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Cell Structure and Function and Response to Chemotherapy in Tumors Heterotransplanted into the Subrenal Capsule of Mice and Rats

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Abstract—*Specimens from 16 freshly biopsied human tumors, two mammary adenocarcinomas, ten ovarian adenocarcinomas, two squamous cell carcinomas, one malignant histiocytoma and one chondrosarcoma of the bone, two human ovarian adenocarcinomas established by transplantation into nude mice and two adenocarcinomas induced in rat mammary gland were transplanted under the renal capsule of 510 normal immunocompetent mice and 180 rats and the effects of chemotherapy were evaluated. The results showed successful transplantation of all types of tumors in both animal species. Morphological analysis revealed preserved glandular structures with surface microvilli, mucin and CEA production and partially preserved basement membranes. Treatment with cyclophosphamide, vinblastine, adriamycin and cisplatin caused cell shrinkage, degradation and partial or total disappearance of the tumor cells. Vascularization was distinct in all specimens. A cellular infiltrate was found frequently but not consistently. A common end stage was a fibrotic scar with no cellular activity, occasionally giving a misleading impression of a growing tumor on gross observation. The results were obtained rapidly and suggest that the subrenal capsule assay would be useful for evaluating the sensitivity of human tumors to therapeutic manipulation, but needs supplementary histological examination.*

INTRODUCTION

CONTINUED growth of excised human tissue and cells is needed in order to study the mechanism and characteristics of transformation, development and progression in tumors as well as to select treatment modalities in cases of malignant disease. Previous attempts to develop reliable, consistent, effective and economical screening tests for the selection of chemotherapeutic agents [1–3] have met with difficulties. *In vitro* sensitivity tests for cancers include the nucleotide precursor incorporation test [4], the stem cell assay [5] and the dye exclusion method [6], and bioluminometric measurements of intracellular adenosine triphosphate [7]. The cells are detached from their natural supportive systems in these techniques; however, drug delivery is artificial, and no metabolic activation occurs. Similarly, the cell-to-cell relationship and host response cannot be studied.

The nude mouse has been the rodent prototype for the transplantation of benign and malignant cells and tissues [8, 9], even though the rate of successful transplantations is only approximately 50% [2, 8], possibly due to the immune state of the animal [10]. Other variables which affect transplantation include the health of the mouse, its handling, and the preparation of the tumor [11, 12] and inoculation site [13]. The frequency of successful growth in the nude mouse also correlates with the degree of malignancy [9, 14] and antigenicity of the tumor [12], possibly causing selection of the growing cell type compared to the original tumor.

Bogden *et al.* [15] recently introduced the subrenal capsule assay (SRCA) for human tumor heterotransplantation. This is based on an *in situ* measurement of small changes with time in the size of biopsy specimens from tumors transplanted under the renal capsule of immunodeficient or normal immunocompetent mice. Results can be obtained from the SRCA within 6–11 days [16]. This method has been applied to studies on transplanted colon carcinomas [17], melanomas [18–21] and ovarian carcinomas [22], although mostly without histological confirmation. We have previously reported some early results on the morphology of transplanted tumors by the SRCA method [23], showing the similarity of transplanted specimens to original tumors. The purpose of this study was to use morphological and immunohistochemical methods to determine the response to chemotherapeutic tumor agents of different types of tumors transplanted by the SRCA method.

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MATERIALS AND METHODS

The material consisted of 20 tumors transplanted into the renal capsule of 510 mice and 180 rats. The human tumors were one chondrosarcoma and one malignant histioclytoma of the bone, two squamous cell carcinomas, one of the cervix and one of the vulva, two mammary adenocarcinomas and ten ovarian adenocarcinomas of a low degree of differentiation, consistent with serous cystadenocarcinoma. Two ovarian adenocarcinomas were transplanted for this study. Two mammary adenocarcinomas were obtained from rats which had received an intragastric dose of 9,10-dimethylbenz(a)anthracene. Chemotherapy was applied as indicated in Table 1.

The samples for the SRCA were taken from solid tumors under aseptic conditions, and were excized from the same areas of the tumors as those taken for histological examination. If a tumor originated from an area which might have been contaminated by micro-organisms, the area was swabbed with antiseptic solutions and/or creams, or else systematic antimicrobial therapy was given at the same time. A sample of approximately 1 cm^3 was removed, taking care to exclude necrotic tissue. This was then fragmented and transferred to sterile test tubes containing culture medium 199. In the laboratory the samples were transferred onto dishes containing the same medium and further cut into pieces measuring 1 mm^3. Normal immunocompetent CDF_1 female mice and Sprague–Dawley rats were used as recipients.

The operative procedure for subcapsular implantation was as follows. An incision was made through the skin and body wall of an anesthesized animal dorsally in the region of the left kidney. The kidney was partially exteriorized and a small slit was carefully made in the renal capsule with a scalpel. A piece of the tumor tissue was inserted beneath the capsule through the slit with the aid of a trocar. The tumor size [(length + width)/2] was expressed in terms of ocular micrometer units (omu), where 10 omu = 1 mm. The kidney was replaced in the body cavity and the body wall and skin incisions were closed with silk sutures. The animals were allowed to eat and drink *ad libitum* after recovering from anesthesia.

Five mice per treatment modality for each tumor were treated with drugs on a daily schedule for 5 days beginning on the day after implantation, with five control animals receiving physiological saline or a solution containing polyethyleneglycol 28.8 g, Tween 80 1.92 g, NaCl 8.65 g, methyl-*p*-hydroxybenzoate 1.73 g and propyl-*p*-hydroxybenzoate 0.19 g in 1 l of distilled water. The drug dosages were adjusted to reach maximum effect within tolerable toxicity levels (Table 1). On the sixth day after implantation the animals were killed by carbon dioxide suffocation and the tumor-bearing kidneys were partially exteriorized. The kidneys were kept moist with physiological saline to prevent shrinkage and the final tumor sizes measured. The kidneys were then excised and processed. The initial and final body weights of the mice were measured. Toxicity was considered tolerable when the weight loss was less than 20%. If the mean weight loss in a particular test group exceeded 20%, the assay for this drug or drug combination was disregarded.

The specimens for histological examination were fixed in a glutaraldehyde–formalin solution [24], embedded in paraffin and routinely stained with hematoxylin and eosin. Periodic acid-Schiff and other stains were also used when necessary.

