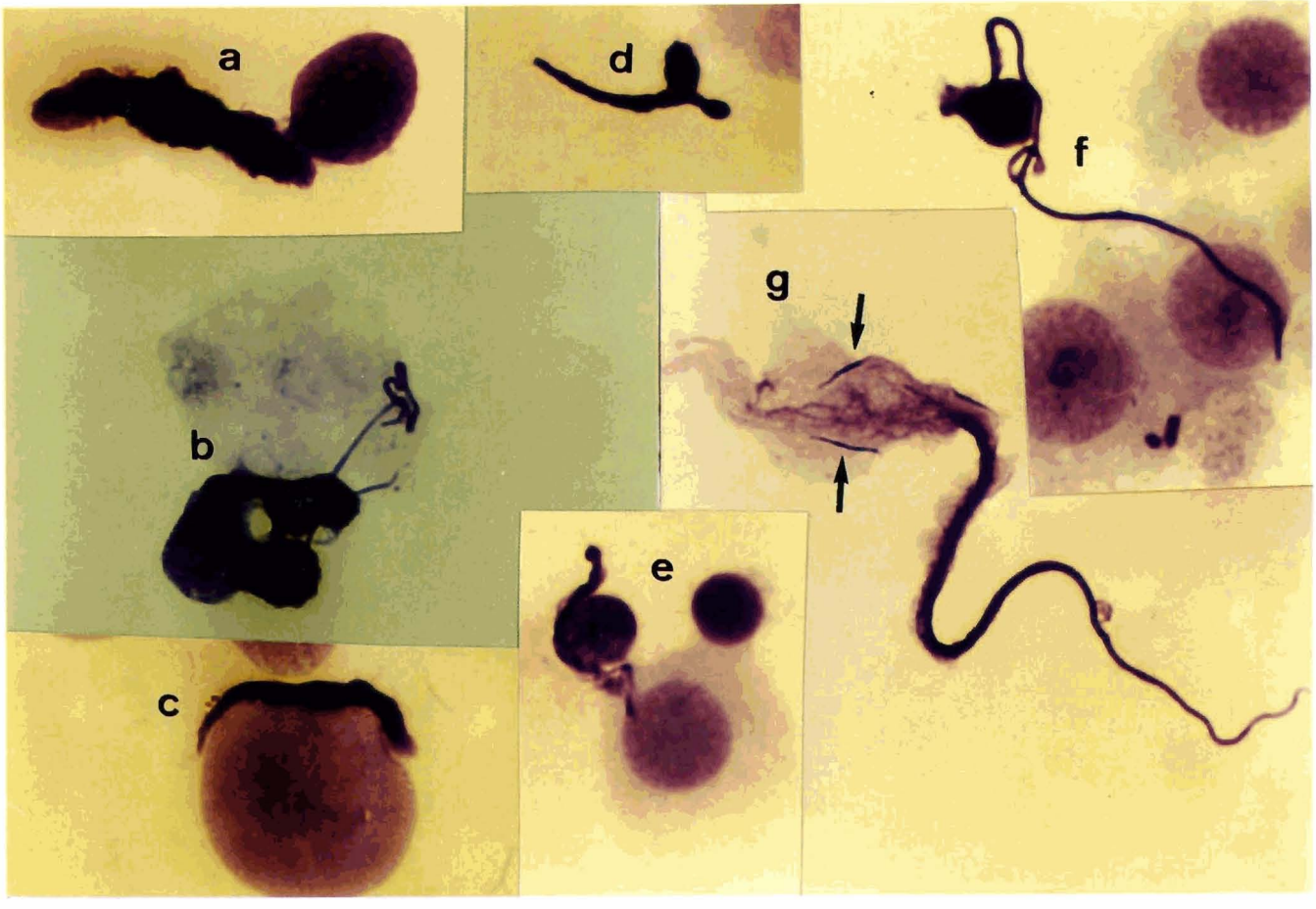


Fig. 7. In situ nuclear segments and buds of malignant cells. **Insets a, b:** Nuclear segments and buds of Pa-cells. Inset **c:** Attached nuclear segment on a Pa-cell nucleus. **Insets d, e, f:** Nuclear segments and buds of Walker rat tumor cells. **Inset g:** A Pa-NV of fibrous chromatin head, containing fine nuclear tail segments (arrows). Giemsa. x 1,000

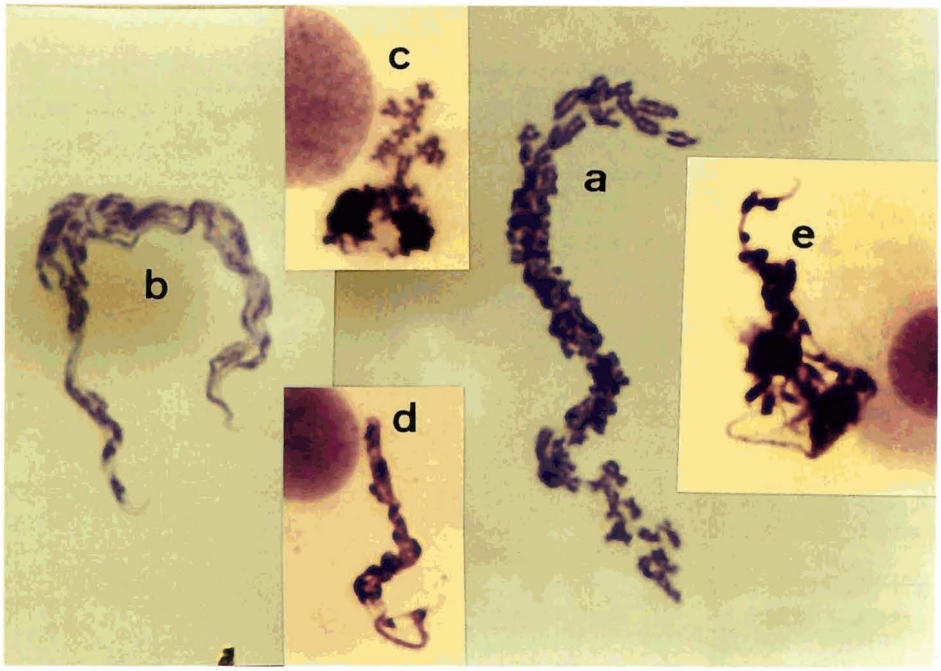


Fig. 8. Inset a, b: In situ Pa-metaphases, exhibiting the morphology of nuclear segments, of regular chromosomes or fibrous chromatin with condensed chromosomes, **Insets c, d, e:** Pa-cell nuclear remnants extruding chromosomes. Giemsa. x 1,000

