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Behavior of Tumors Produced by Transplantation of Human Mammary Cell Lines in Athymic Nude Mice*

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Abstract—*The behavior of malignant tumors produced by subcutaneous injections of 4 human mammary cell lines (BT 20, MCF-7, CaMa 15, MaNo 4) in athymic nude mice was studied over several serial passages. BT 20 and MCF-7 tumors became manifest after latency periods of variable length. They displayed a slow infiltrative growth, had metastatic rates of 9% and 15% respectively, and seldom recurred after surgical excision. Active growth of CaMa 15 and MaNo 4 tumors began a few hours after grafting; they were very aggressive undifferentiated carcinomas which recurred frequently (69% and 70%) and metastasized in 50% and 37% of the cases respectively. The commonest sites of metastases were the regional lymph nodes, lungs and mediastinum. Thoracic metastases were more frequent in CaMa 15 and in MaNo 4 tumors than in the 2 other groups. Whether and to what extent these tumors were influenced by uncontrolled host factors was not determined. Therefore, their behavior should be evaluated on the basis of the present experimental conditions rather than as fixed characteristics particular to each cell population.*

INTRODUCTION

ALTHOUGH neoplasms are frequently produced by transplantation of human mammary carcinoma cell lines into nude thymus-deficient mice [1], very little is known about their behavior. Indeed, in some studies the heterotransplantation has been carried out only to determine the tumorigenicity of cultured cells and no follow-up has been mentioned [2–4]. In other studies with a follow-up limited to the original graft [5–7] or to the first 7 serial passages [8], no metastases have been observed grossly or microscopically. Metastases from tumors produced by injected mammary cell lines have only been reported in lymph nodes of 3 out of a nonspecified number of mice bearing tumors of 11 lines followed for unknown periods of time [9] and in the lungs of 2 out of 6 newborn mice with original transplants [10]. Furthermore, no data have been published concerning the evolution of these tumors after they have been surgically excised. Therefore, it appeared important to study the long term behavior of neoplasms resulting from the transplantation of cultured human mammary cells into nude mice and to determine their potential to recur and to metastasize over numerous serial passages. Detailed information on the evolution of these tumors appeared all the more interesting in view of the usefulness of this model in experimental studies of human breast cancers.

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MATERIALS AND METHODS

Cell lines

Of the 4 lines used in this study, the BT 20 and the MCF-7 have been extensively characterized [1]. Two other lines were kindly provided by Dr. G. Fossati (Istituto Nazionale Tumori, Milan, Italy) who started them from a primary infiltrating ductal carcinoma of the breast (CaMa 15) and from non-neoplastic mammary tissue next to a medullary carcinoma of the breast (MaNo 4). Originally, both of these lines were typically epithelial by light and electron microscopic criteria, but in time their cells became elongated and no

longer arranged themselves in mosaic-like colonies (G. Fossati: personal communication). Although these 2 lines have not been fully characterized as yet, their mammary epithelial origin was corroborated by the demonstration of α-lactalbumin in the tumors they produced in nude mice (see below).

Prior to transplantation all 4 lines had human karyotypes. HeLa cell contamination was excluded by glucose-6-phosphate dehydrogenase mobility.

Nude mice

Adult inbred BALB/c nu/nu mice raised in our laboratory were used throughout this study. Both breeders and experimental animals were uninterruptedly kept under pathogen-limited conditions without antibiotic coverage. They were fed a pelleted diet sterilized by irradiation (4 MR, U.A.R., Villemoisson-sur-Orge, France). Drinking water and all material coming in contact with the animals were autoclaved. Under these conditions the mice survived in good health for about 1 year, individual mice surviving up to 19 months.

Transplantations

For the original transplants $5–7 \times 10^6$ trypsinized cells were suspended in 0.3 ml of medium RPMI 1640 and injected unilaterally or bilaterally in the subcutaneous tissue (s.c.) of the dorso-lateral region of the mice next to the thoracic mammary glands. Subsequent passages were carried out by mincing 2–3 mm^3 of tumor tissue in RPMI 1640 and implanting it s.c. in the dorso-lateral region of 1 or more mice by means of a trocar. Cells and tumors of the BT 20, CaMa 15 and MaNo 4 lines were transplanted into male or female mice without any additional manipulation. Cells and tumors of the MCF-7 line were injected into virgin females to whom pellets of 1.25 mg 17-β-estradiol in cholesterol were implanted s.c. The estrogen effect was monitored by weekly vaginal smears. A detailed study of the hormonal dependence of MCF-7 xenografts will be reported elsewhere.