For immunohistochemical studies on basement membrane components the 7-S collagen domain of human type IV collagen was purified from human kidney and the fragment P1 of laminin from human

Table 1. Daily doses of drugs in single and combination therapy

Drug	Manufacturer	Dose (mg/kg) Mouse Single	Mouse Combination	Rat Single	Rat Combination
Cyclophosphamide	Lääke, Turku, Finland	30–50	30–50	10	10
Mitolactol	Chinoin, Budapest, Hungary	180	—	50–120	—
Adriamycin	Farmitalia Carlo Erba, Milano, Italy	—	3–4	—	2–3
Cisplatin	Lääke, Turku, Finland	2	1–2	1	0.5–1
Vincristine	Lilly, Indianapolis, IN, USA	—	0.5–1	—	0.25–0.5
Methotrexate	Farmos, Turku, Finland	—	4	—	3
Bleomycin	H Lundbeck & Co A/S, Copenhagen, Denmark	30	30	—	—
Tamoxifen	ICI, Macclesfield, England	125	—	3	—

placenta [25, 26]. Antisera to these proteins were raised in rabbits. The antibodies were purified by immunoabsorption with the relevant antigen coupled to Sepharose 4B. The laminin antibodies were cross-absorbed with 7-S collagen and vice versa, and there was no cross-reaction between the two antibodies in the radioimmunoassay. Rabbit antibodies to human factor VIII and carcinoembryonic antigen (CEA) were purchased from Dako Immunoglobulins A/S, Copenhagen, Denmark.

The avidin–biotin modification of the peroxidase–antiperoxidase method was used for the immunohistochemical studies [27]. Five-micrometer paraffin sections were deparaffinized and treated with 0.4% pepsin (Merck, Darmstadt, F.R.G.). Endogenous peroxidases were blocked by incubating with H_2O_2 in methanol. The sections were successively treated with rabbit antibodies, biotinylated anti-rabbit immunoglobulin (Vector Laboratories, Burlingame, CA), dilution 1:500, avidin (Vector), dilution 1:1000, and biotinylated horseradish peroxidase complex (Vector). The peroxidase reaction was performed using 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, MO) as a substrate.

The specimens for ultrastructural studies were fixed in McDowell's solution [24], post-fixed in 1% osmium tetroxide in the same buffer, dehydrated and embedded in Epon. Sections of 1 μm were cut and stained with 1% toluidine blue for orientation by light microscopy. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a Jeol JEM 100 B electron microscope.

RESULTS

The success rate was 92%, indicating a high number of growing tumors obtained by this method. The growth was considerable regardless of the original cell type, whether mesenchymal (chondrosarcoma or malignant histiocytoma) or epithelial, the histological type (sarcoma, adenocarcinoma or squamous cell carcinoma), the degree of differentiation (well-differentiated or anaplastic), the origin (induced in a rat or spontaneous in a human subject) or the intermediate host (nude mouse or primary).

The histological structure was similar to the primary tumor in many aspects when transplanted to either mice or rats. Mammary tumors retained their glandular features and mucin production with a distinct epithelial component when transplanted to mice, regardless of whether they had been primary in humans (Fig. 1) or induced in rats (Fig. 2). The epithelial features were clearly distinguishable in electron-microscopy (Fig. 3). Ultrastructural analysis revealed secretory features in epithelial cells, and also tubular structures covered by numerous surface microvilli (Fig. 4). The same tumors also grew progressively when transplanted to rats. Immunohistochemical stainings showed preserved CEA staining activity (Fig. 5) and preserved basement membranes which were positive for collagen IV and laminin (Fig. 6).

The ovarian tumors, mostly of a high degree of malignancy, showed polymorphous cells in solid arrangements. The cells varied in size and shape, the nucleoli were prominent and the nucleo/cytoplasmic ratio was increased. Mitotic activity was also commonly observed with some abnormal mitoses. The tumor cells also retained their malignant characteristics when transplanted subsequently to nude mice, and this transplant was grown in the renal capsule (Fig. 7). The functional characteristics were preserved with basement membrane production found using stains for collagen IV and laminin. Undifferentiated tumors progressing as peritoneal carcinosis did not acquire any specific features reflecting their original cell type upon transplantation (Fig. 8).

Other tumor types transplanted included squamous cell carcinomas of the cervix which grew progressively with a distinct inflammatory infiltrate when transplanted to mice (Fig. 9). Ultrastructural analysis revealed preserved keratinization (Fig. 10) seen as tonofilaments and keratohyalin granules in the cytoplasm. Mesenchymal tumors such as histiocytoma and chondrosarcoma also retained their morphological characteristics after transplantation (Fig. 11), though cartilage formation was not seen.

Chemotherapeutic treatment was effective, as shown in Table 1, causing significant decrease in tumor transplant size. The morphological effects were subdivided into mild, moderate and severe. Mild changes included cellular ballooning or shrinkage and cytoplasmic homogenization (Fig. 12). A slight loss of cellular organization was visible in electron-microscopy (Fig. 13). A moderate response included altered cytoplasmic staining properties, cytoplasmic vacuolization, karyorhexis and cytolysis (Fig. 14). Severe effects involved advanced degeneration in a large number of cells (Fig. 15). Some cells were elongated, retaining their nuclei, while others consisted of anucleated debris and occasionally only remnants of tumor cells were found. The vascular structures were preserved in drug-treated transplanted tumors, with surviving tumor cells located in adjacent areas (Fig. 16).

Fibrosis was a prominent feature in drug-treated tumors. In some specimens tumor cells were intermixed with thickened collagenous fibers, in others tumor cell aggregates were laying side-by-side with collagenous plaques. A common end stage comprised a fibrous scar (Fig. 17) with minimal cellu-

lar activity (Fig. 18). A cellular infiltrate composed of lymphocytes, not granulocytes varying in intensity, was occasionally prominent in untreated transplants (Fig. 9) but often completely absent in treated tumors.

DISCUSSION

As shown in this study, the SRC assay proved a reliable and consistent method of propagating human tumors under conditions similar to the human situation. Previous studies [28–30] report success rates of 81–100%, depending partly on fibrosis and necrosis in the original tumor and the handling of specimen [16, 18, 30]. The growth potential was a fairly constant property of the tumor cells, and no subpopulation selection took place [18]. The significance of growth regulation is seen in nude mice transplants [31], where significant alterations in the transplant occur as compared with the original tumor.