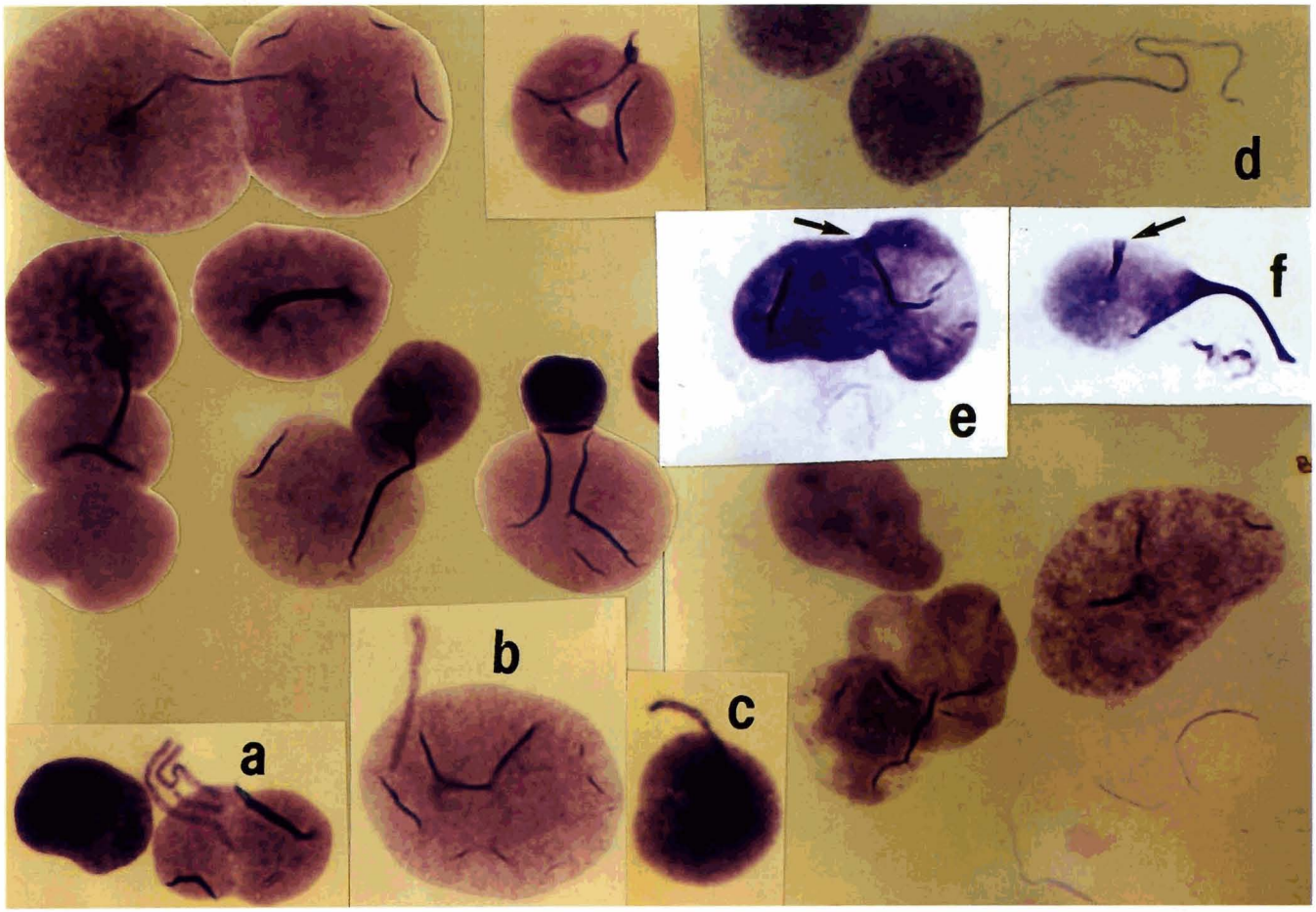


Fig. 9. In situ nuclear segments of NV tail morphology and texture in the cytoplasm, nucleus or passing from nucleus to nucleus in Pa-cells. **Insets a, b, c:** Extrusion of chromosomes in lines by Pa-cell nuclei which do not show condensed areas of the massive chromosomal extrusion. **inset d:** Extrusion of fine chromatin by Pa-cells. **Insets e, f:** Decondensation of thin nuclear segments into chromosomes. Arrows point at the two arms of a chromosome. Giemsa. x 1,000

1-3 sets is shown in Table 2. All chromosomes including atypical (with structural aberrations) and sex chromosomes are carried by NVs (Figs. 15-18). Polyploid metaphases are also meiotic (Fig. 19).

Chromosomal preparation slides besides chromosomal analysis and morphology were also evaluated for nuclear morphology. It was observed that invasion of NVs into host cells was followed by nuclear fusion. Nuclear projections with subsequent fusion occurred between two or more variably-sized nuclei, forming a round vacuole in the middle of fused nuclei (Fig. 20a-c). The nuclear membrane of fused nuclei was irregular to smooth (Fig. 20d,e). Nuclear fusion resulted in huge nuclei which then produced and extruded NVs and buds of variable size and number (Fig. 20). Among the three cell lines, Pa-cells showed the highest degree of cellular and nuclear fusion, nuclear budding and NV formation (Fig. 20) and M-cells the lowest.

Several stages of the malignant cell life cycle were identified in a tissue section from recurrent human peripheral neuroectodermal tumor (PNET)-myeloblastoma (Figs. 21, 22) and undifferentiated skin tumor

Table 2. Repetitiveness of each chromosome in hypohaploid sets.

CHROMOSOME No.	OF 1-10 CHROMOSOMES Cell line		OF 1-3 CHROMOSOMES Cell line	
	M	Pa	M	Pa
X	10	1	5	0
Y	0	0	0	0
1	14	6	12	6
2	14	4	8	1
3	5	6	3	5
4	6	2	3	1
5	10	1	8	1
6	10	3	6	1
7	3	1	1	0
8	10	3	8	1
9	15	7	9	4
10	12	1	5	0
11	5	7	4	4
12	5	6	5	3
13	13	4	9	3
14	8	3	7	1
15	7	2	3	1
16	8	4	4	1
17	10	6	7	3
18	7	3	3	1
19	6	4	5	2
20	6	6	2	1
21	9	4	5	0
22	10	4	5	2
Atypical		61	0	33

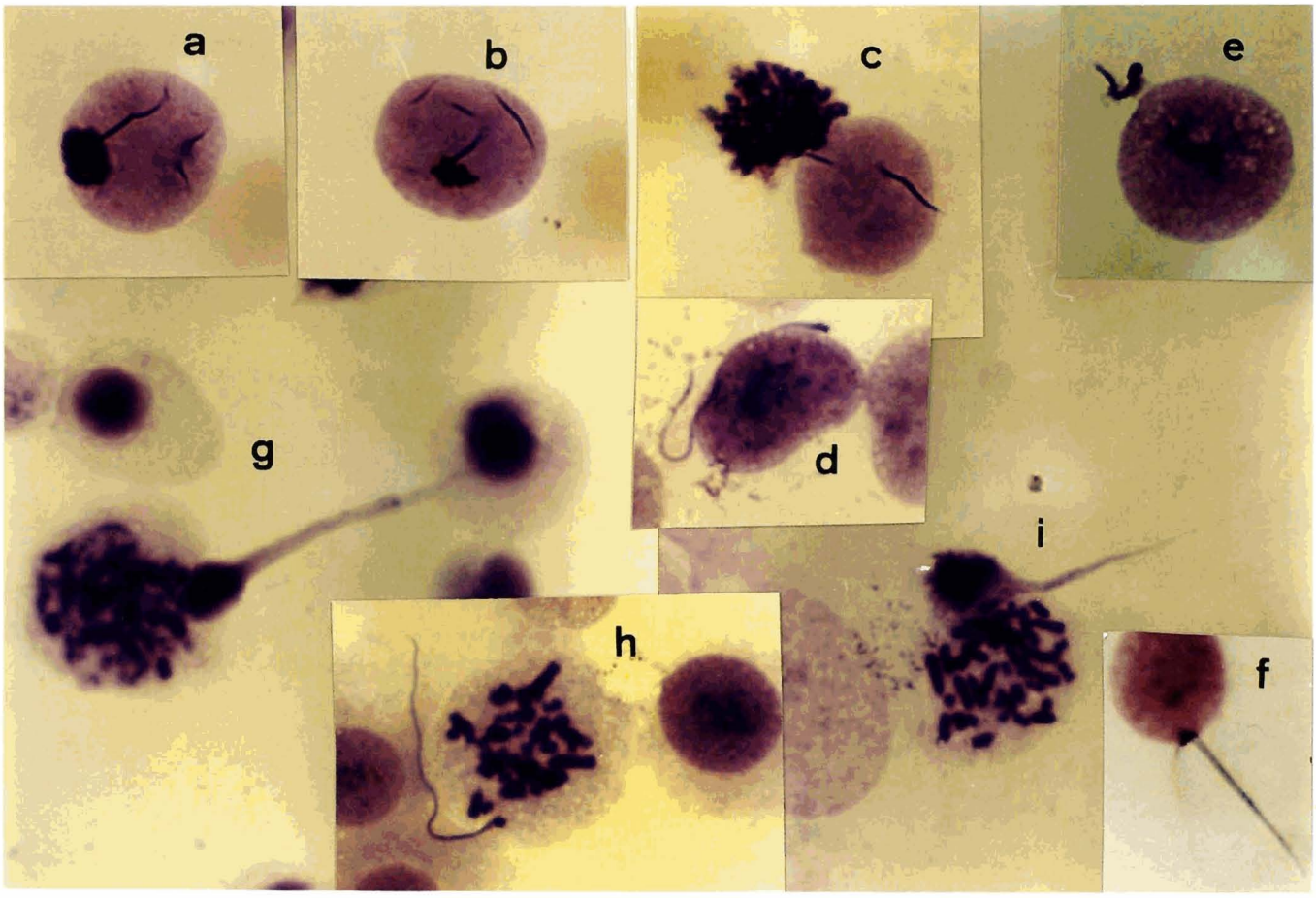


Fig. 10. Insets a, b: Intranuclear Pa-NVs and NV-tails. **Inset c:** Pa-NV passing through a host nucleus. **Insets d, e, f:** Small-sized Pa-NVs attached on host cell nucleus. **insets g, h, i:** Walker rat tumor NVs directed at a host cell metaphase. Giemsa. x 1,000

