Europ. J. Cancer Vol. 15, pp. 659–670.
© Pergamon Press Ltd. 1979. Printed in Great Britain
0014-2964/79/0501-0659 $02.00/0

Establishment and Characterization of a Cell Line of Human Breast Carcinoma Origin*

I. KEYDAR,† L. CHEN,† S. KARBY,† F. R. WEISS,† J. DELAREA,† M. RADU,† S. CHAITCIK§ and H. J. BRENNER§

†*The George S. Wise Center for Life Sciences, Department of Microbiology, Tel Aviv University, Ramat Aviv, Israel*
§*Chaim Sheba Medical Center, Tel Hashomer Hospital, Department of Oncology, Tel Aviv University, Tel Aviv, Israel*

Abstract—*A cell line (T-47D) has been established from the pleural effusion of a patient with breast carcinoma. These cells exhibit epithelial morphology and form monolayers in culture. The mammary epithelial characteristics are also supported by immunohistologic detection of intracellular casein and the presence of steroid receptors characteristic of mammary tissue. Electron microscopy reveals the presence of desmosomes, tonofilament bundles and tubular-like structures which are characteristic of epithelial cells. In addition, the cultured cells exhibit an aneuploid karyotype with a mode of 66 chromosomes, including an extra-long subtelocentric chromosome. The cytosol of the T-47D cells contains specific high affinity receptors for estradiol, progesterone, glucocorticoid and androgen. Sedimentation studies indicated that the four hormone-receptor complexes were all 7-8S. Competition studies showed that steroid binding to estrogen and progesterone receptors was inhibited only by the homologous hormone, whereas binding of dexamethasone and dihydrotestosterone to their respective receptors was inhibited by certain other steroids as well.*

INTRODUCTION

ESTABLISHED human mammary tumor cell lines obviously constitute an important tool for immunological, genetic, virological and hormonal studies of human breast cancer. Cultivation of long-term cell lines from solid breast tumors has proven to be quite difficult and few such human cultures have been reported [1–5]. However, pleural effusion fluid from breast carcinoma patients has provided an excellent source of malignant cells usually free of fibroblasts. Attempts to establish cell lines from pleural effusion fluids have met with some success in the past few years [6–10].

Several investigators have shown that cells of certain mammary tumors in rodents and humans possess specific steroid binding proteins [11, 12]. These high affinity steroid binding proteins presumably serve as receptors which mediate cellular responses to the corresponding hormone. Most of the information available on the steroid responses of tumors *in vitro*, and on the cellular components which bind steroids, stem from studies performed on heterogeneous mammary tumor samples shortly after their surgical resection and short-term organ culture [13–15].

Steroid receptors in human mammary cells grown in long-term culture have only been demonstrated in the MCF-7 line [16, 17]. Other human mammary tumor cell lines that have been investigated show little or no steroid receptor activity [5].

This paper reports the successful establishment of a new human cell line obtained from the pleural effusion of a patient with disseminated carcinoma of the breast. Cytologic and ultrastructural studies indicate its epithelial origin. The presence in these cells of receptor proteins specific for estrogen, progesterone, glucocorticoids and androgens, the ability of these tumor cells to produce casein in culture, their ability to be cultured *in vitro*, and the presence of a characteristic extra-long subtelocentric chromosome, indi-

Accepted 25 October 1978.
*This study was conducted under Contract N01 CP-2-3237 within the Virus Cancer Program of the National Cancer Institute, NIH, PHS.

cate that this cell line will be a useful model for exploring the properties, nature and chemotherapy of this important human neoplastic disease.

MATERIALS AND METHODS

Establishment of cell lines

In November, 1974, pleural effusion fluid was obtained by thoracocentesis of a 54-yr old patient with inoperable infiltrating duct carcinoma of the right breast. Cytopathologic examination revealed red blood cells, leukocytes, histiocytes and tumor cell clusters. After centrifugation at 800 ***g*** for 10 min, the cells were suspended in 10 ml of RPMI-1640 medium supplemented with 10% calf serum, 10% fetal calf serum, 0.2 i.u./ml insulin (Wellcome), 20 μg/ml hydrocortisone hemisuccinate (Dyosynth, OSS, Holland), 100 μg/ml streptomycin, 100 U/ml penicillin and 25 μg/ml mycostatin. After incubation for 24 hr at 37°C in 5% CO_2, the floating cells were transferred and the adhered cells refed. After 7 weeks of incubation, an epitheloid colony was isolated which gave rise to a cell line which formed monolayers and retained differentiated epitheloid morphology on subculture. For routine propagation, the hydrocortisone was omitted from the medium. This cell line was designated T-47D.

Electron microscopy

Cells from monolayer cultures were washed with 0.05% versene and collected by centrifugation at 800 ***g*** for 10 min. The pelleted cells were fixed in 5% glutaraldehyde-cacodylate and postfixed in 1% osmium tetroxide and embedded in Epon. Thin sections on grids were stained overnight with uranyl acetate, poststained with 0.5% lead citrate and examined in a Joel 100-B electron microscope.

Karyotype determination

Three-day old monolayer cultures were treated with 0.5 μg/ml colchicine, incubated at 37°C for 2 hr, trypsinized, washed, fixed in methanol–acetic acid (3:1) and stained with Giemsa.

Immunofluorescence

The casein content of sections of the original tumor and T-47D cell line was evaluated by the Coon's immunofluorescence direct method employing fluorescein isothiocyanate conjugated-goat anti-human casein kindly supplied by Dr. J. Gruber (Office of Program Resources and Logistics, National Cancer Institute of the Virus Cancer Program. Sections of lactating mammary gland and non-mammary tissues served as positive and negative controls.

Steroids

The following labeled hormones were used: estradiol 17β [2,4,6,7(n)-^{3}H], 83 Ci/mmole (Amersham-Searle Co.), R-5020 [17,21-dimethyl-19-nor-4,9-pregnandiene-3, 20 dione-6, 7-^{3}H], 51.4 Ci/mmole (Roussel-UCLAF), dexamethasone [1(2)-^{3}H], 28 Ci/mmole (Amersham-Searle Co.), 5 alpha-dihydrotestosterone [1,2,4,5,6,7(n)-^{3}H], (DHT) 140 Ci/mmole (Amersham-Searle Co.).