Tumors

After variable periods of time (depending upon the rapidity of growth of each neoplasm) the tumors were excised under general anesthesia, the surgical wounds closed with silk sutures, and the mice allowed to survive. The regional lymph nodes were not removed except for a few cases in which they were adherent to the tumors. The tumors removed surgically were used for grafting into other animals and for various examinations. Inoperable tumors were allowed to grow until the animals died naturally or were sacrificed. A complete autopsy was then performed. Recurrent tumors were handled in the same manner.

All tumors and autopsy specimens were examined histologically using conventional techniques. The presence of α-lactalbumin was checked by Dr. J. Hurlimann (Department of Pathology, University of Lausanne) using indirect immunofluorescence on tumor tissue fixed in alcohol. The antigens and antisera were prepared according to Hurliman and Dayal [11]. Selected tumors of each group were re-explanted *in vitro* by seeding finely minced neoplastic tissue in plastic flasks and using the original media.

Table 1. *Tumors produced by transplantation of human mammary cell lines*

Cell line	Transpl./ takes	Serial passages	Latency period (days)	Tumors		
				Volume (cm^3)*	Age of tumors (days)	GI†
BT 20	53/46	28	7–79	2.34±0.53	21–183	5.62±1.23
MCF-7	74/70	13	7–18	0.44±0.07	21–182	0.76±0.08
CaMa 15	76/76	36	0	2.65±0.27	10–32	16.52±1.36
MaNo 4	70/70	34	0	2.88±0.35	11–42	16.43±1.39

*In cm^3 according to the formula $V=(\pi/6)-x(d_1 . d_2 . d_3)$ where $d_{1,2,3}$ represent the 3 largest diameters. Mean±standard error (S.E.).

†Growth Index as an estimation of the daily increase in size.

$$GI=\frac{\text{Tumor volume}}{\text{Duration of tumor in days}} \times 100.\ \text{Mean} \pm \text{S.E.}$$