Even though the animals in this study were all immunologically competent and no efforts were made to alter their immune status, the take rate of the transplants was high. Immune depression has been advocated for animals using the SRCA method [32, 33] and nude mice have also been used [15, 16], but the choice of mice or rats for the SRCA did not significantly affect the present results. The original method of Bogden *et al.* [15, 16] used immunocompetent mice, and a few studies [32] have used rats, but with similar results.

As shown here, tumors retain their morphological characteristics, such as glandular configuration, mucin secretion or CEA production, when transplanted by the SRCA method. Previous studies involving the use of histological examination of transplanted melanomas report preserved cell type and shape, pigmentation [21] and a positive Fontana–Massons stain for melanin [20]. Soft tissue sarcoma grafts showed the same storiform pattern as the original tumor [20], and the glandular structures of original colon carcinoma [18, 34] and ovarian tumor [23, 35] were retained together with mucin production and production of CEA. The basic functional characteristics, i.e. production of CEA and basement membrane components collagen type IV and laminin, were preserved. Xenografts in nude mice also exhibit CEA production [36] and retain tumor-specific antigens [37]. Extreme heterogeneity was nevertheless observed for the expression of the colon-specific antigen CEA and colon mucin antigen by Gold *et al.* [38]. Biochemical changes have also been reported in transplants [37, 39, 40] in nude mice in tumors explanted by the SRCA method [23], with original antigenic characteristics preserved.

These results showed the reliability of the SRCA for chemotherapy studies; as the tumors were histologically similar to tumor of origin, selection of cell type did not occur. Also, when using nude mice in this study as intermediate hosts the structures of the original tumor were preserved. Early reports using nude mice emphasized the histological and functional similarity of xenografts to the original tumor [39], while later studies report changes in tumor differentiation [41] and histological differences [14, 42]. The extrapolation of results obtained with nude mice to humans involves problems of strain heterogeneity, dosage and toxicity of drugs [43]. The applicability of results obtained using nude mice to the human situation is questionable [41] due to abnormal immune response, as well as difficultics in transplantation of certain tumor types [3]—a problem not encountered here.

Chemotherapy was found here to induce distinct gross and morphological changes. Histopathological evaluation of drug treatment given to cell lines in nude mice has pointed to nuclear cytoplasmic degenerative changes and an arrest of cell division, stromal edema and fibrosis [44] similar to those reported here. The cytological findings of nuclear degeneration, karyolysis and karyohexis as well as cytolysis and cytoplasmic vacuolation approximated the decrease in tumor mass and size of transplant. Histological studies on transplants in the SRCA report cellular degeneration and necrosis [34]. Tissue necrosis from drug treatment reported in nude xenografts [41] was minimal in this study. Analysis of the effects of chemotherapy in general is hampered by the problems inherent in animal models, namely the structures and functions of the tissues and cells differ from those in humans. Likewise, drug dosage schedules are difficult to relate with some studies employing concentrations equivalent to the LD_{10} or LD_{50} of the animals [32].

A commonly used measure of drug effects in the SRC assay is measurement of size of transplanted tumor, as advocated by Bogden *et al.* [15, 16] and also used in this study. However, fibrosis and cell infiltration obliterated gross estimates of tumor shrinkage in some instances in this study, as also shown before [35]. A frequent but not consistent finding in this study was a cellular infiltrate in the transplanted tumor tissue adjacent to the host renal tissue. The infiltrate consists mainly of T lymphocytes [33]. Drug treatment affects the inflammatory reaction, with cyclophosphamide being more effective than others [35]. This inflammation may be due to contamination when performing the transplantation. Some authors consider gross measurements adequate [33, 45], other reports [34] emphasize histological studies and the need to identify inflammation and the host reaction. Aamdal *et al.* [18] showed that mouse cells contribute 5–10% of the cells in SRC grafts, making gross estimates of drug effects inaccurate.