Nonradioactive estradiol, dexamethasone and testosterone were purchased from Sigma Chem. Co., and R-5020 from Roussel-UCLAF.

Cell culture for receptor determination

For the experiments described, cells were grown for six days at an initial concentration of 10^7 cells/Roux bottle. Twenty-four hours before collection of cells, the medium was supplemented with 2.5% FCS and 10 μg/ml insulin.

Assay of cytoplasmic receptors

Aliquots (200 mg) of cytosol were incubated with either TES (Tris-HCl 0.01 M, EDTA 1.5 mM, sucrose 0.5 M, dithiothreitol 0.5 mM, glycerol 10%, pH 7.4) buffer or with 100-fold excess of nonradioactive hormone for 30 min. Labeled hormone [^{3}H]-estradiol 5 nM, [^{3}H]-R5020 40 nM, [^{3}H]-dexamethasone 30 nM or [^{3}H]-5-alpha-dihydrotestosterone 5 nM, was then added and the mixtures incubated at 0°C. After 2 hr, 50 μl per sample of dextran-coated charcoal (DCC) suspension (charcoal 100 mg/ml, dextran T-10 1 mg/ml in 0.01 M Tris–HCl, 1.5 mM EDTA, pH 7.4) was added. After a 15 min incubation with occasional stirring, the charcoal was pelleted by centrifugation; aliquots of the supernatant were added to 3 ml of Insta Gel and the radioactivity was measured in a Beckman scintillation counter.

Sucrose gradient analysis

The cytosol was incubated with labeled steroid in the absence or presence of non-labeled competitor and treated with charcoal-

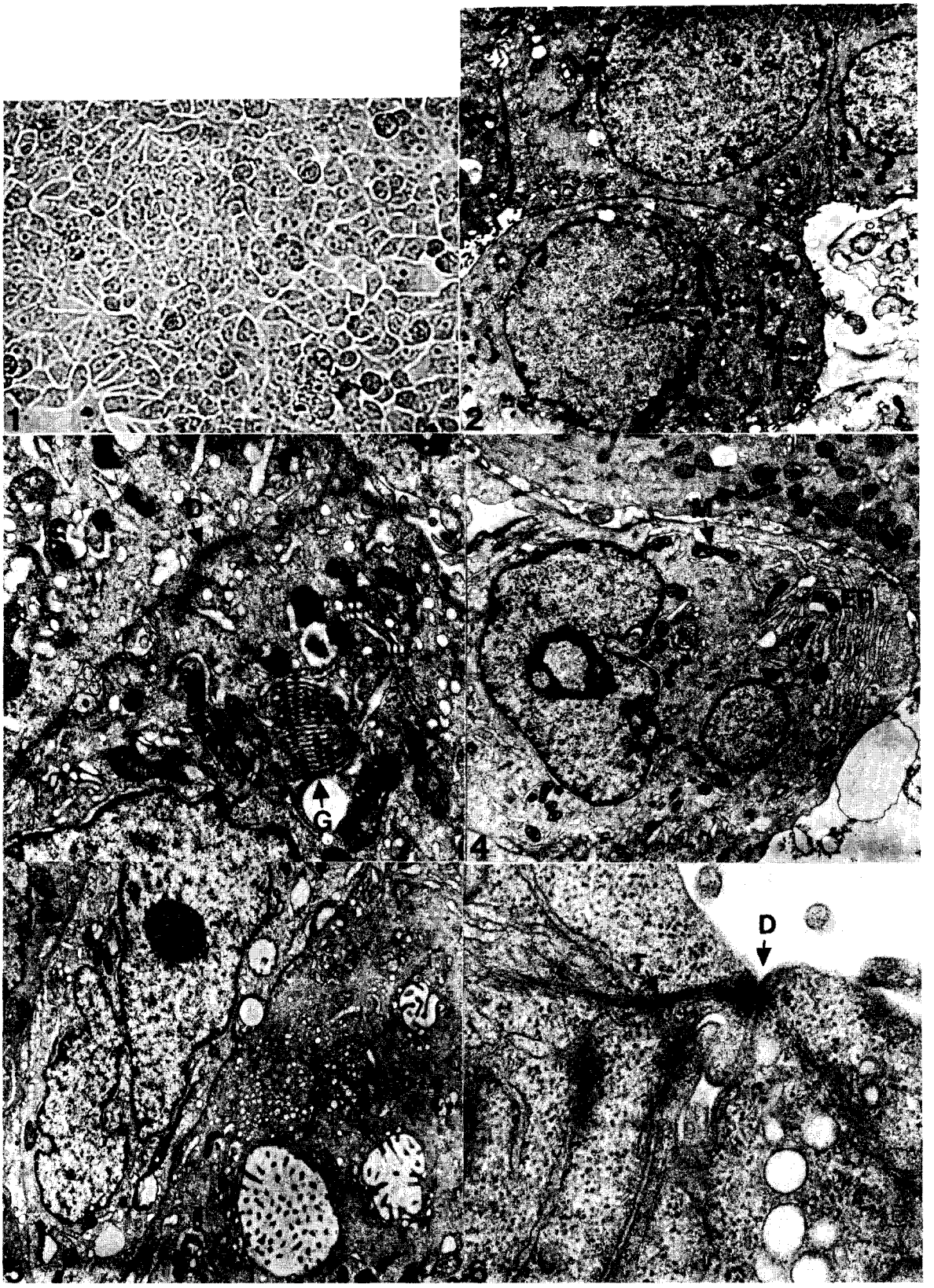


Fig. 1. *T-47D differentiated epithelial cell line growing in monolayer culture. Nonstained preparation* × 200.

Fig. 2. *Electron micrograph of T-47D culture showing three adjacent cells irregularly shaped with large nuclei and few microvilli* × 6200.

Fig. 3. *Electron micrograph of T-47D cell illustrating well-defined Golgi apparatus (G). Note dicytosomes composed of several lamellae with small coated vesicles and desmosomes (D)* × 11,700.