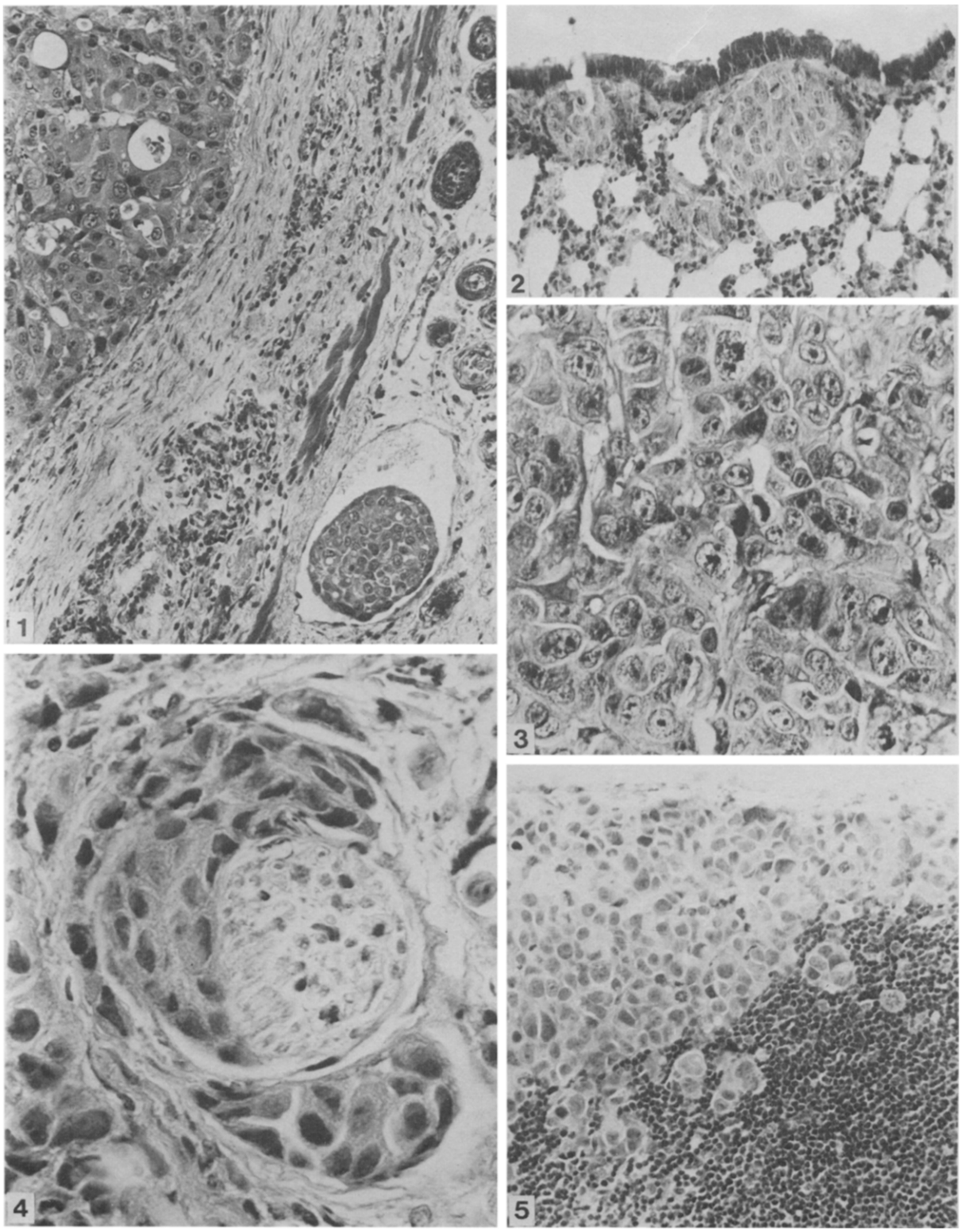

Fig. 1. *BT* 20 *tumor with involvement of a dermal lymphatic. H & E.* ×160.

Fig. 2. *Small BT* 20 *pulmonary metastases next to bronchiolar lining. H & E.* ×230.

Fig. 3. *MCF-7 tumor showing a trabecular pattern. H & E.* ×400.

Fig. 4. *Perineural invasion by MCF-7 tumor cells. H & E.* ×320.

Fig. 5. *Metastasis of MCF-7 tumor cells in the peripheral sinus of an axillary lymph node, H & E.* ×230.