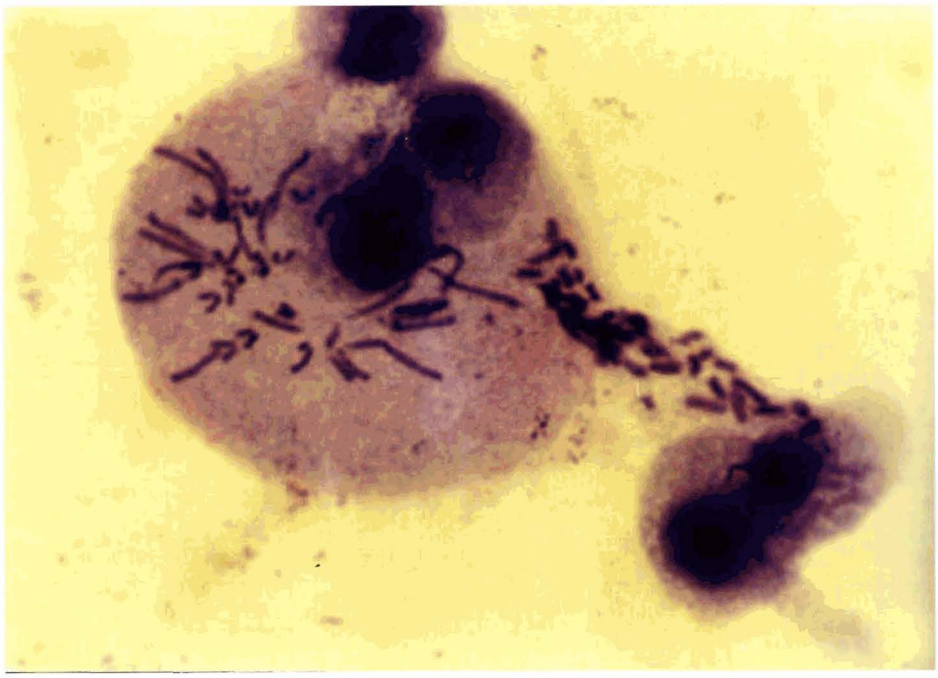


Fig. 11. In situ Walker rat tumor NV metaphase being implanted on a host cell, also undergoing meiosis. Giemsa. x 1,000

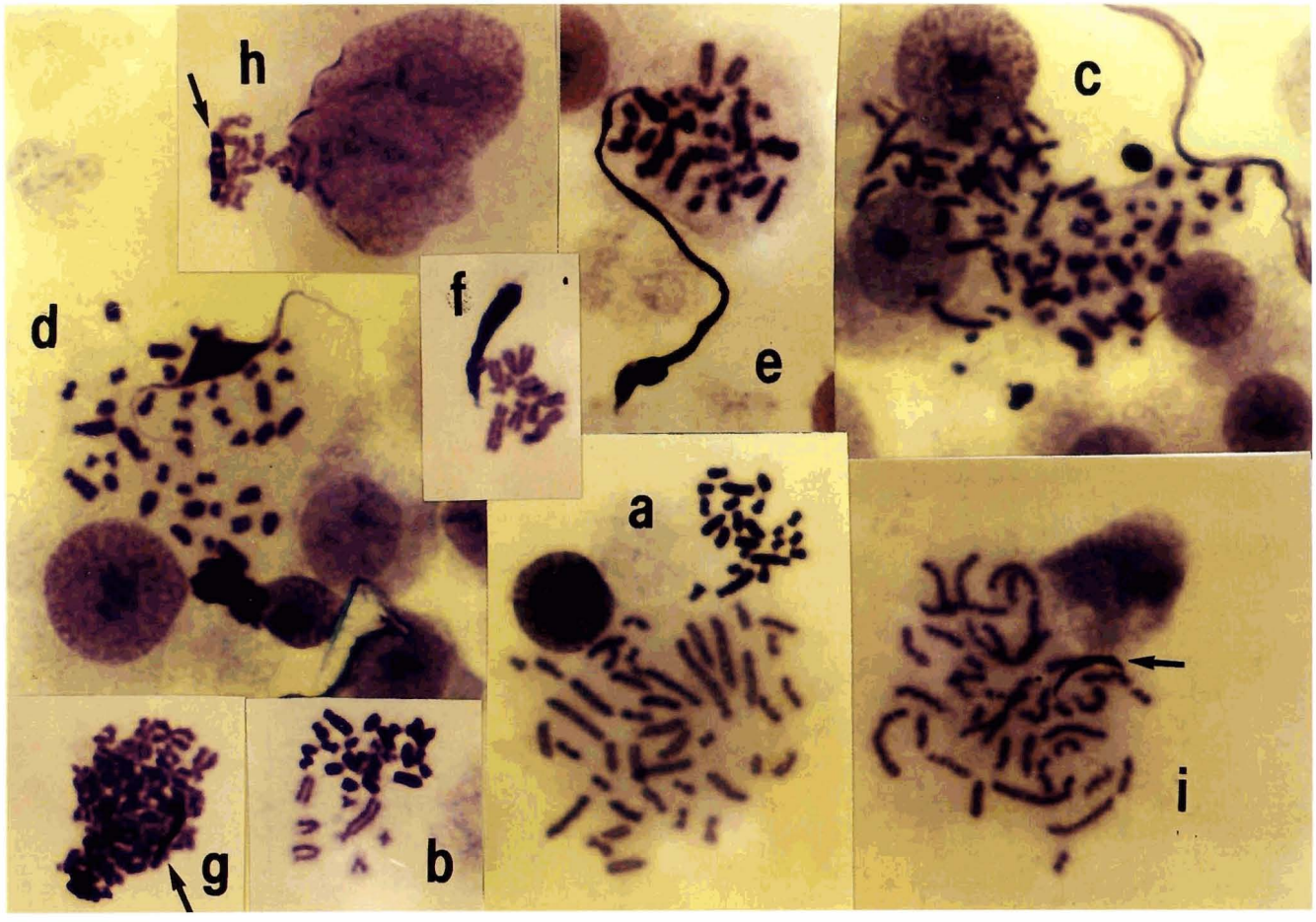


Fig. 12. In situ cytogenetic morphology of malignant cells. **Inset a:** Two Walker rat tumor cell metaphases side by side. One exhibits the morphology of an NV, of hyperchromatic, condensed chromosomes, and the other is cytoplasmic of regular chromosomes. **Inset b:** A mixed Pa-cell metaphase of hyperchromatic condensed with regular chromosomes. **Inset c:** Simultaneous extrusion of chromosomes by two Walker rat tumor cell nuclei resulting in a mixed cytoplasmic metaphase (hybrid). **Insets d, e:** Walker rat tumor cell metaphases containing NVs. **Inset f:** Pa-nuclear segment extruding chromosomes. **Insets g, h:** Pa-NV metaphases containing nuclear segments (arrow). **Inset i:** Walker rat tumor cell metaphase containing nuclear segment (arrow). Giemsa. x 1,000

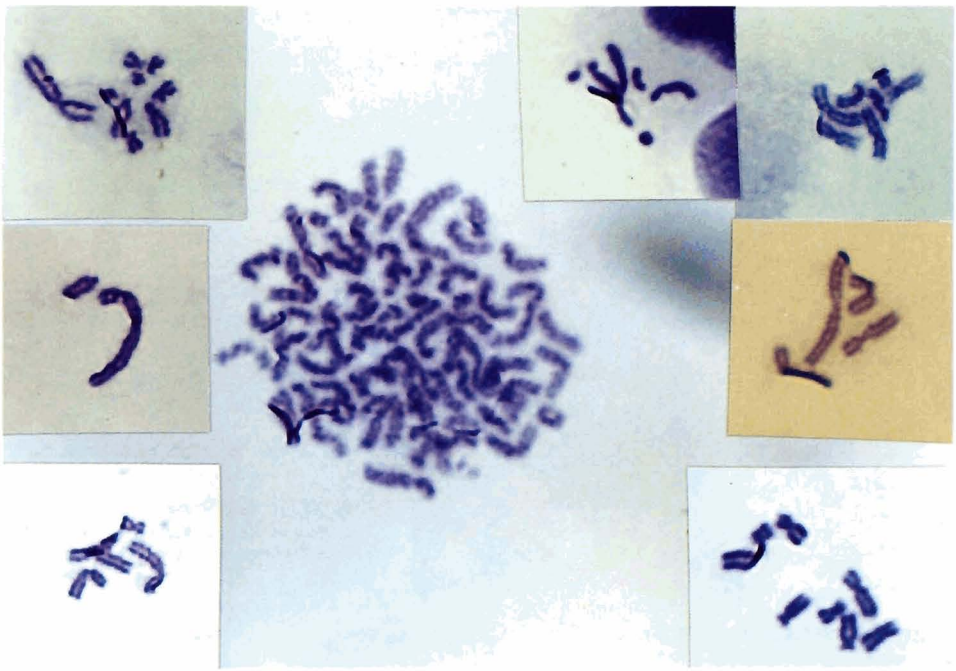


Fig. 13. Fine condensed, hyperchromatic, nuclear tail segments episomatic in metaphases or attached to chromosomes of Pa-cells. Giemsa. x 1,000