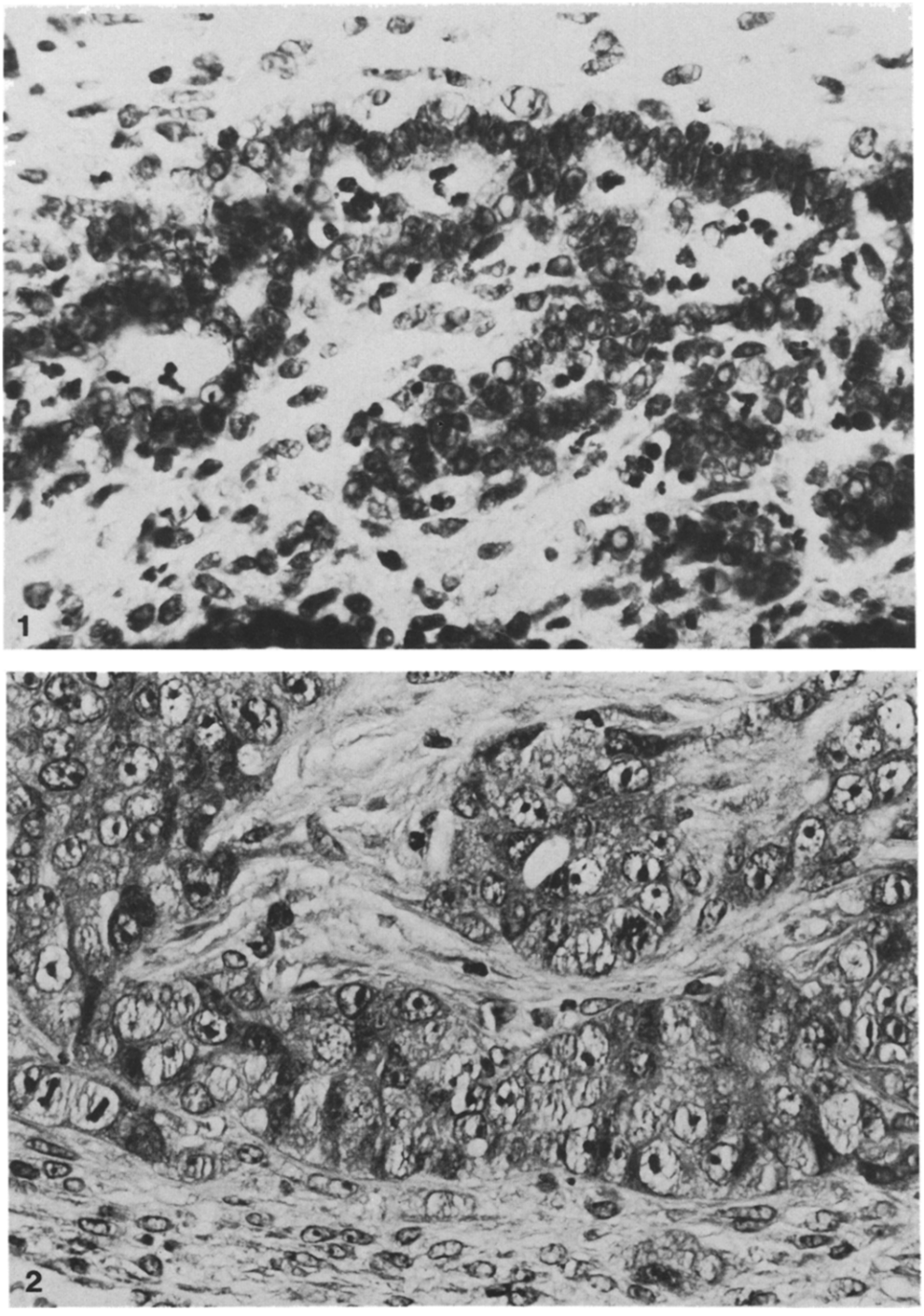


Fig. 1. A human mammary tumor in a mouse SRCA, with preserved glandular structures. HE, × 360.
Fig. 2. A mammary tumor induced in a rat and transplanted to mice SRCA, showing malignant cells with prominent cytological atypia and a distinct stromal reaction. HE, × 360.

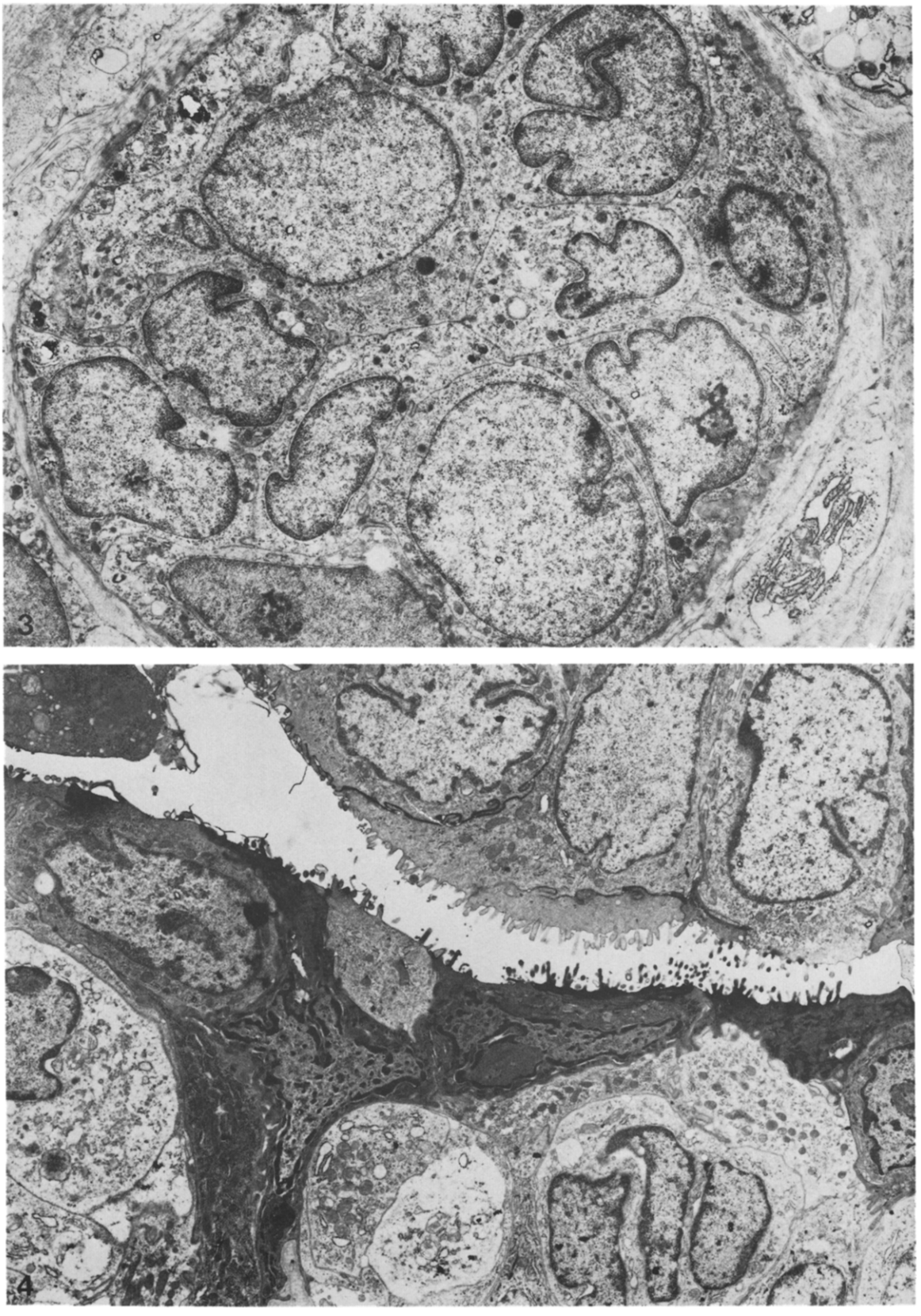
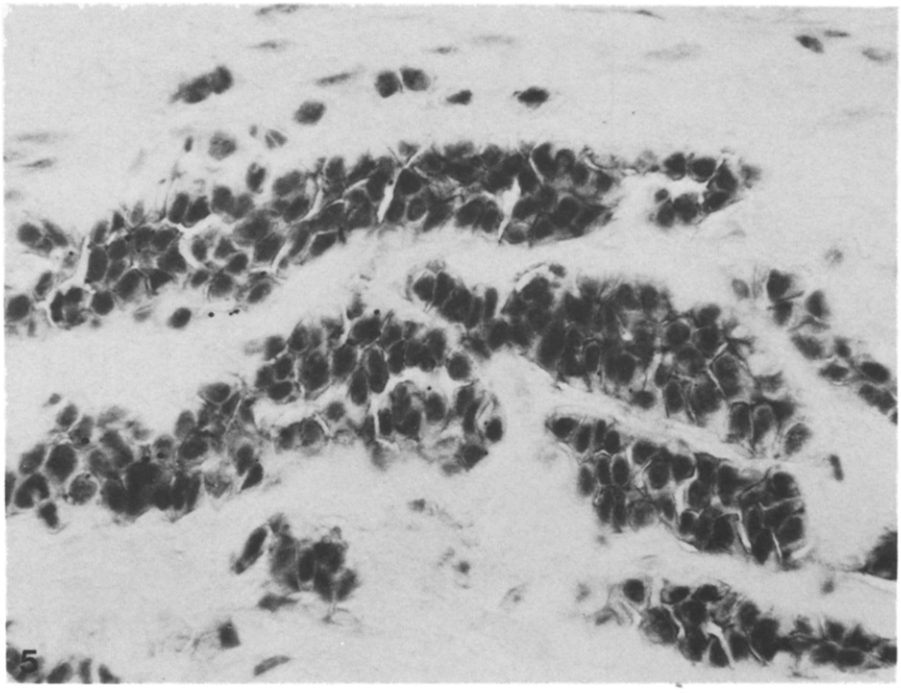