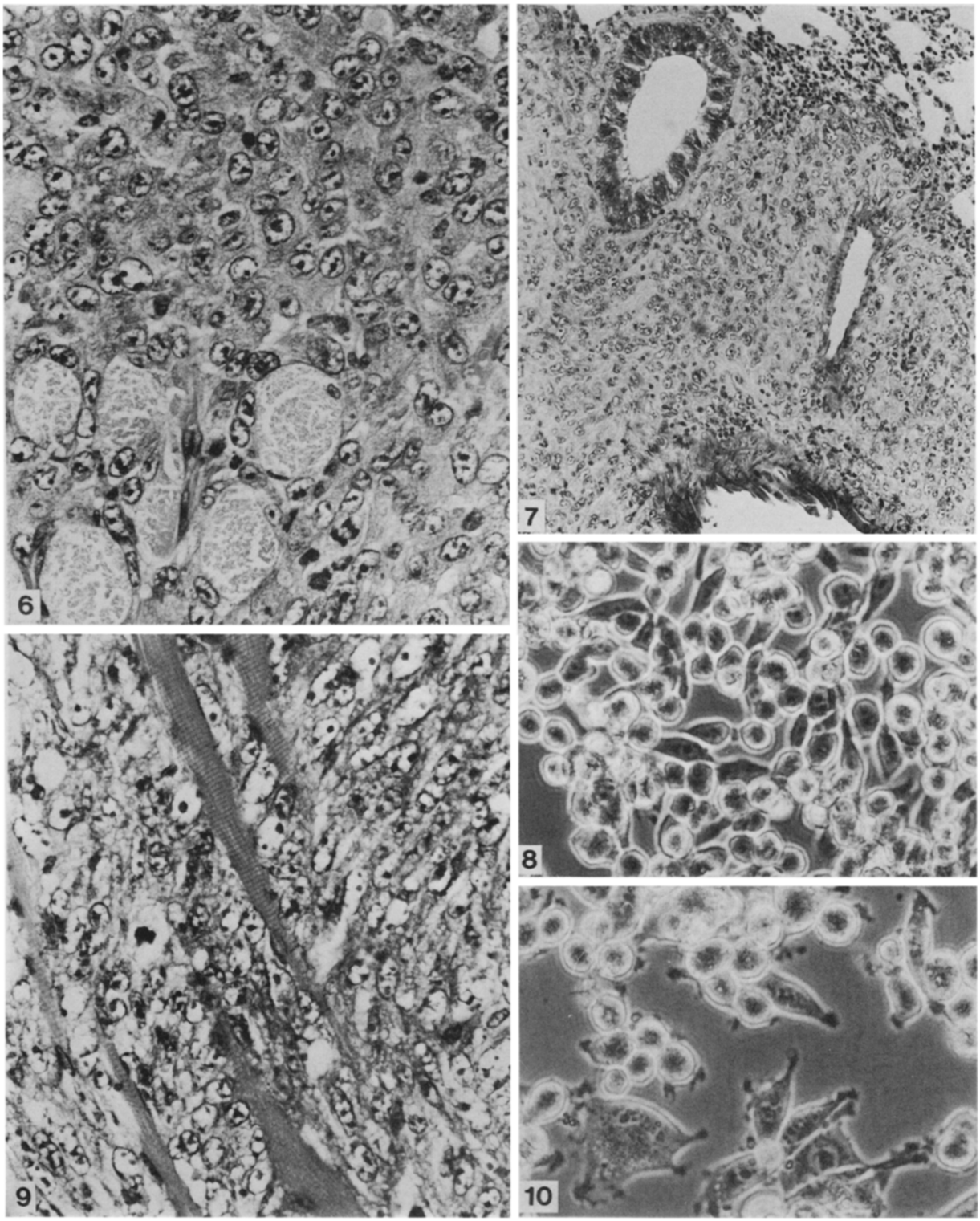

Fig. 6. *CaMa* 15 *tumor showing undifferentiated cells invading skeletal muscle, H & E.* × 400.

Fig. 7. *Pulmonary metastasis of a CaMa* 15 *tumor surrounding a bronchiole and blood vessels. H & E.* × 130.

Fig. 8. *Living culture of a CaMa* 15 *tumor at the* 22*nd passage in the mouse.* 5 days in vitro. × 500.

Fig. 9. *MaNo* 4 *tumor composed of poorly differentiated cells invading striated muscle. H & E.* × 400.

Fig. 10. *Living culture of a MaNo* 4 *tumor at the* 49*th passage in the mouse.* 8 days in vitro. × 500.

*Table 2. Follow-up**

Cell line	Total No. of mice	Total No. of mice with metastases	Surgical excisions				
				Mice with recurrences		Mice without recurrences	
			No. of mice	With metastases	Without metastases	With metastases	Without metastases†
BT 20	44	4	14	2	2	2	8
MCF-7	38	6	8	1	0	1	6
CaMa 15	63	32	52	24	12	5	11
MaNo 4	61	23	46	20	16	0	10

*To death (14–487 days after transplantation) or to a minimum of 25 post-operative days.
†Tumor-free survivals in days: BT 20, 49–179; MCF-7, 25–118; CaMa 15, 53–294; MaNo 4, 61–280.

Table 3. Location of metastases

Cell line	Regional lymph nodes	Upper mediastinal lymph nodes	Mediastinum	Lungs	Others
BT 20	2	2	0	1	0
MCF-7	5	0	2	1	0
CaMa 15	9	5	13	22	11*
MaNo 4	2	4	14	12	5†

*Pleura, pericardium, myocardium, chest wall and spine, peritoneum, retroperitoneum, retroperitoneal lymph nodes and sub-cutaneous tissue.
†Pleura, peritoneum and retroperitoneum.

RESULTS

The main features of the tumors and their follow-up are summarized in Tables 1–3 and in Figs. 1–10. In addition, a few points need clarification.

Histology

BT 20 and MCF-7 tumors were composed of carcinoma cells arranged in cords separated by scanty stroma (Figs. 1 and 3) and were comparable to earlier descriptions [6, 8]. CaMa 15 and MaNo 4 tumors were made up of undifferentiated cells that were frequently spindle shaped, especially in the MaNo 4 tumors, and were arranged in ill-defined bundles or in no particular pattern (Figs. 6 and 9). α-Lactalbumin was demonstrable in the cytoplasm of some neoplastic cells, supporting their mammary epithelial origin and their being undifferentiated carcinomas with pseudosarcomatous features.

Tumor size and growth rate

The volume and the growth index (GI) of the tumors varied considerably from mouse to mouse of every line. For instance, 50 CaMa-15 tumors studied 14 days after grafting had volumes ranging from 0.53 to 8.02 cm^3 (mean 2.29 ± 0.22) and GIs of 3.78–57.29 (mean 16.37 ± 1.59). Such variability was also noticed in the recurrences whose GI was frequently different from that of the preceding tumors. If should be noted that the mean GI of CaMa 15 tumors increased from 13.09 during the first year to 21.79 during the subsequent 6 months, whereas the mean GI of the other tumors remained essentially constant.