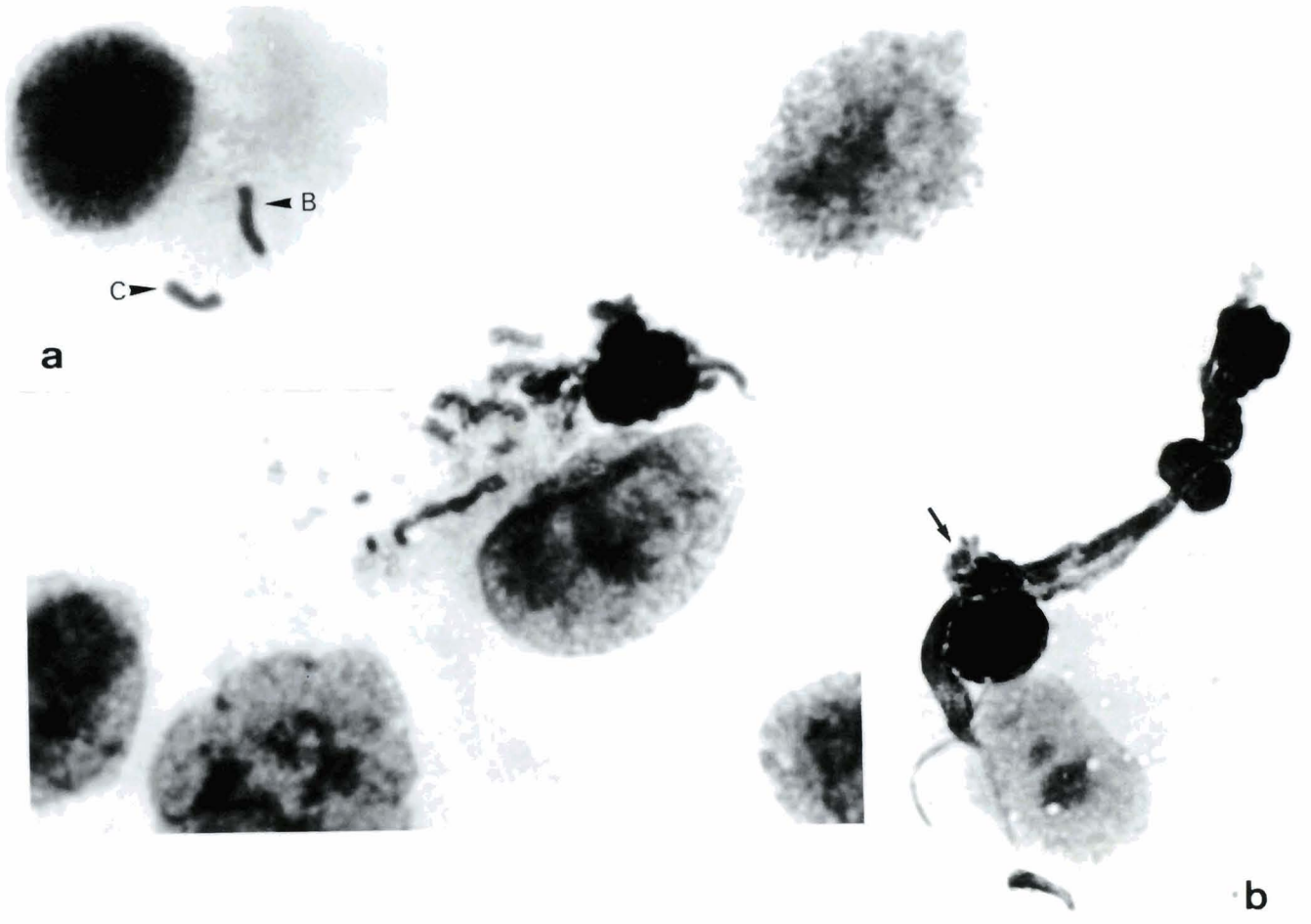


Fig. 14. In situ cytogenetic morphology of Pa-cells. An NV extruding and implanting its chromosomes in the cytoplasm of a host cell. **Inset a:** Two cytoplasmic chromosomes are not artefactual. **Inset b:** Nuclear segment with buds extruding chromosomes (arrow). Giemsa. x 1,000

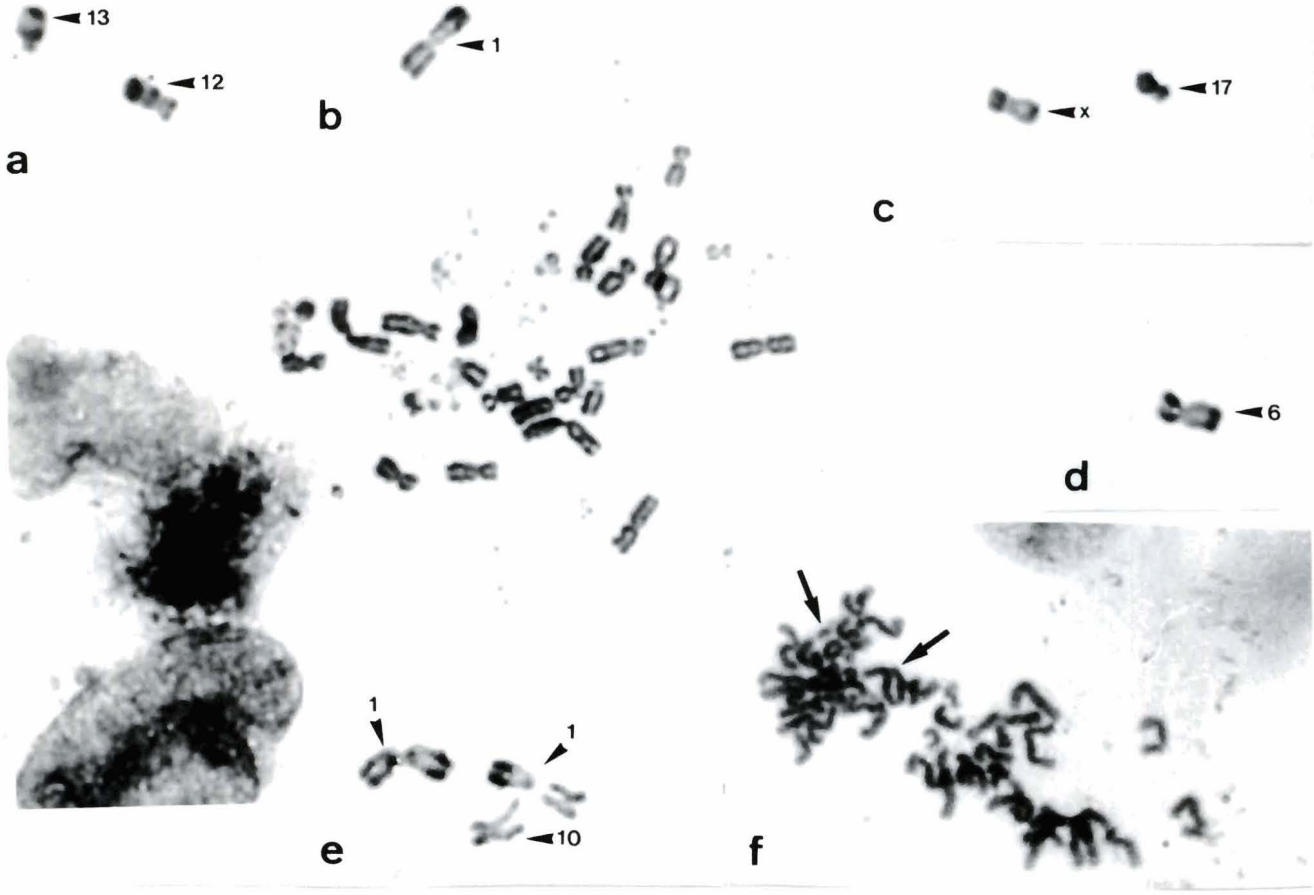


Fig. 15. In situ cytogenetic analysis of M-cells. A hypodiploid cytoplasmic metaphase with broken chromosomes. Giemsa x 1,000. **Insets a, b, c, d, e:** Sets of 1, 2 or 3 chromosomes belong to different NVs. Giemsa-RhG banding. x 1,000. **Inset f:** A meiotic, in situ, NV metaphase containing «0» chromosome (arrows). Giemsa. x 1.000

