cant variation in plant height between mycorrhizal and nonmycorrhizal plants was observed, but at later stages the variation was significant. The low inoculum produced less and late mycorrhizal association (table 2) whereas a high inoculum (40 and above endophytes per plant) developed fast and more VAM (table 1). A spore inoculum per plant of 40 or above produced about 3-fold more shoot dry weight than nonmycorrhizal plants. Non-inoculated plants exhibited the symptoms of chlorosis at later stages of harvestings (35 days).

A different pattern of growth and development in maize plants was obtained in response to different inoculum densities. An inoculum of 40 or more endophytes per plant showed a significant variation in growth of maize compared with nonmycorrhizal plants and mycorrhizal plants inoculated with a low level of endophyte. A positive correlation between inoculum density and growth of plants is reported[10]. In some studies[11] spore inoculation has been reported to delay the mycorrhizal association for various reasons, like the time required for spore germination, germ tube elongation and penetration into roots, but a higher inoculum may overcome such problems. The 3-fold increase in dry weight of mycorrhizal plants compared with nonmycorrhizal plants may be attributed to an increase in uptake of nutrients[1,12], by providing an additional absorbing surface for the root system of the host plant. Mycorrhiza not only absorbs phosphorus but also accumulates more phosphate and can solubilize the 'unavailable' phosphorus which is not available to nonmycorrhizal plants[13]. The present study suggests that mycorrhizal association is necessary for better growth. The inoculum level necessary for the enhanced growth, however, may vary in different plants and for various endophytes under different ecological conditions.

Table 2. Establishment of mycorrhiza in maize with respect to spore inoculation

Spore number inoculated	Mycorrhiza establishment (%)		
	1st harvesting (20 days)	2nd harvesting (35 days)	3rd harvesting (60 days)
2	-	6	8
4	-	7	12
16	-	15	22
40	8	30	68
60	12	36	70
Control	-	-	-

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Double minutes in fibroblast-like cells isolated from human tumors[1]

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Summary. Double minutes (DM) were found in several fibroblast-like lines isolated from human tumors. DM varied in size and morphology within the same metaphase.

Double minutes (DM) have been observed in metaphases of many tumor-derived epithelial cells[2-9] and never, to our knowledge, in normal cells[2,7]. In this report, we present our data on the incidence of DM in fibroblast-like cells originating from neoplastic and non-neoplastic tissues.

Materials and methods. Surgical specimens were obtained from patients who had not received chemotherapy or radiotherapy. Specimens from solid tumors were excised from the interior of the tumor in order to avoid possible contamination with surrounding normal tissue. Tissue pieces were trypsin-dissociated and transferred to culture flasks with McCoy's 5a medium supplemented with 15% fetal bovine serum, penicillin, streptomycin and amphotericin B. Most specimens gave growth of long spindle-shaped cells within 10–20 days. At this stage, supernatants were replaced with fresh medium and cells were allowed to form confluent monolayers; these were trypsinized and cell suspensions were transferred in new flasks. Epithelial cell growth was never noticed in flasks from which supernatant medium had been removed immediately after the first appearance of fibroblast growth. Cell morphology was examined on coverslip cultures stained with Giemsa. Chromosome preparations were made by conventional methods[10], after the 1st and up to the 10th passage of each finite cell line. Due to variable response of cell lines, colcemid doses which ranged from 0.06 to 0.3 μg/ml given for 3–8 h, were required in order to obtain reasonable preparations. All cells were free of contamination as shown by microscopic examination and by ^{3}H-thymidine labelling and autoradiography[11,12].

Results. 16 finite fibroblast-like cell lines were produced from tissue specimens obtained from patients with infiltrating ductal carcinoma (7 cases), chronic mastitis (4 cases), lymph node metastatic lesion of primary infiltrating ductal carcinoma (2 cases), nipple melanoma (1 case), trachelitis (1 case) and tonsillitis (1 case); samples of normal breast epidermis (2 cases) were also obtained from 2 patients with infiltrating ductal carcinoma. All lines used in this work consisted entirely of diploid or near-diploid fibroblast-like cells as confirmed by detailed microscopic examination. Several lines produced from malignant tissues exhibited a peculiar morphological pattern of growth: 'periodically appearing piling-up (PAPU) colonies' (fig. 1). Most of these colonies, observed in cell populations growing in culture flasks or on coverslips, resembled those observed by Giraldo et al.[13] in cultured sarcoma cells. These results will be presented in detail elsewhere.