Fig. 4. *Electron micrograph of T-47D cell showing endoplasmic reticulum (ER), irregularly shaped nucleus and abundant mitochondria (M)* × 7800.

Fig. 5. *Electron micrograph of T-47D showing well-defined intracytoplasmic duct-like vacuoles (DLV)* × 7800.

Fig. 6. *Electron micrograph to T-47D cells illustrating a cytoplasmic junction with well-defined desmosomes (D) and parallel arrays of tonofilament bundles (T)* × 31,000.

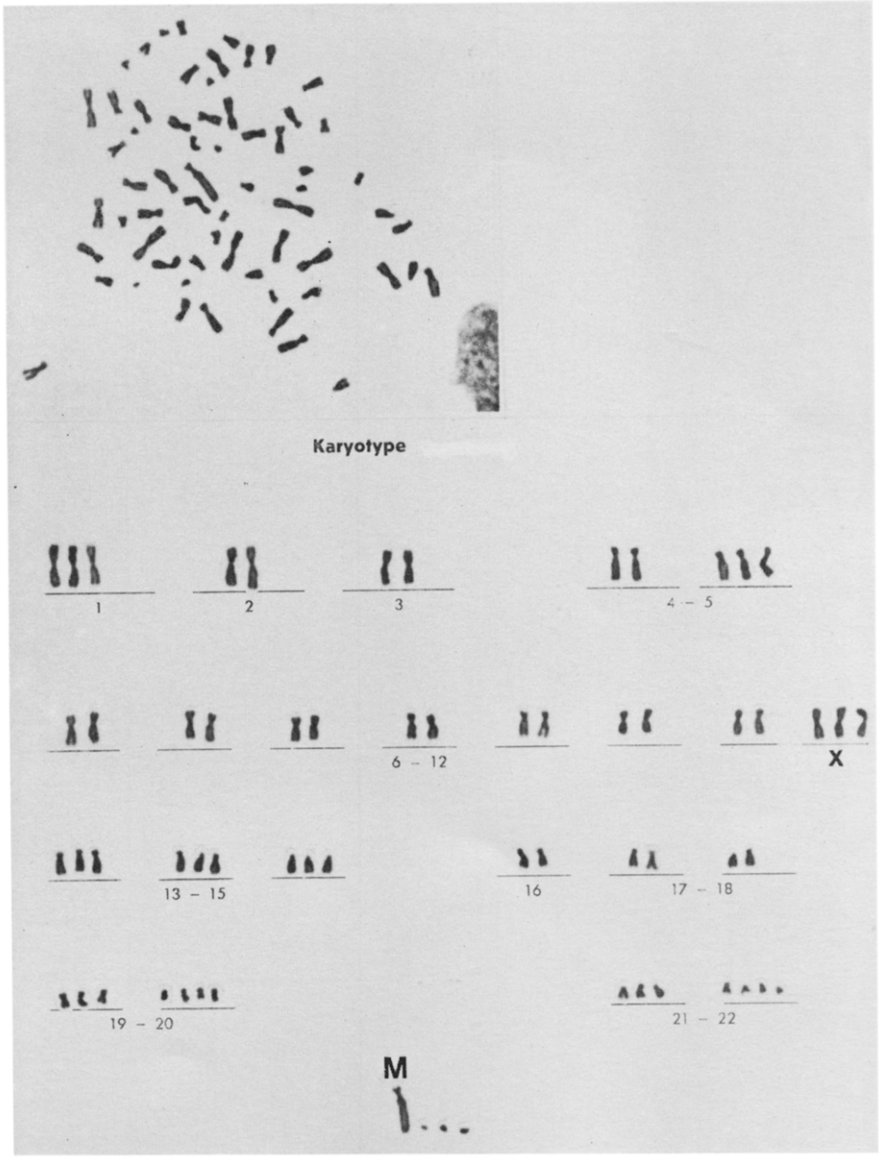


Fig. 7. A representative karyotype of a T-47D cell showing 62 chromosomes including markers (M).