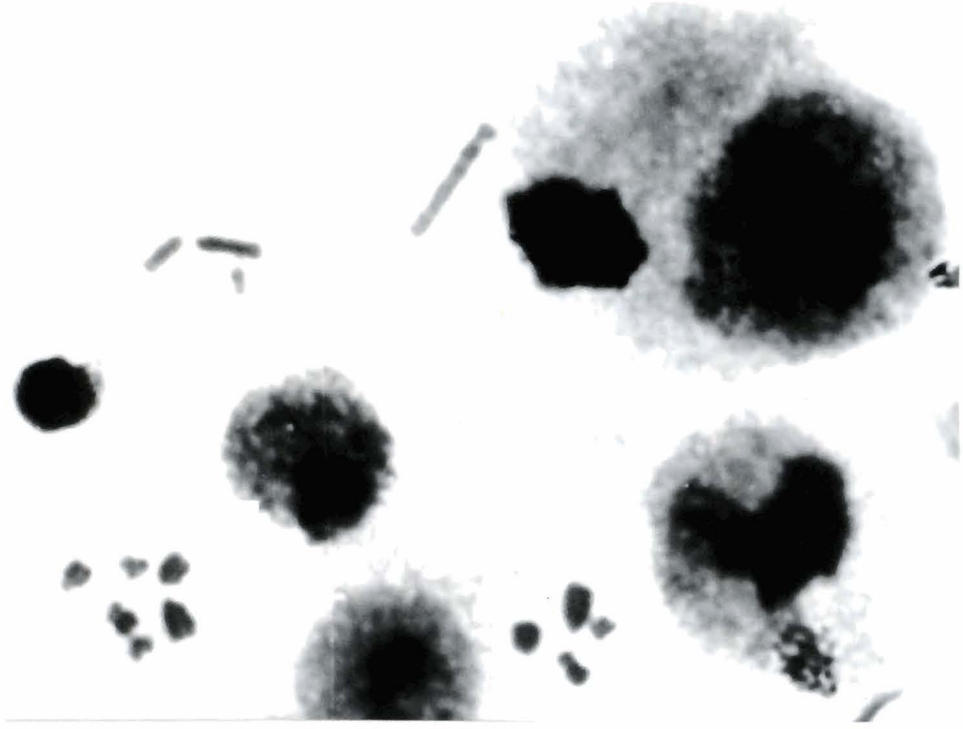


Fig. 16. In situ, hypohaploid sets of chromosomes of different Waker rat tumor cell NVs. Giemsa. x 1,000

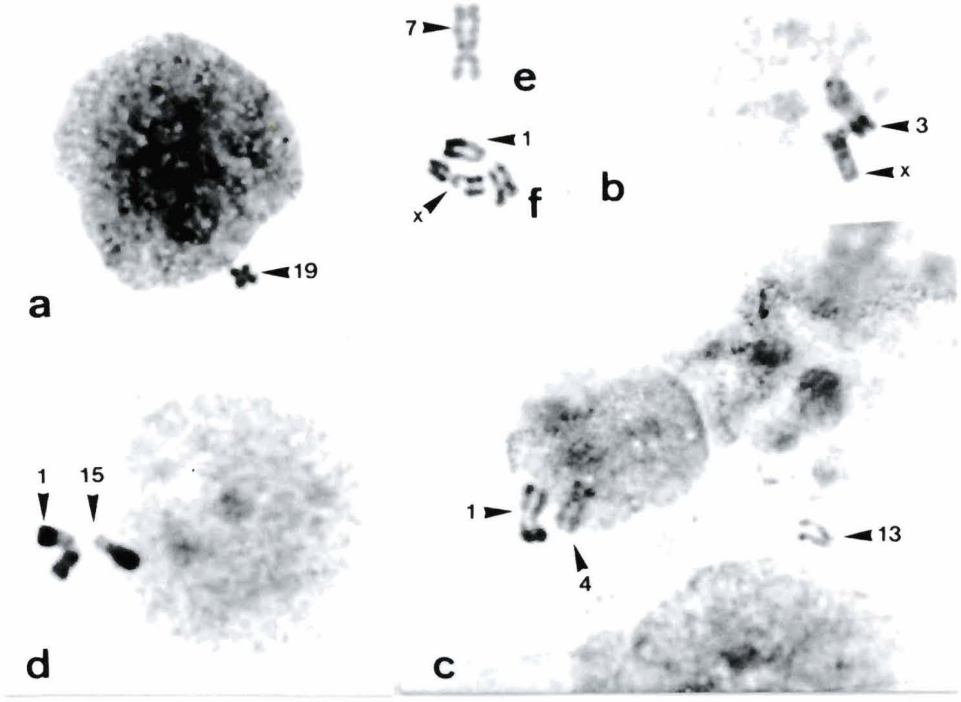


Fig. 17. Insets a, b, c, d: Pa-cell nuclei extruding different number of chromosomes by the chromosomal spreading technique. **Insets e, f:** Single and double structurally normal chromosomes of Pa-NVs. Giemsa-RhG banding. x 1,000

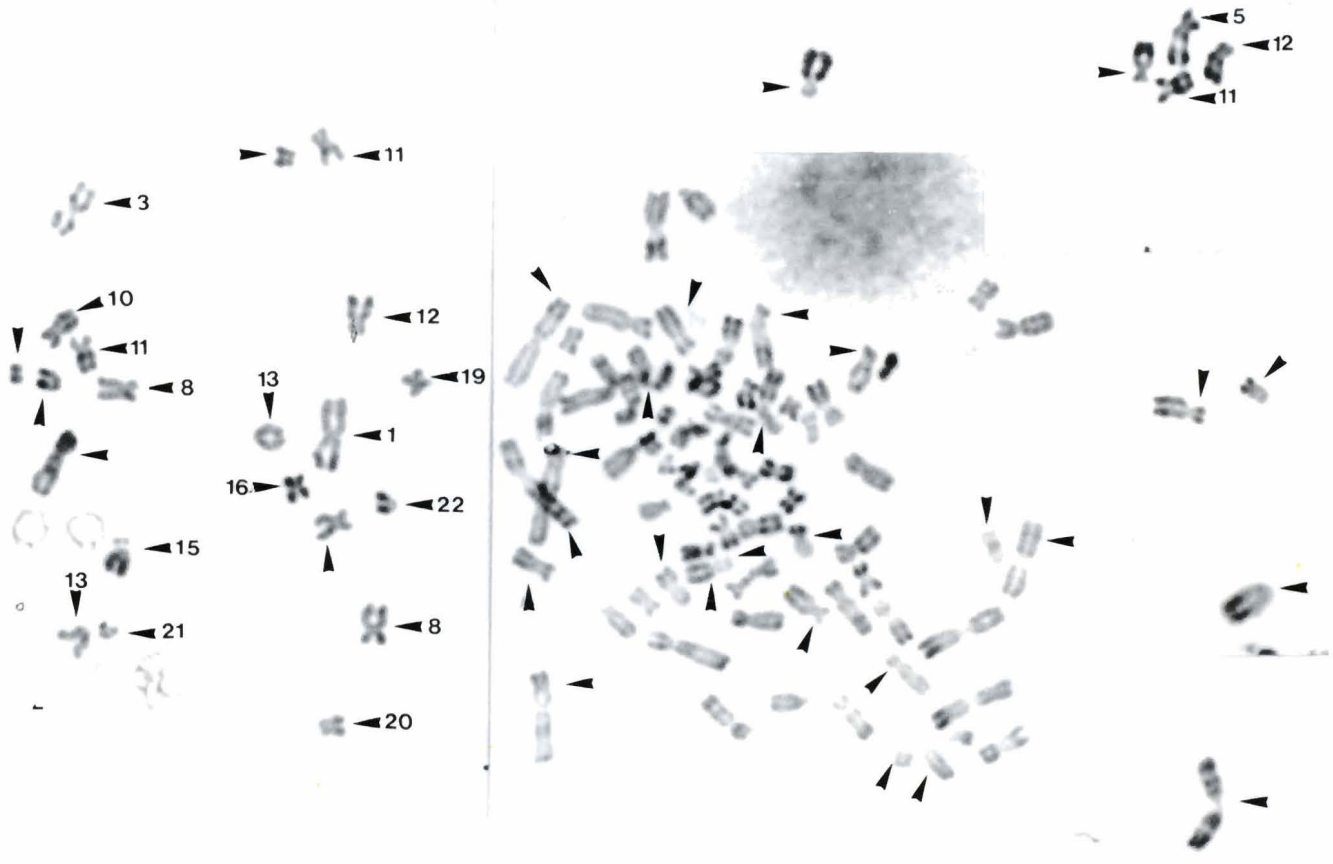


Fig. 18. Hyperdiploid and hypohaploid Pa-cell metaphases with structurally abnormal chromosomes shown by arrows without numbers. Giemsa-RhG banding. x 1,000