Fig. 3. A rat mammary tumor in a mouse SRCA, showing tightly arranged epithelial cells with scanty mucin production. Uranyl acetate/lead citrate, × 2400.

Fig. 4. Tubular structures in rat mammary adenocarcinomas transplanted to mice SRCA, covered by dark and light cells with prominent microvilli. Uranyl acetate/lead citrate, × 3000.



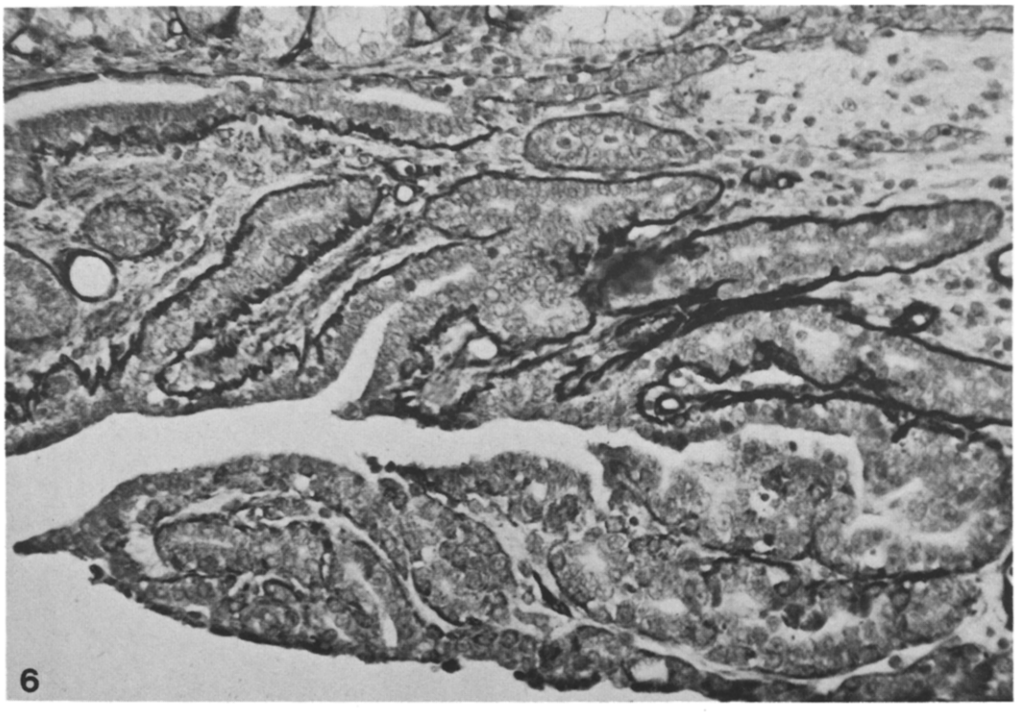


Fig. 5. *Human mammary adenocarcinomas transplanted to rat SRCA, showing preserved CEA production. CEA,* × 360.
Fig. 6. *A rat mammary adenocarcinoma transplanted to a rat SRCA, with prominent glandular structures and preserved basement membrane. Laminin,* × 180.