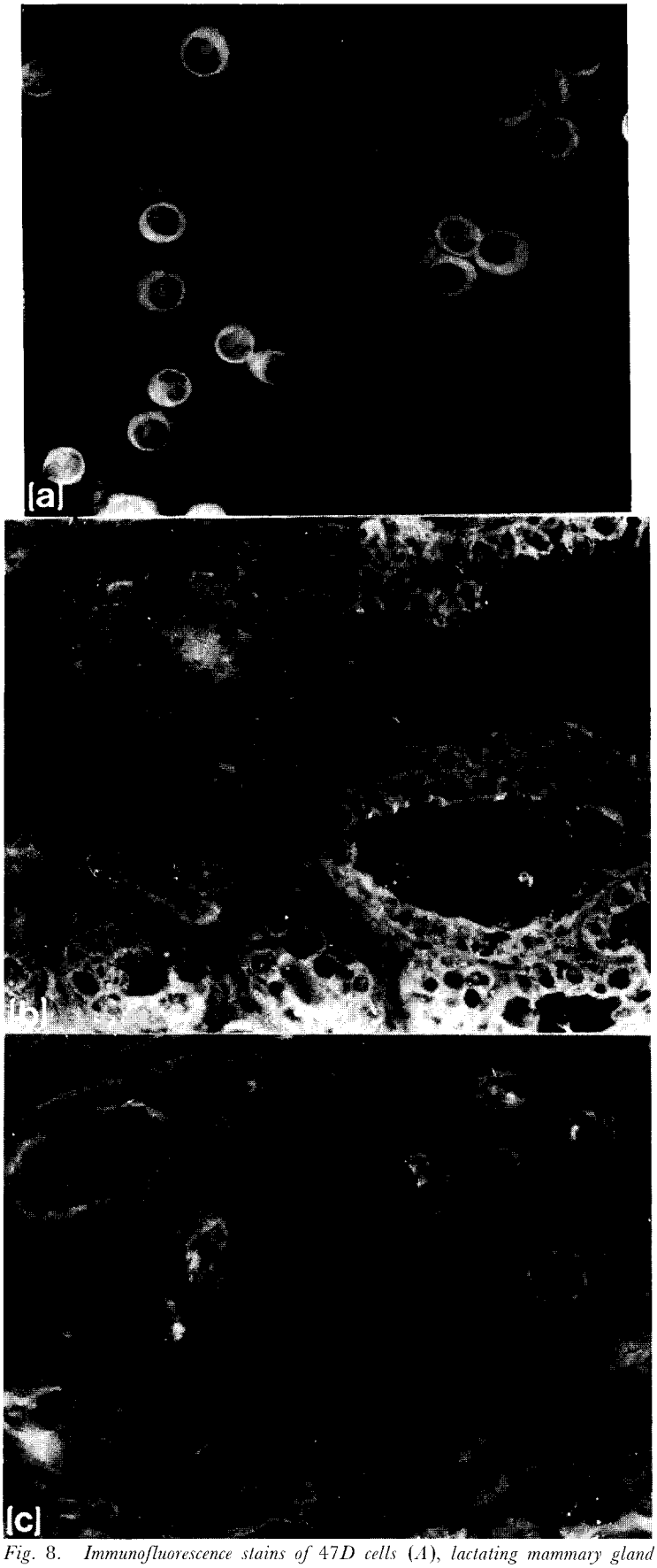


Fig. 8. Immunofluorescence stains of 47D cells (A), lactating mammary gland (B), and parent breast tumor of the 47D cell line (C) with anticasein serum. Note bright cytoplasmic fluorescence in A and similar staining pattern in B and C where fluorescence is seen primarily at the apical border of the epithelial cells lining the acinic and tumor glands and their sections $\times 500$.

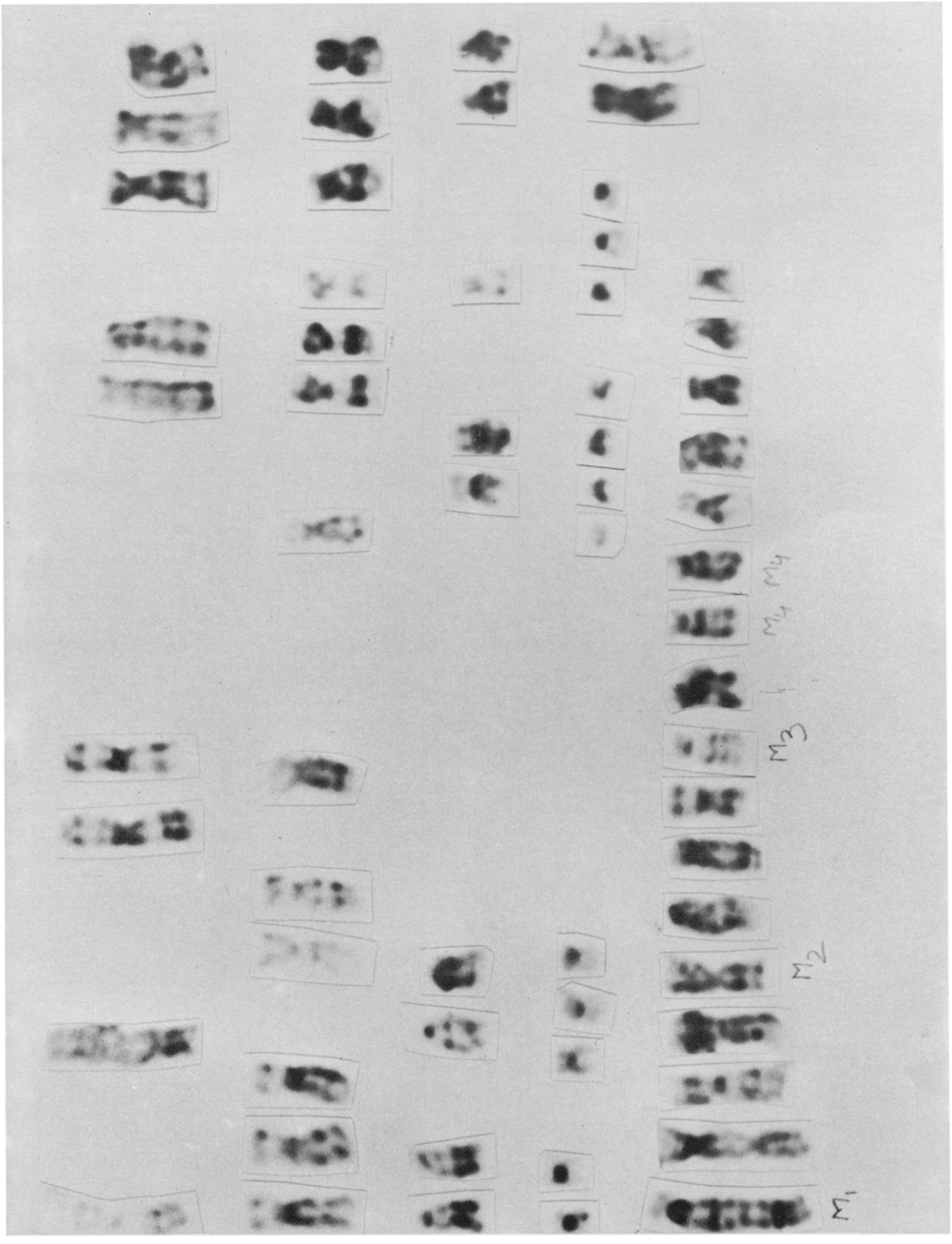


Fig. 15. Trypsin–Giemsa banded karyotype of T-47D cell line.

dextran as described above. Aliquots of 150 μl of the reaction mixtures were layered on a 5–20% sucrose gradient prepared in Tris–EDTA buffer, pH 7.4, containing 10% glycerol and centrifuged in an SW-56 rotor (180,000 ***g***, 18 hr at 4°C), in a Beckman L2-65 ultracentrifuge. Purified rabbit IgG (10 mg/ml) served as a 7S sedimentation marker. Fractions were collected from the bottom of the tubes directly into scintillation vials and the radioactivity counted.