Fig. 19. A polyploid, in situ meiotic, Pa-cell metaphase of condensed chromosomes. Giemsa. x 1,000

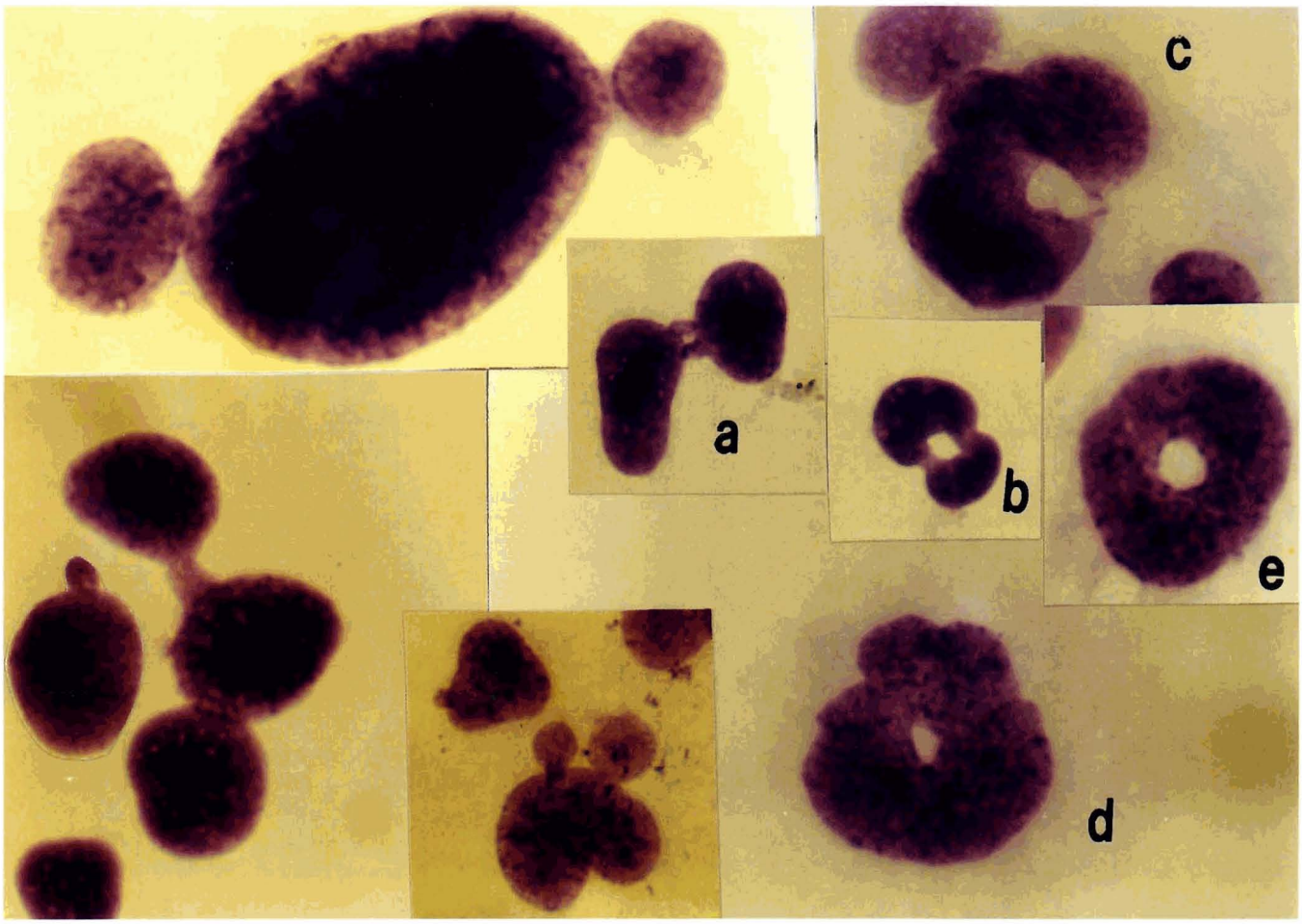


Fig. 20. NV formation and variably-sized nuclear buds of Pa-cells. **Insets a, b, c:** Pa-cell nuclear fusion through nuclear projections. **Inset d:** Pa-cell fused nuclei with vacuole and irregular nuclear membrane. **Inset e:** Fused nuclei with smooth nuclear membrane. Giemsa. x 1,000

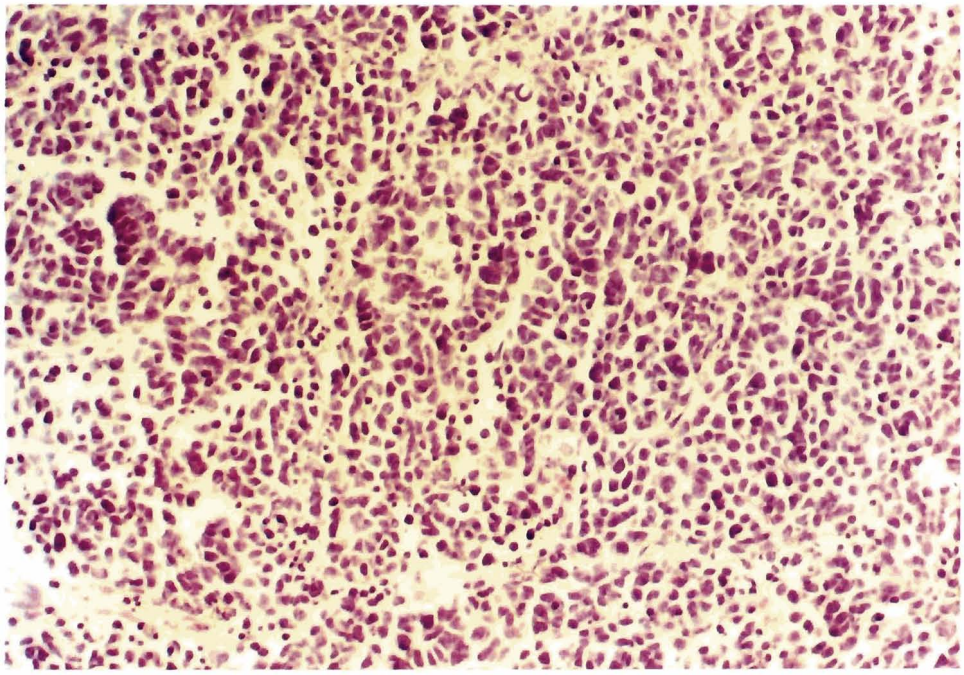


Fig. 21. Histological picture of a human peripheral neuroectodermal tumor. H&E. x 200