DM were found in 5 of the above mentioned cases in varying frequencies: 13.3% and 4% respectively in 2 cases of ductal infiltrating carcinoma, 7% in the case of nipple melanoma, 2% in the case of trachelitis and 2.5% in 1 case of lymph node metastasis. In all cases, except trachelitis, cells containing DM exhibited PAPU colonies. In the case of lymph node metastasis, cells with as many as 22 DM were found, while in all other cases the range of DM per cell varied from 1 to 4. The diameter of each dot-body of DM contained within the same metaphase may vary from 0.2 to 0.7 μm. Also, the distance of the unstained space between the two dot-bodies of a DM may vary from 0.1 to 0.7 μm among DM of the same metaphase, independently of their size. These morphological features are shown in a single metaphase of a SE-L cell (fig. 2). In 2 metaphases of SE-L cells with numerous DM, several dicentric chromosomes and rod-like DM were observed (fig. 2). No direct correlation was noticed between a) high colcemid doses, chromosomal aberrations, aneuploidy or polyploidy and b) presence of DM.

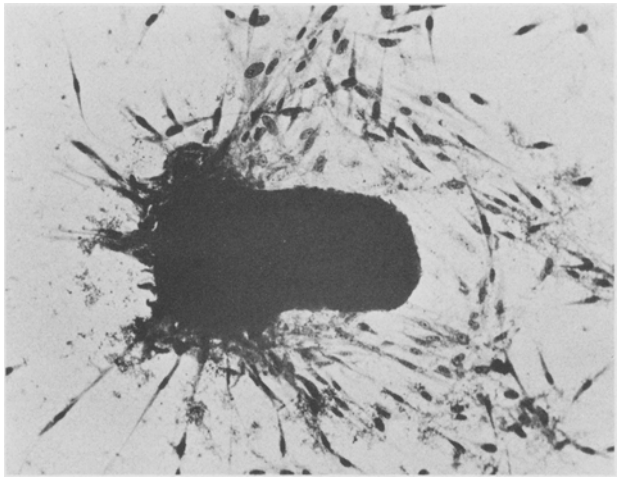

Fig. 1. A piling-up colony of SE-L cells. SE-L line was derived from an explant of a lymph node metastatic lesion. Giemsa, × 170.

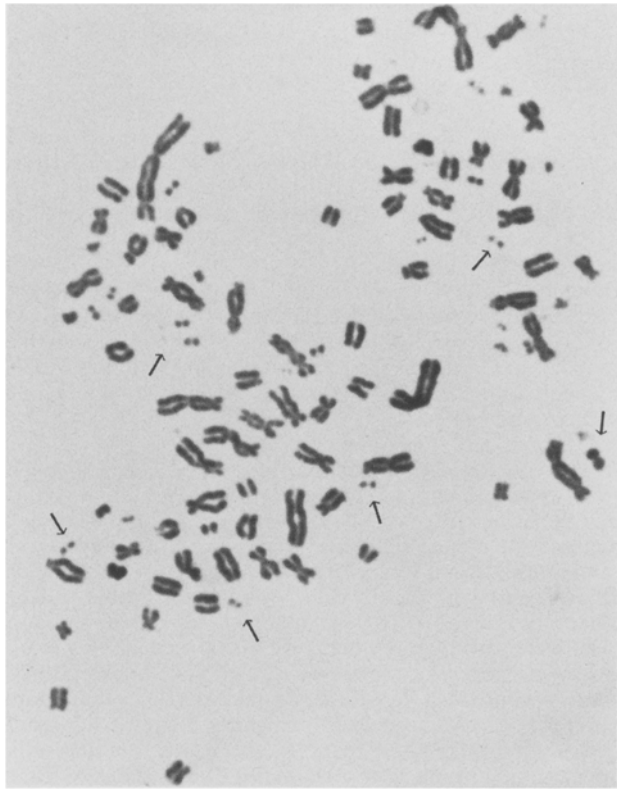

Fig. 2. Metaphase of a SE-L cell containing numerous DM of various sizes and several dicentric chromosomes. Several large rod-like DM, similar to those observed recently by Levan and Levan[18], are also shown. Giemsa, × 3875.

Discussion. These results suggest that other than epithelial type cells may be susceptible to the same factor that causes DM formation in epithelial malignant cells. The presence of DM in fibroblast-like cells produced from neoplastic tissues and their absence from 'preneoplastic' (e.g. mastitis[14,15]) tissues substantiates the necessity for a more detailed examination of the stromal cells, which support and interact with the tumor cells. Stromal cells, to our knowledge, have never been implicated in the tumorigenesis process of epithelial tissues and they are not supposed to possess the properties of malignant cells; therefore, considering the hypothesis that DM represent genes favorable for cancer growth[3], a new hypothesis of a 'DM-causing-factor' present in malignant tissues may be introduced.

DM differ from single dot chromosomes which may be present in both malignant or normal cells as a result of colcemid treatment[16], specific cytotoxic agents, or due to reconstruction processes of the chromosomal material in hybrid cells[17]. Although DM and dots have both been considered as fragments of preexisting chromosomal regions[5], their function may be quite different, since DM showed a potential for changes in shape and size[18]. This potential has been recently related to acquired differential gene function[7,19,20] and malignancy[3,5,18].

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