RESULTS

Morphology and ultrastructure

After repeated subculturing for over 3 yr, the cells remain pleomorphic epithelioid cells with basophilic cytoplasm. The nuclei are large, irregularly shaped with loose chromatin pattern and contain one to three nucleoli. On electron microscopic examination, microvilli, desmosomes, tonofilament bundles and intracytoplasmic duct-like vacuoles characteristic of epithelial cells are evident. Also evident are large numbers of mitochondria, a well developed Golgi apparatus, granular endoplasmic reticulum and abundant-free ribosomes (Figs. 1–6). Mycoplasmas were not detected by standard culture methods or by electron microscopy.

Karyotype analysis

Within the cell population there is a considerable variation of chromosome number ranging from 60 to 70, with a mode of 66 (Fig. 7). Furthermore, 2–3% of the cells have a chromosome number close to 100. Dicentric chromosomes and an extra long subtelocentric chromosome are characteristic. Centromere elongation breaks (chromatidic and isochromatidic) with resulting acentric fragments and translocated chromosomes are also frequently observed. No HeLa marker chromosomes are present (Dr. W. A. Nelson-Rees [Pers. Commun.]).

Immunofluorescence

When the cells were incubated with fluorescein-conjugated antihuman casein, intense cytoplasmic and membrane fluorescence was observed in more than 60% of the T-47D cells (Fig. 8A). In sections of human lactating mammary gland, the fluorescence was seen in the secreting acini as expected (Fig. 8B). No fluorescence was seen in either cells of human colonic epithelium or of a human lymphocyte cell line (NC-37). Casein was also evident in the sections of the original breast carcinoma (Fig. 8C).

Estrogen receptors

In all cases, estradiol binding was assayed on the day the cytosol was prepared although the activity was not detectably affected by storage of the cytosol at −70°C for 2 months. Specific binding was measured by incubating various concentrations of cytosol with 5 nM [^{3}H]-estradiol for 2 hr at 4°C. Binding of the receptor by [^{3}H]-estradiol was a linear function of protein concentration (Fig. 9). The time course of binding, measured at 1.6 mg cytosol protein per 200 μl

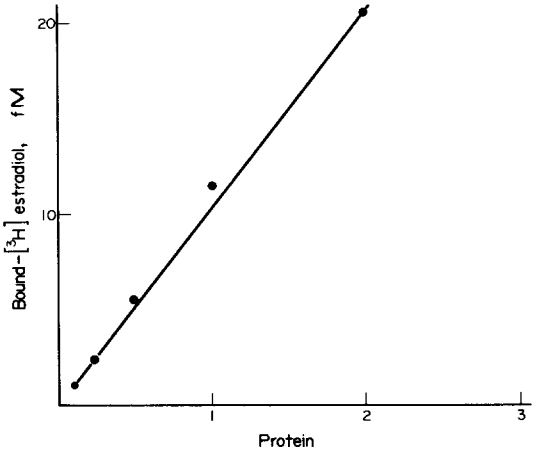


Fig. 9. Specific binding of [^{3}H]*-estradiol in cell cytosol, with increasing protein concentration, determined following* 2 hr *incubation.*

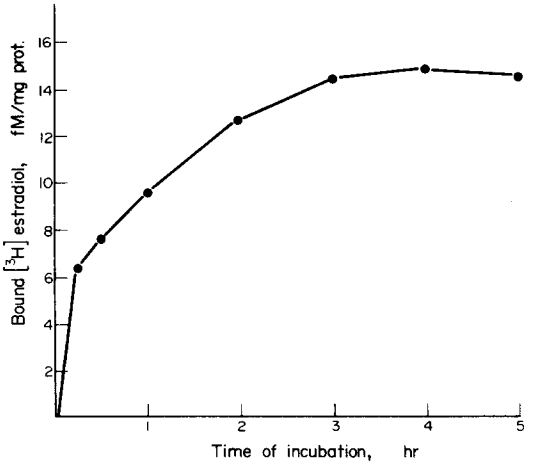


Fig. 10. Specific binding of [^{3}H]*-estradiol with time, determined at a constant cytosol protein concentration* (8 *mg/ml*).

assay mixture (8 mg/ml), showed that an equilibrium was reached after 3 hr (Fig. 10).

Specific binding was calculated from the difference in the amount of [^{3}H]-estradiol bound in the presence and absence of an excess of unlabeled estradiol. The receptor content of the T-47D cells was 17 fmole bound estradiol/mg protein.

The sedimentation pattern of the T-47D estradiol receptors on sucrose density gradients is shown in Fig. 11A. A radioactive peak was obtained in the 8S region. The binding is totally suppressed by the addition of an excess of unlabeled estradiol but not of unlabeled R-5020 progesterone or dexamethasone. The dissociation constant for specific estradiol binding, obtained from the Scatchard plot was 2.98×10^{-9} M (Fig. 11B).

Progesterone receptors

We have found that T-47D cells have 8S receptors for progesterone as measured with [^{3}H]-R-5020, a highly potent progestin (Fig. 12A). Competition studies with unlabeled hormones show that only R-5020 or progesterone were able to compete for binding of [^{3}H]-R-5020. No significant competition was detected with unlabeled estradiol or dexamethasone. The dissociation constant of R-5020 for this receptor was found to be 3.57×10^{-9}M (Fig. 12B) and the average receptor concentration was 254 fmole/mg cytosol protein.

Glucocorticoid receptors

Glucocorticoid receptors were measured by radioactive dexamethasone, either alone (40 nM) or in combination with 100-fold excess of nonradioactive steroid.

The results shown in Fig. 13A indicate that dexamethasone, R-5020 and progesterone effectively inhibited [^{3}H]-dexamethasone binding, while estradiol only partially competes for the glucocorticoid receptor. The glucocorticoid receptor sediments in the 7-8S region of the gradient. The Kd was found to be 3.29×10^{-9} M (Fig. 13B) and the cells contained 29 fmole/mg protein of glucocorticoid receptors.