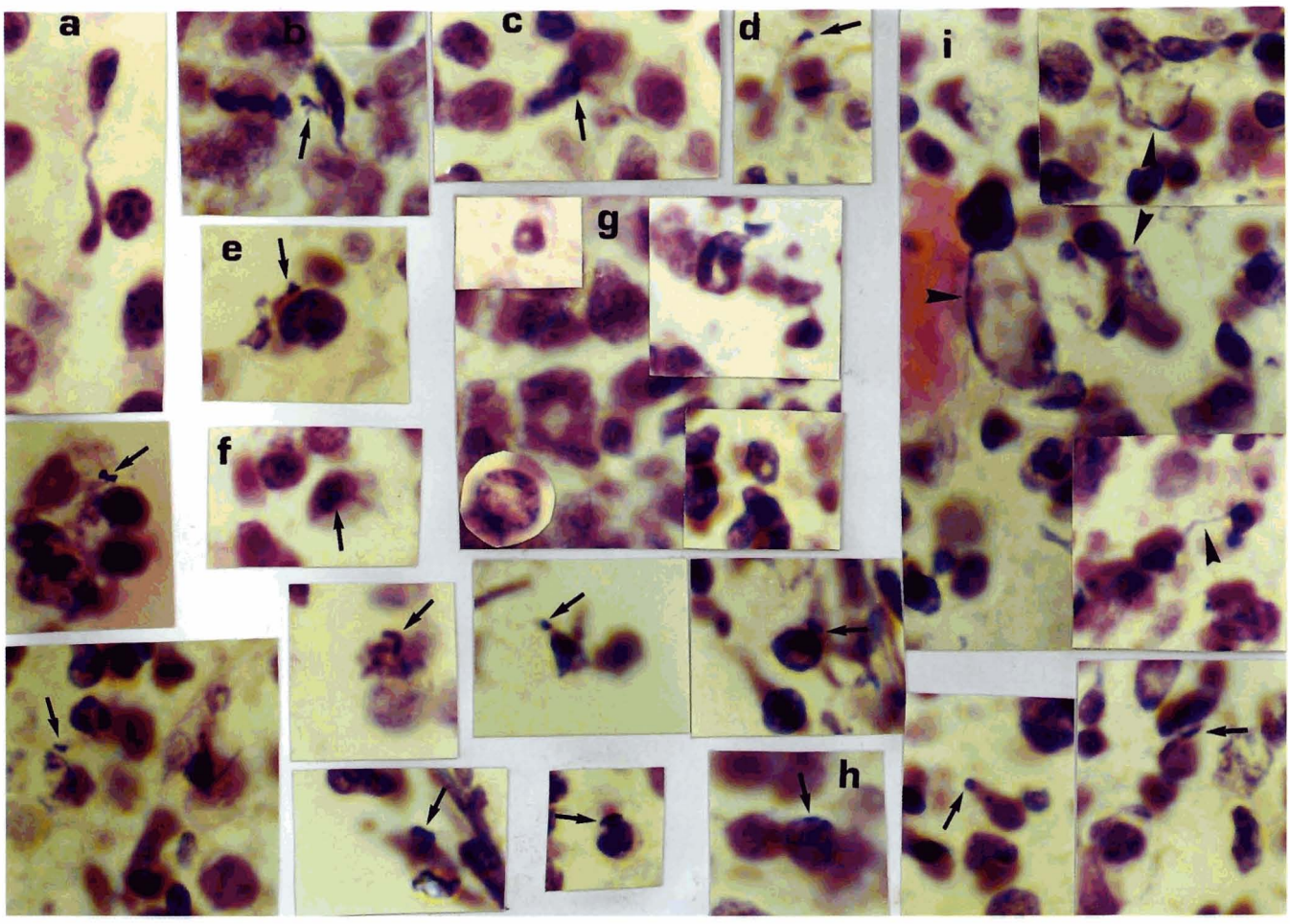


Fig. 22. Stages of malignant cell life cycle identified in peripheral neuroectodermal tumor section of figure 21. **Inset a:** Condensed head NVs connected by nuclear bridge. **Inset b:** NV extruding chromosomes. **Insets c, d, e, f:** NVs carring and implanting chromosome. **Inset g:** Fused nuclei with vacuole. **Inset h:** Nucleus with condensed chromosomes. **Inset i:** Nuclear tails and segments. Thin arrows point at extruded or implanted chromosomes. H&E. x 1,000

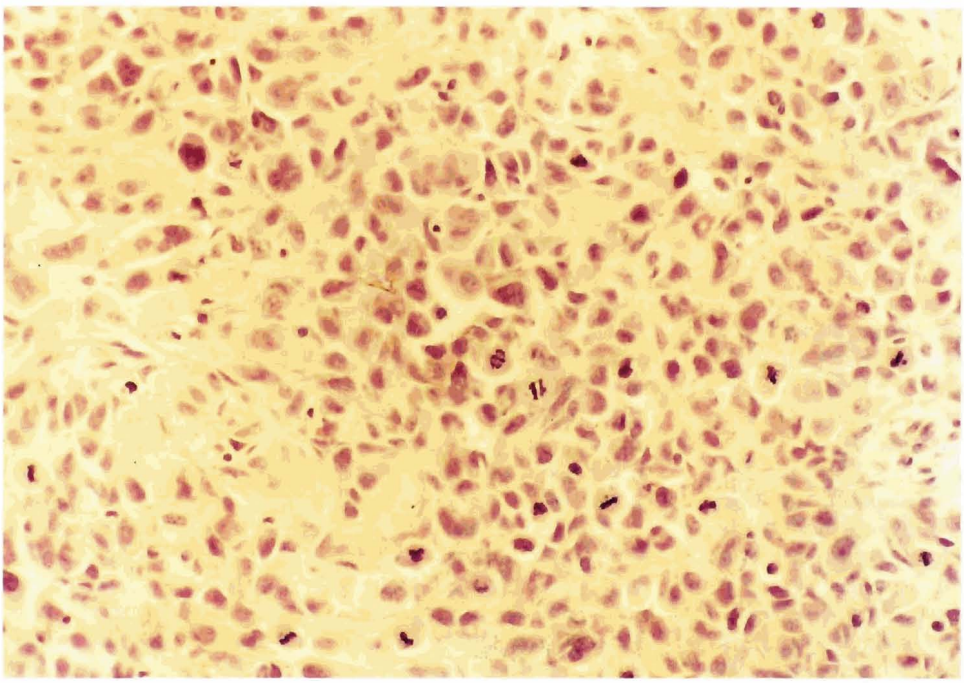


Fig. 23. Histological picture of a human undifferentiated skin tumor. H&E. x 200

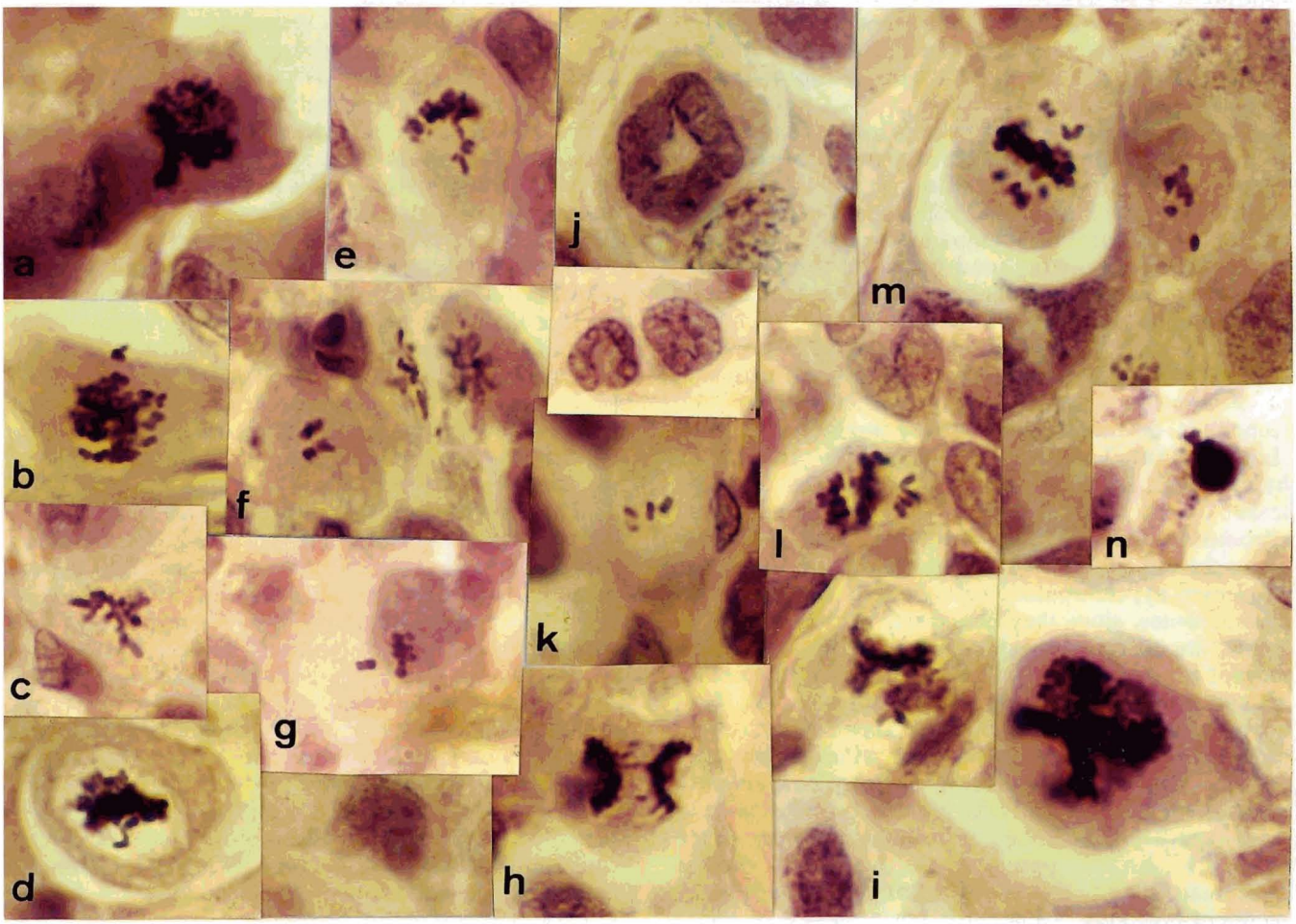


Fig. 24. Stages of malignant cell life cycle identified in the undifferentiated skin tumor section of Fig. 23. **Insets a-g:** cytoplasmic NV metaphases. **Inset h:** meiotic anaphase exhibiting nuclear bridge. **Inset i:** metaphase fusion. **Inset j:** fused nuclei with central vacuole. **Insets k-n:** aneuploid metaphases and chromosomal extrusion. H&E. x 1,000

(Figs. 23, 24). Condensed head NVs connected by the nuclear bridge, or extruding chromo-somes, or carring chromosome, fused nuclei with vacuole, condensed chromo-somes in the nucleus and nuclear tails were observed (Figs. 21-24).