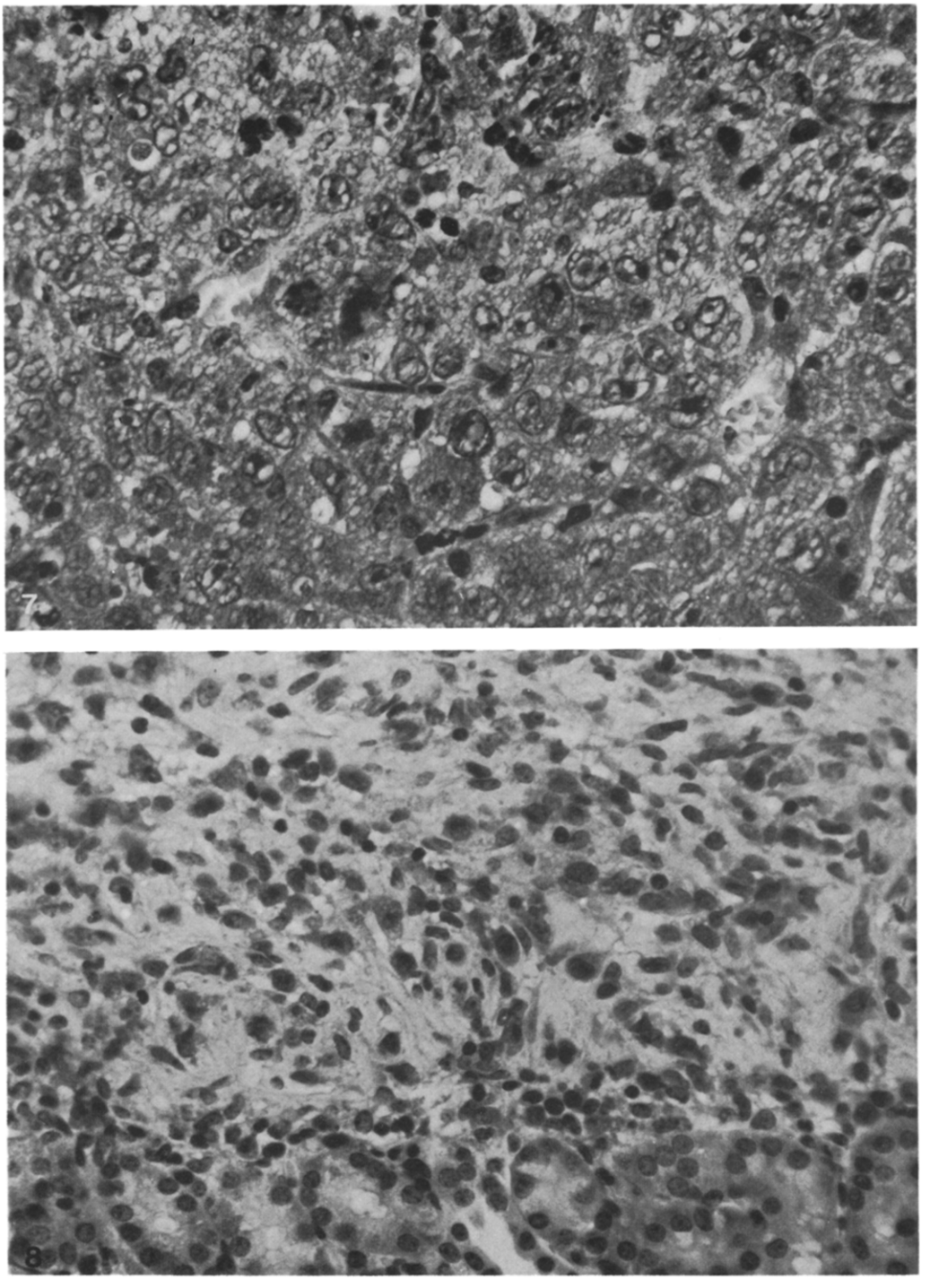
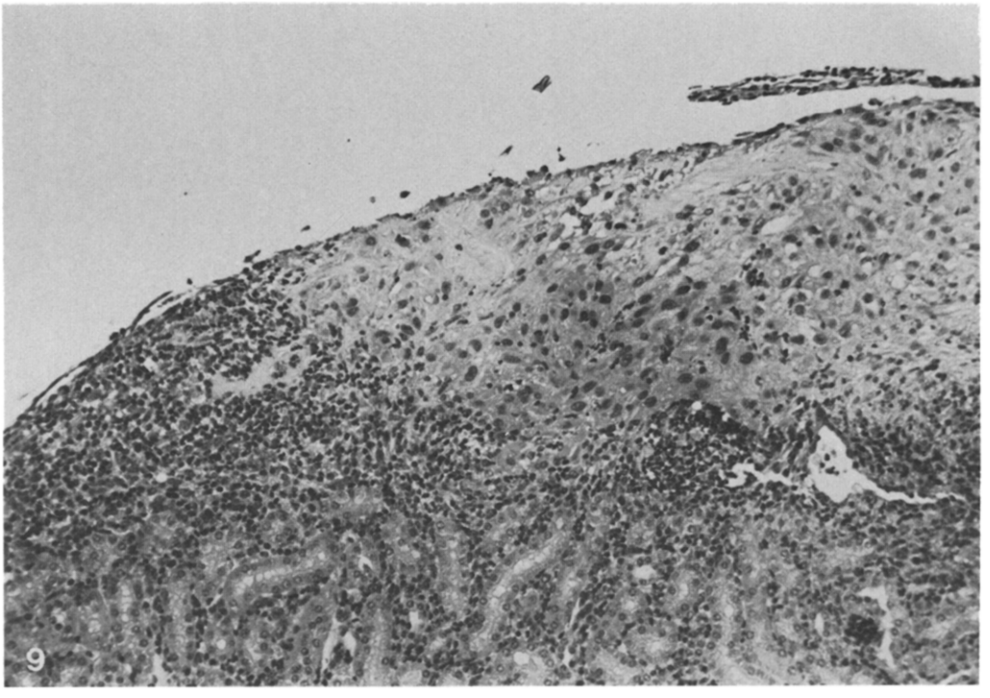


Fig. 7. A human ovarian adenocarcinoma transplanted to a mouse SRCA, with prominent cytological alterations, atypical nuclei and large nucleoli. HE, × 360.

Fig. 8. Carcinosis peritonei transplanted to mouse SRCA showing undifferentiated polymorphic cells. HE, × 360.



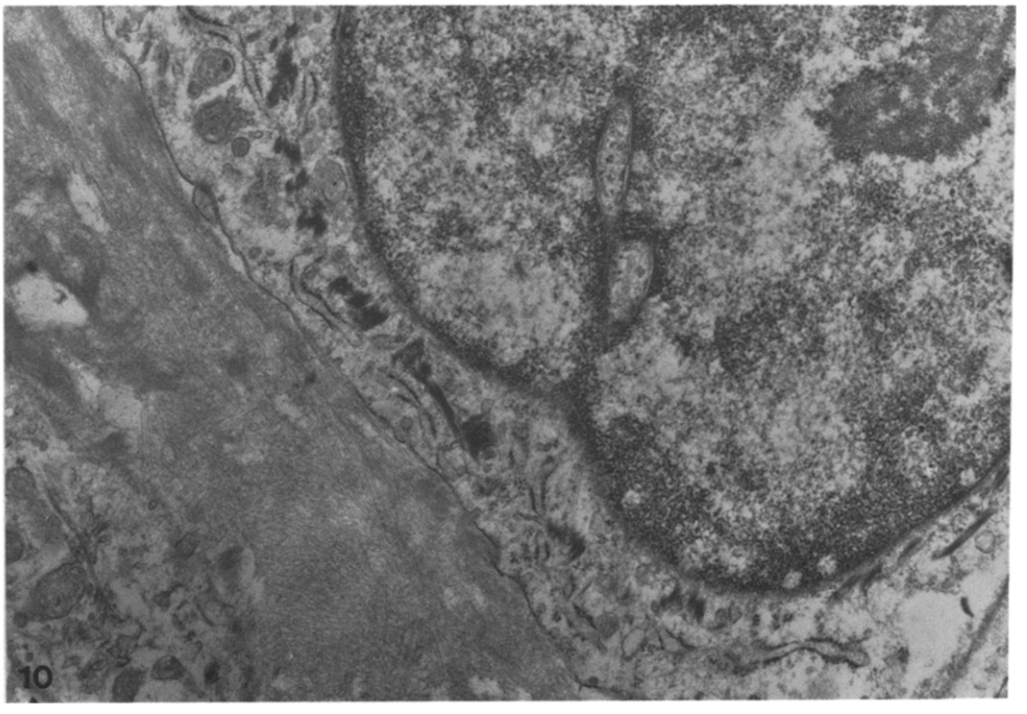
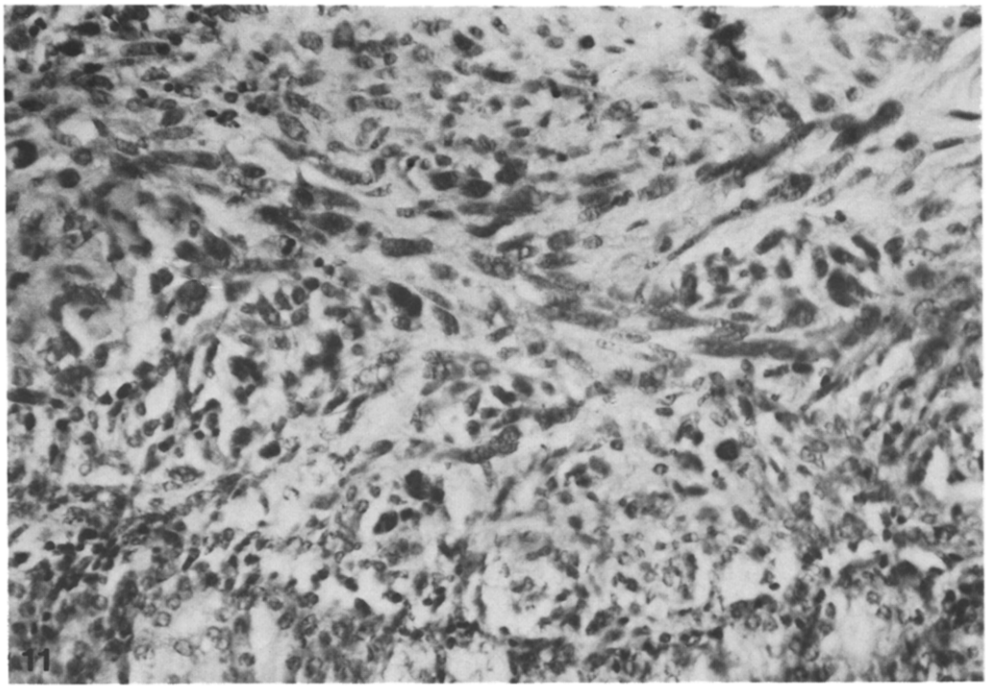