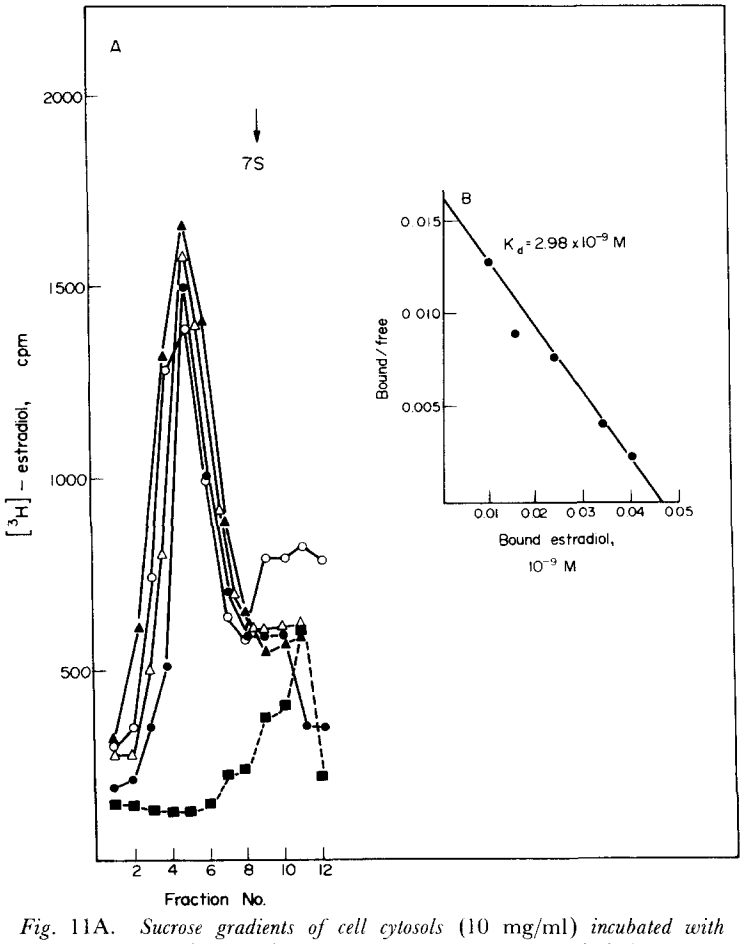


Fig. 11A. *Sucrose gradients of cell cytosols* (10 mg/ml) *incubated with labeled *estradiol* (○ - - ○) *in the presence or absence of* 100*-fold unlabeled estradiol* (■ - - ■) *R*-5020 (△ - - △), *progesterone* (▲ - - ▲) *or dexamethasone* (● - - ●);

Fig. 11B. *Scatchard plot of the binding of varying concentrations of* [^{3}H]-*estradiol with a constant cytosol level* (9.3 mg/ml).

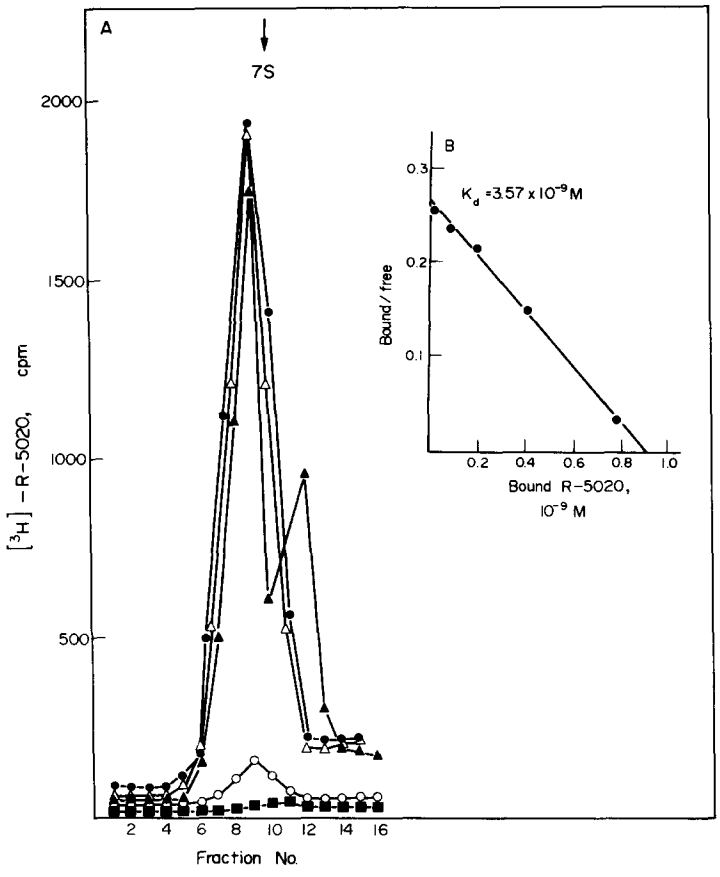


Fig. 12A. *Sucrose gradients of cell cytosols* (5 mg/ml) *incubated with labeled *R-5020* (▲--▲) *in the presence or absence of* 100*-fold unlabeled R-5020* (■--■), *progesterone* (○--○), *estradiol* (△--△) *or dexamethasone* (●——●);
Fig. 12B. *Scatchard plot of the binding of varying concentrations of* [^{3}H]-*R-5020 with a constant cytosol level* (4 mg/ml).

Androgen receptors

The results shown in Fig. 14A indicate that these cells have androgen receptors, as measured by their ability to bind [^{3}H]-5-α-dihydrotestosterone (DHT). The gradients revealed a decrease in androgen binding in the 7-8S region with DHT and R-5020 and a partial inhibition with estradiol and dexamethasone. The dissociation constant for androgen binding was found to be 4.8×10^{-9}M (Fig. 14B). The cytosol contains approximately 3 fmole/mg protein of DHT-receptor.

DISCUSSION

We describe here the isolation and characteristics of a human mammary tumor cell line which was established and has been maintained in our laboratory for over 4 yr.