Discussion

A cytological study has recently been reported on the morphology and function of malignant NVs (Logothetou-Rella, 1993). This study provides further cytogenetic documentation about NVs, as a cytological study is considered subjective and artefactual.

NV metaphases were easily recognisable by the head with tail morphology in the in situ cytogenetic analysis. This morphology was not always maintained by the conventional chromosomal spreading technique. Thus, the in situ cytogenetic analysis was used as a guide for the detailed chromosomal evaluation and morphology by the chromosomal spreading technique. Cytogenetic morphology defines the morphology of cells after treatment with hypotonic solutions. Chromosomal preparation slides provide useful genetic morphological information, as hypotonic cell swelling does not alter but renders visible genetic events. The in situ technique cannot cause artefacts because it does not involve procedures such as cell trypsinization, cell transfers, centrifugation, pipetting, chromosomal suspension spreading on slides or banding. It involves direct treatment of growing attached cells (on the culture vessel surface) with hypotonic solution, fixation and staining. It is therefore not influenced by room temperature, humidity or handling errors and can be used as a guide for cellular and genetic events.

The presence of «0» chromosome, fibrous chromatin, condensed, hyperchromatic chromosomes, the extrusion of chromosomes and nuclear bridges, shown by both techniques document that NVs are product of meiosis (Logothetou-Rella, 1994a). Malignant NVs carry mainly hypodiploid, hypohaploid chromosomal sets and to a lesser extent hyperdiploid or polyploid ones. Most chromosomes are located in the NV head and fewer along the tail in line. NV chromosomes are regular or condensed hyperchromatic or even atypical. Condensed chromosomes, since they co-existed with «0» chromosomes, are classified meiotic (Plachot et al., 1987). NV metaphases in the cytoplasm of host cells, further document the parasitic property of malignant NVs. NVs not only contain and carry, but also donate chromosomes to host cells. Observations show that within a malignant cell line, there are cells which play the role of chromosomal donors or recipient host cells. Recipient host cells were identified at interphase or meiotic metaphase of the cell cycle. An NV, upon contact, breaks its nuclear membrane, releases and implants its chromosomes in the recipient host cell. Apparently, when an NV, at metaphase, invades a host cell metaphase, a new mixed metaphase is the result (hybrids), possessing the genetic information of the donor and recipient cells, like mammalian fertilization or viral cell infection. In most mammals, fertilization occurs at the oocyte metaphase of the second meiotic division (Beier and Lindner, 1983). This is exactly what happens within a malignant cell line. It cannot be excluded that NV penetration of a diploid meiotic host cell nucleus may trigger meiosis of the host cell. So far meiosis occurred during gametogenesis. Recently, meiosis was also observed in PHA-activated normal human lymphocytes associated with calcium-activated neutral proteinase (CANP) (Logothetou-Rella, 1994a). This study documents that malignant cells undergo uncontrolled meiosis. Since malignant cells and NVs were sensitive to CANP inhibitor (Logothetou-Rella, 1994b), meiosis of malignant cells is also associated with the presence of CANP.

Every fragment of the malignant cell nucleus, so far considered debris, contains and extrudes chromosomes, and has a high attachment affinity on host cell nucleus like episomes. Very small nuclear tail segments show attachment affinity for chromosomes. Fragments of NV tails, enclosing chromosomes, present in host nuclei and metaphases, are also capable of passing from nucleus to nucleus. NV tail fragments keep getting thinner, smaller and upon decondensation give rise to chromosomes. The malignant cell nucleus has the ability to donate and implant its genetic information into other cells in the form of NVs, buds and nuclear tail segments; all nuclear products of meiosis. It is possible that smaller, microscopically invisible NVs may exist and function in a malignant cell line. The function of NVs indicates that these meiotic products of malignant cells are not apoptotic. Preliminary cytological observations in this laboratory have shown that apoptotic cells exhibit high nuclear shrinkage and vacuolation (unpublished data), alterations which are not shown by malignant cells and their meiotic cellular products. The terminology of NV penetration, fertilization or infection seems more appropriate in neoplasia than apoptosis, since NVs play an identical role to spermatozoa in fertilization or virus in viral cell infection.

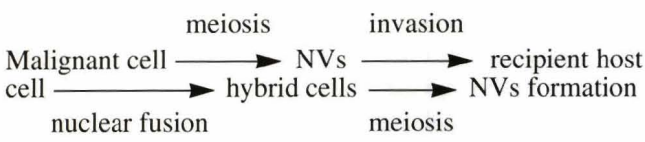
The karyotype of primary M and Pa-cells, by conventional evaluation, has previously been reported. M-cells consisted of 25% tetraploid and 75% diploid metaphases (Logothetou-Rella et al., 1992). Pa-cells consisted of polyploidies up to 147 chromosomes with structural aberrations (Logothetou-Rella, 1993). In those studies though, hypodiploid and hypohaploid chromosomal sets were not evaluated as they were considered artefactual. The observation of common NVs in human neoplasia and the lymphocytic NV production by meiosis (Logothetou-Rella, 1994a) necessitated re-evaluation of all aneuploid metaphases of these cell lines in terms of NV content. The present karyotype of M-cells shows reduction of the original percent diploid and tetraploid metaphases upon serial subcultivation, leading to karyotype instability. Since there is no repetitiveness of a particular metaphase, meiosis of these malignant cells is random and uncontrolled.

The karyotype of Walker rat tumor cells showed

different percentage of diploidy and hypodiploidy than Nicholson et al. (1968) and Hartley et al. (1987). Specific banding for rat chromosomal typing was not performed. Nevertheless, karyotype instability has been shown to be characteristic of most transplantable tumors (Ohno et al., 1960) and emerged sublines were speculated to occur via structural rearrangements and chromosome loss from polyploid cells (Nicholson et al., 1968). This study documents that this happens within a malignant cell line. The ability for malignant cell fusion and especially nuclear and metaphase fusion leads to hybrid cells. Pa and Walker rat tumor cells showed fusion even of two hypohaploid metaphases resulting in a hypodiploid hybrid metaphase. Then hybrid cells produce hypodiploid, hypohaploid and even hyperdiploid NVs by meiosis. Newly formed NVs continue the cycle of nuclear invasion, fusion, hybrids and NV formation. If there were no host cells and nuclear fusion did not occur within a malignant cell line, then all malignant diploid and hyperdiploid cells, within a given cell population, would be reduced to zero by continuous hypodiploid and hypohaploid NV formation with subsequent necrotization because NVs are unstable, parasitic cellular elements. This occurs with lymphocytic NVs. Normal lymphocytic NVs are morphologically, cytologically and cytogenetically identical to malignant NVs. However, lymphocytic NVs invade and fuse with each other to a limited extent (preliminary work). Under the conventional growth conditions, lymphocytic hyperdiploid metaphases were not detected, and the diploid ones kept decreasing with PHA activation. Consequently, the karyotype descended from diploid to hypohaploid, upon PHA-activation, leading to gradual necrotization of all the lymphocytic population in one month (Logothetou-Rella, 1994a). Also, the haploid spermatozoa, since they do not fuse with each other, are unstable and show a short life span unless fused with the oocyte nucleus. Capability for nuclear fusion differs among malignant cell lines. Immortal Pa- and Walker rat tumor cell lines showed a higher degree of nuclear fusion than M-cells which have exhibited senescence after many subcultivations. Hybridization of two different cell lines has previously been reported (Conzelmann et al., 1982). This work reports hybridization within the same cell line.

From the literature, hypodiploid and hypohaploid metaphases of malignant cells have been neglected as artefactual and there can be no cytogenetic comparison of previous studies with this one. Hence generalization of this study is based on morphological identification of NVs common in human neoplasia (Logothetou-Rella, 1993).

In conclusion, this study documents the life cycle of malignant cells as follows:



Such a life cycle, cell to cell collision and interaction justifies karyotype instability, heterogeneity, structural aberrations, propagation and invasion of malignant cells.

Recognition of several stages of the malignant cell life cycle in malignant tissue section denies the possibility of artefactual observations due to cell cultivation.

More research is in progress to further elucidate the function of malignant NVs.

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