Fig. 9. Cervical squamous cell carcinoma in mouse SRCA with distinct cellular infiltrate adjacent to renal tubuli. HE, × 180.
Fig. 10. Cervical squamous carcinoma in mouse SRCA with keratohyalin granules and tonofilaments in the cytoplasm. Uranylacetate/lead citrate, × 10,000.



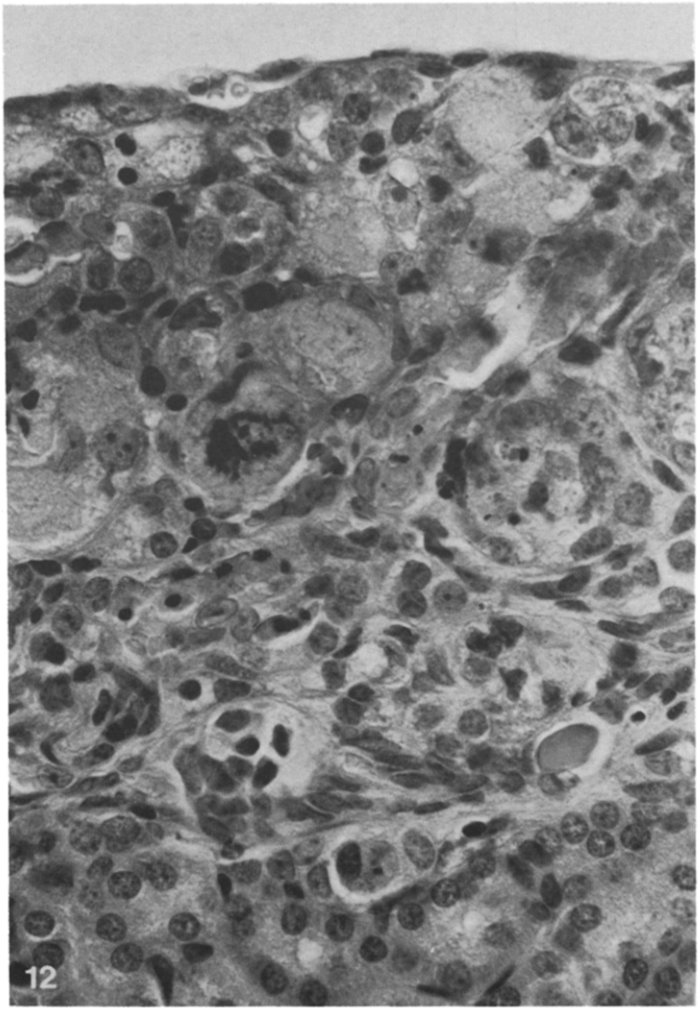
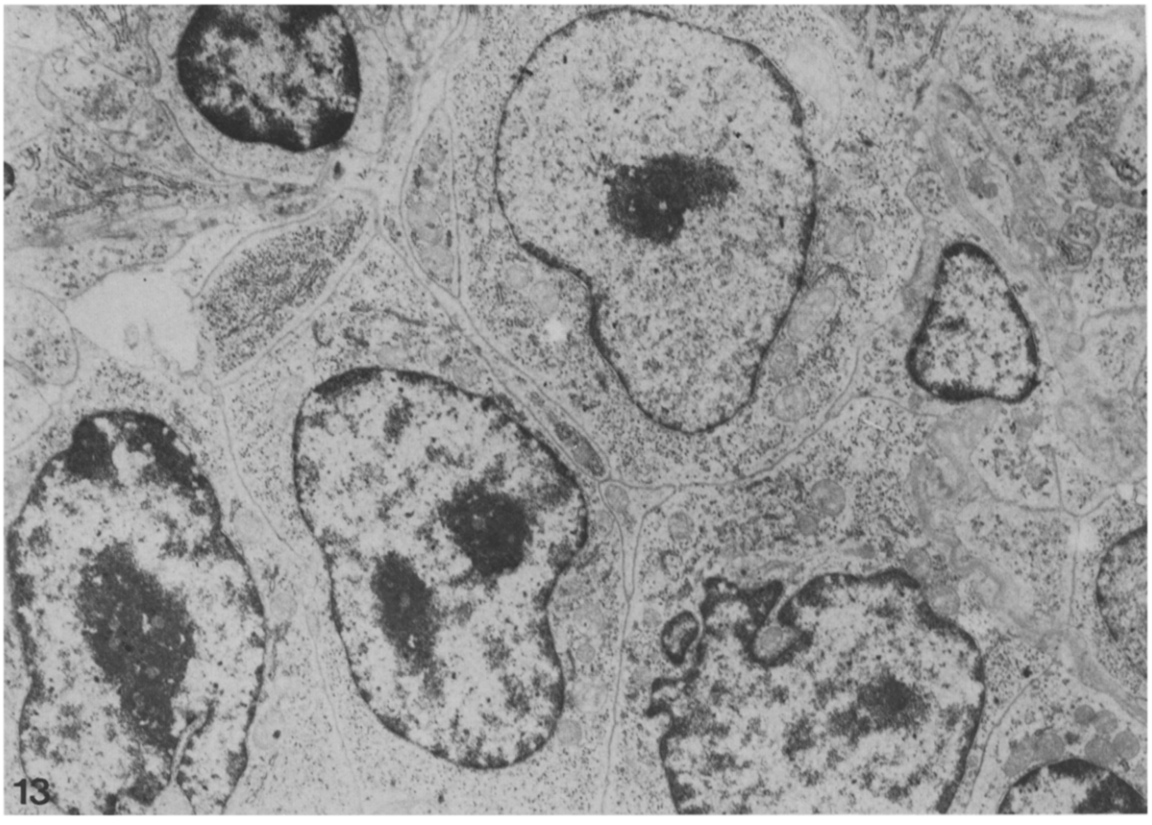