As suggested by Buehring *et al.* [18] and Seman *et al.* [19], the ultrastructure of cells in culture may reflect their tissue of origin. Even after several years of passage the T-47D cell line contains desmosomes, tonofilaments and intracytoplasmic duct-like vacuoles which indicate an epithelial origin. Strong evidence for the mammary origin of the T-47D cells, is that like the original carcinoma, the cell line exhibits fluorescence when tested by the direct immunofluorescence technique with goat anti-human casein serum. Further evidence for the mammary origin of the T-47D cells is also provided by the presence of cytoplasmic steroid receptors which are characteristic of mammary cells. The high affinity nature of this hormone receptor is evident from the finding of a radioactive 7-8S binding component which is markedly suppressed by the addition of an excess of the unlabeled homologous hormone. The Scatchard plot of the interaction of cytosol with increasing hormone concentrations reveals a high affinity binding component in cytosol for each of the four steroids tested. The steroid receptors in T-47D cells share

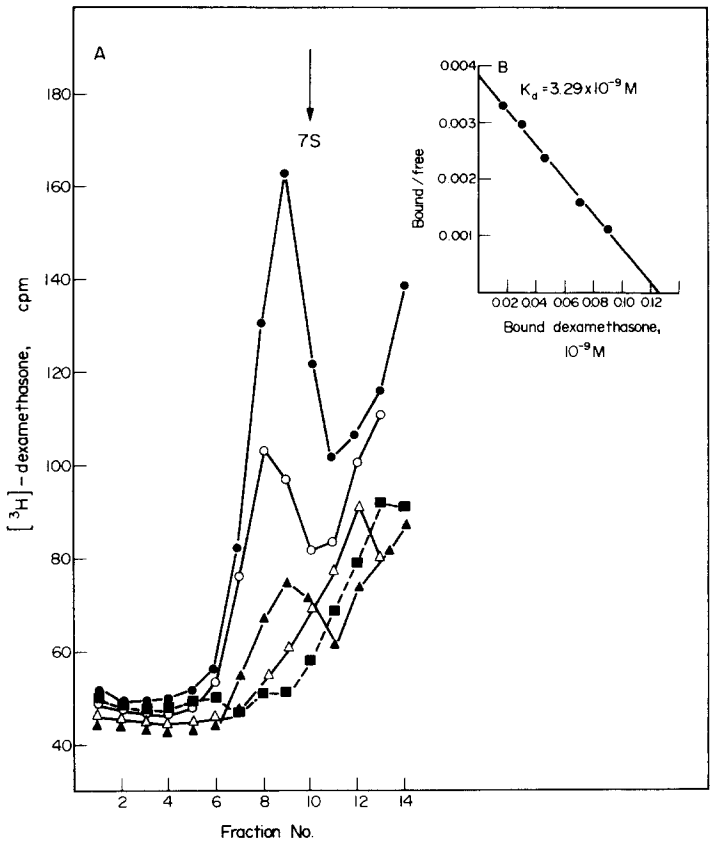
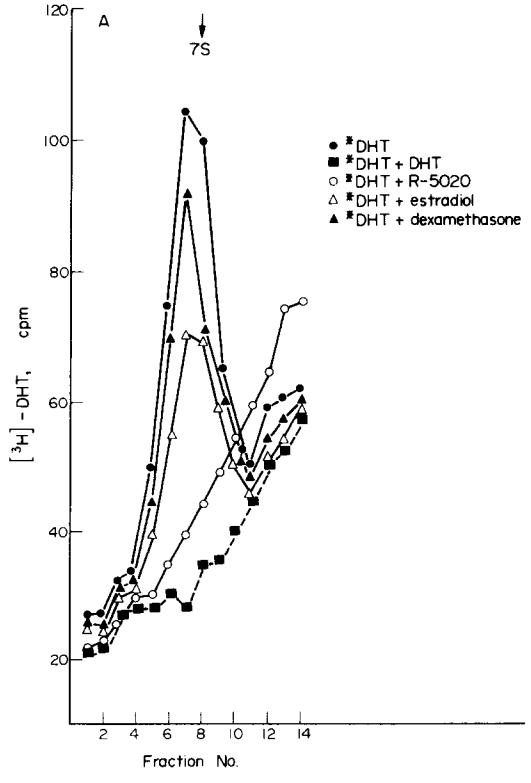


Fig. 13A. *Sucrose gradients of cell cytosols* (8.5 mg/ml) *incubated with labeled *dexamethasone* (●--●) *in the presence or absence of* 100*-fold unlabeled dexamethasone* (■--■), *estradiol* (○--○), *R*-5020 (△--△) *or progesterone* (▲--▲);

Fig. 13B. *Scatchard plot of the binding of varying concentrations of* [^{3}H] *dexamethasone with a constant cytosol level* (6.2 mg/ml).



several similar properties with the receptors reported in the MCF-7 line [17]. The studies on the specificity of the receptor sites using competition by various hormones show that estradiol and progesterone receptors bind only the respective homologous hormone. Dexamethasone and DHT receptors, however, were able to bind other steroids as well, though less effectively than the homologous steroids. The fact that dexamethasone and DHT binding molecules sediment on sucrose density gradients at about 8S suggests that these are true receptors. Similar results of cross-competition for glucocorticoid and DHT receptors by various steroids have also been

Fig. 14A. *Sucrose gradients of cell cytosols* (6 mg/ml) *incubated with labeled *5-alpha-dihydrotestosterone* (*DHT*) (●--●), *in the presence or absence of* 100*-fold unlabeled DHT* (■--■), *R*-5020 (○--○), *estradiol* (△--△), *or dexamethasone* (▲--▲);

Fig. 14B. *Scatchard plot of the binding of varying concentrations of* [^{3}*H*]*-DHT with a constant cytosol level* (6 mg/ml).

reported for other tissues and cells [17, 20, 21].

In view of the data reported for MCF-7 cells [17] and from our results in T-47D cells, the assumption suggested by Wittliff [22] that two different hormones may become associated with a single receptor site appears unlikely. In both types of cells, progesterone competes for glucocorticoid receptors, while dexamethasone is ineffective in competing for progesterone receptors. In T-47D cells, estradiol also competes for the glucocorticoid receptor but no effective competition was observed with dexamethasone for estradiol receptor.

This line of human mammary carcinoma cells may provide a novel system to explore fundamental aspects of hormone-tumor interactions, including clarification of how hormones regulate receptor levels [5, 13] and modify cell metabolism. They also offer possible opportunities for the evaluation of chemotherapeutic agents.