Aggressiveness

Although the tumors of all 4 lines often appeared well delimited grossly they did infiltrate the surrounding structures (Figs. 1, 6 and 9). Penetration of the abdominal wall was a frequent occurrence especially with the CaMa 15 and the MaNo 4 tumors, whereas invasion of the chest wall was seldom observed. Perineural invasion was often seen at the periphery of MCF-7 tumors (Fig. 4).

Complications

Many mice were not severely affected by the presence of their tumors. Complications were more frequent among mice with CaMa 15 and MaNo 4 tumors and included: mechanical disturbances by sizable tumors,

hemorrhages, respiratory impairment and superior vena cava syndrome due to intrathoracic metastases, cervical edema, and paralysis of hind legs secondary to metastases to the spine. As the mice were generally sacrificed when debilitation set in, it was not possible to determine how many of them would have been killed by their tumor. Wasting, however, appeared to be hastened in tumor bearing animals. In addition, the estrogen stimulation in mice with MCF-7 tumors frequently led to squamous metaplasia of the endometrium with plugging of the cervical canal and uterine dilatation, weight loss, and early wasting.

Tissue cultures

When portions of BT 20 and MCF-7 tumors were re-explanted *in vitro*, epithelial outgrowths comparable to those of the original cultures [8, 12] were observed. Likewise, cultures of CaMa 15 tumors displayed cells similar to those of the cultures prior to transplantation; no mosaic pattern was seen, but many cells remained round as others became polygonal or spindly (Fig. 8). MaNo 4 tumors had a pattern of outgrowth similar to that of CaMa 15, but with greater cellular pleomorphism (Fig. 10). Although the doubling times were not determined, the cultures prepared from CaMa 15 tumors grew more rapidly that the original ones, whereas no appreciable differences were noted in the other groups.

Primary murine tumors

Of a total of 273 mice used in this study, one was found to have a generalized undifferentiated malignant lymphoma when 218 days old and two presented small pulmonary adenomas when sacrificed at 127 and 256 days of age.

DISCUSSION

The tumors produced by the s.c. transplantation of these 4 cell lines are unquestionably malignant as judged by their ability to metastasize. It would appear that CaMa 15 and MaNo 4 tumors are more aggressive than those of BT 20 and MCF-7 cells as, by and large, they grow more rapidly after no appreciable latency periods, they invade more extensively, and more frequently they give rise to recurrences and metastases. Moreover, the surgical excision of the neoplasms results in tumor-free survivals in a larger proportion of mice of the BT 20 and MCF-7 than in the CaMa 15 and MaNo 4 groups. A direct comparison between these groups of tumors, however, is hazardous. Firstly, CaMa 15 and MaNo 4 lines are not as fully characterized as the widely used BT 20 and MCF-7 cells. Secondly, the behavior of the tumors varies not only from one line to another, but also between tumors of any one line. The variations could be due to multiple cell clones within any one tumor, but one wonders to what extent host and environmental factors beyond our control may influence and modify the genetic make up, the biochemical properties, the morphology, and the neoplastic behavior of transplanted tumor cells. Therefore, the malignant characteristics of these cell lines must be evaluated in the light of the experimental conditions used.

The tumors of all 4 lines developed lymphatic as well as blood-born metastases which were accepted as such only when the tumor cells formed a nodule even though of microscopic size. Isolated tumor cells in lymph nodes or in the lungs were not included. It should be noted that the number of metastases was in part related to the extent of the histological examination: for instance, we routinely examined all pulmonary lobes at three different levels and found microscopic metastases in only 1 level on several occasions. Of particular interest was the presence of metastases in the upper mediastinal lymph nodes which were probably the starting point of dissemination in the rest of the mediastinum in several animals. The metastatic spread of these tumors probably began early as attested to by metastases observed 14 days after transplantation of CaMa 15 tumors and by those animals that underwent excision of their tumor 10–14 days after grafting and died with metastases without having developed local recurrences over long periods of time. These cases also point out that, as in the human, metastases may require a long time to become manifest.

Investigations currently in progress indicate that CaMa 15 and MaNo 4 cells are highly undifferentiated. Although the material of origin of these lines, their history, the karyotypes prior to transplantation, and the presence of α-lacatalbumin in some of their tumors speak in favor of a human mammary nature, some of the immunological, enzymatic and Giemsa banding findings are puzzling as they do not fit standard patterns and incite further study. Should their putative human mammary nature remain unconfirmed, they would nevertheless be of considerable interest as xenografts of undifferentiated neoplastic cells in nude mice with a very aggressive behavior.

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