ADDENDUM

Recently an analysis of the 47D cell line was carried out at the Child Research Center of Michigan under Contract N01-CP-3-3333 from the Division of Cancer Cause and Prevention, National Cancer Institute.

The following information was obtained: The 47D cell line is of human origin as shown by reaction with human antiserum and by isoenzyme and cytogenetic analysis. The glucose-6-phosphate dehydrogenase mobility is that of human type B, the LDH mobility pattern is human, and shows only the lactate dehydrogenase band 5. Cytogenetically, all metaphases examined have human chromosomes, and a characteristic large subtelocentric marker chromosome. The modal chromosome number is 66.

The trypsin–Giemsa banded karyotype is shown in Fig. 15. No normal No. 9 chromosome could be found. Unassignable chromosomes comprise the last row with M indicating marker chromosomes. M_1— the long subtelocentric chromosome appears to be an inverted No. 1 chromosome, with the *p* arms of the marker chromosome being the upper portion of the *q* arms of the No. 1 chromosome The middle third has an insertion of a portion of the *q* arms of a 7, 8 or 9 chromosome attached to a portion of the *p* arms of the No. 1 chromosome. The lower third of the chromosome has the lowest four bands found in the normal No. 1 chromosome. The M_3 is most likely derived from the No. 9 chromosome. M_4 is probably an altered No. 10 chromosome.

Note added in proof

Prior to our receiving the galley proofs of this manuscript, a report by Horowitz *et al. Cancer Res.* **38,** 2434 (1978) was published confirming the presence of steroid hormone receptors in the T-47D cell line.

REFERENCES

1. E. Y. Lasfargues and L. Ozzello, Cultivation of human breast carcinoma. *J. nat. Cancer Inst.* **21,** 1131 (1958).
2. R. E. Nordquist, R. D. Ishmael, C. A. Lovig, D. M. Hyder and H. F. Hoge, The tissue culture and morphology of human breast tumor cell line BOT-2. *Cancer Res.* **35,** 3100 (1975).
3. E. Y. Lasfargues, *New Approaches to the Cultivation of Human Breast Carcinomas in Human Tumor Cells* In Vitro. (Edited by J. Fogh) p. 51. Plenum Press, New York (1975).
4. A. J. Hackett, H. S. Smith, E. L. Springer, R. B. Owens, W. A. Nelson-Rees, J. L. Riggs and M. G. Gardner, Two syngeneic cell lines from human breast tissue: the aneuploid mammary epithelial (Hs578T) and the diploid myoepithelial (Hs578Bst) cell lines. *J. nat. Cancer Inst.* **58,**1795 (1977).
5. M. E. Lippman, G. Bolan and K. Huff, The effect of glucocorticoid and progesterone on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res.* **36,** 4602 (1976).
6. M. V. Reed and G. O. Gey, Cultivation of normal and malignant human lung. *Lab. Invest.* **11,** 638 (1962).
7. H. D. Soule, A. Vasquez, S. Albert and M. Brennan, A human cell line from a pleural effusion derived from a breast carcinoma. *J. nat. Cancer Inst.* **51,** 1409 (1973).
8. R. Cailleau, B. Mackay, R. K. Young and W. J. Reeves, Jr., Tissue culture studies on pleural effusions from breast carcinoma patients. *Cancer Res.* **34,** 801 (1974).

9. R. Cailleau, R. Young, M. Olive and W. J. Reeves, Jr., Breast tumor cell lines from pleural effusions. *J. nat. Cancer Inst.* **53,** 661 (1974).
10. R. K. Young, R. M. Cailleau, B. Mackay and W. J. Reeves, Jr., Establishment of epithelial cell line MDA-MB-157 from metastatic pleural effusion of human breast carcinoma. *In Vitro* **9,** 239 (1974).
11. L. Terenius, Parallelism between oestrogen binding capacity and hormone responsiveness of mammary tumors in GR/A mice. *Europ. J. Cancer* **8,** 55 (1972).
12. H. A. Young, E. M. Scolnick and W. P. Parks, Glucocorticoid-receptor interaction and induction of murine mammary tumor virus. *J. biol. Chem.* **250,** 3337 (1975).
13. G. G. Rousseau, J. D. Baxter and G. M. Tomkins, Glucocorticoid receptors: relations between steroids binding and biological effects. *J. molec. Biol.* **67,** 99 (1972).
14. M. D. Lagios, Hormonally enhanced proliferation of human breast cancer in organ culture, *Oncology* **29,** 22 (1974).
15. U. Spaeren, S. Olsnes, I. Brenhovd, J. Efskind and A. Pihl, Content of estrogen receptors in human breast cancers. *Europ. J. Cancer* **9,** 353 (1973).
16. S. C. Brooks, E. R. Locke and H. D. Soule, Estrogen receptor in a human cell line (MCF-7) from breast carcinoma. *J. biol. Chem.* **248,** 6251 (1973).
17. K. B. Horwitz, M. E. Costlow and W. L. McGuire, MCF-7, A human breast cancer cell line with estrogen, androgen, progesterone and glucocorticoid receptors. *Steroids* **26,** 785 (1975).
18. G. C. Buehring and A. J. Hackett, Human breast tumor cell lines; identity evaluation by ultrastructure. *J. nat. Cancer Inst.* **53,** 621 (1974).
19. G. Seman and L. Dmochowski, Ultrastructural characteristics of human tumor cells *in vitro*. In *Human Tumor Cells in Culture*. (Edited by J. Fogh) p. 395. Plenum Press, New York (1975)
20. L. Terenius, A. Rirmsten, L. Thoren and A. Lindgren, In *Estrogen Receptors in Human Breast Cancer*. (Edited by W. L. McGuire, P. P. Carbone and E. P. Vollmer) p. 237. Raven Press, New York (1975).
21. W. L. McGuire, P. P. Carbone, M. E. Sears and G. C. Escher, In *Estrogen Receptors in Human Breast Cancer: An Overview*. (Edited by W. L. McGuire, P. P. Carbone and E. P. Vollmer). Raven Press, New York (1975).
22. J. L. Wittliff, Specific receptors of the steroid hormones in breast cancer. *Semin. Oncol.* **1,** 109 (1974).