Fig. 11. *Malignant histocytoma of bone in mouse SRCA with elongated cells in collagenous stroma. HE,* × 360.
Fig. 12. *Ovarian adenocarcinoma in mouse SRCA treated with mitolaktol showing slight disorganization of mitotic figures, cytoplasmic homogenization and cell shrinkage. HE,* × 360.



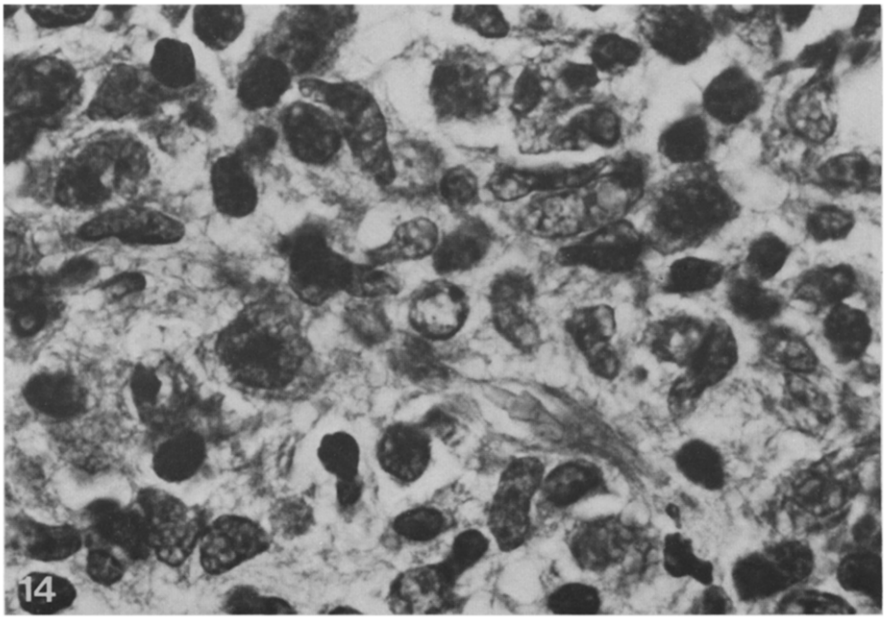
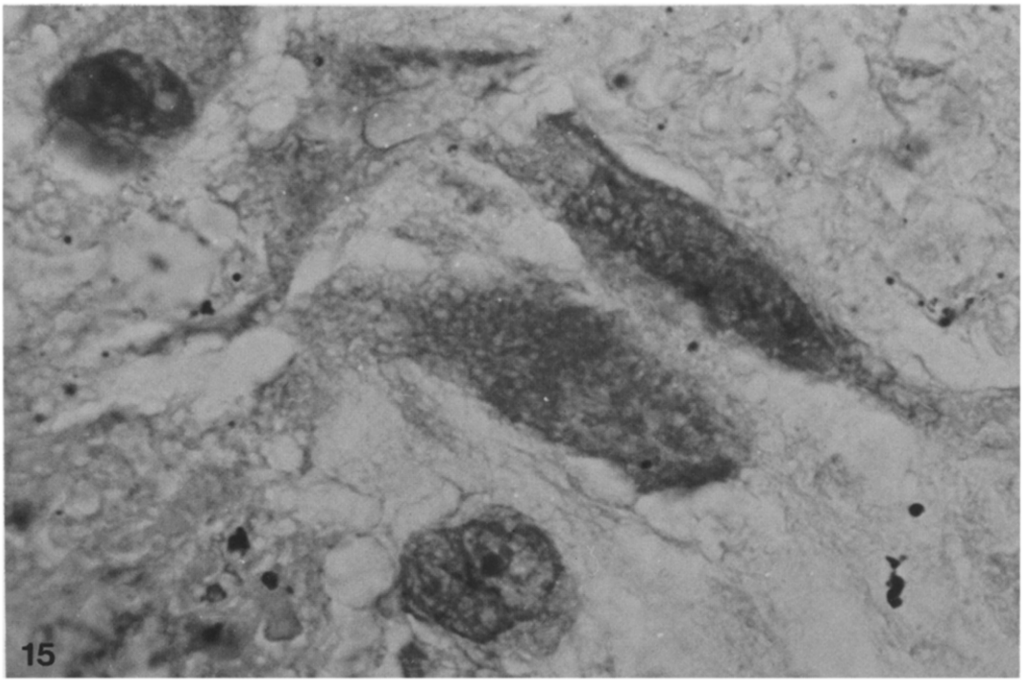


Fig. 13. *Mammary adenocarcinoma in mouse SRCA with tamoxifen treatment-induced changes. Uranylacetate/lead citrate,* × 2000.
Fig. 14. *Ovarian adenocarcinoma in mouse SRCA treated with adriamycin, cyclophosphamide and cisplatin showing nuclear disorganization, cytoplasmic vacuolation and cytolysis. HE,* × 360.



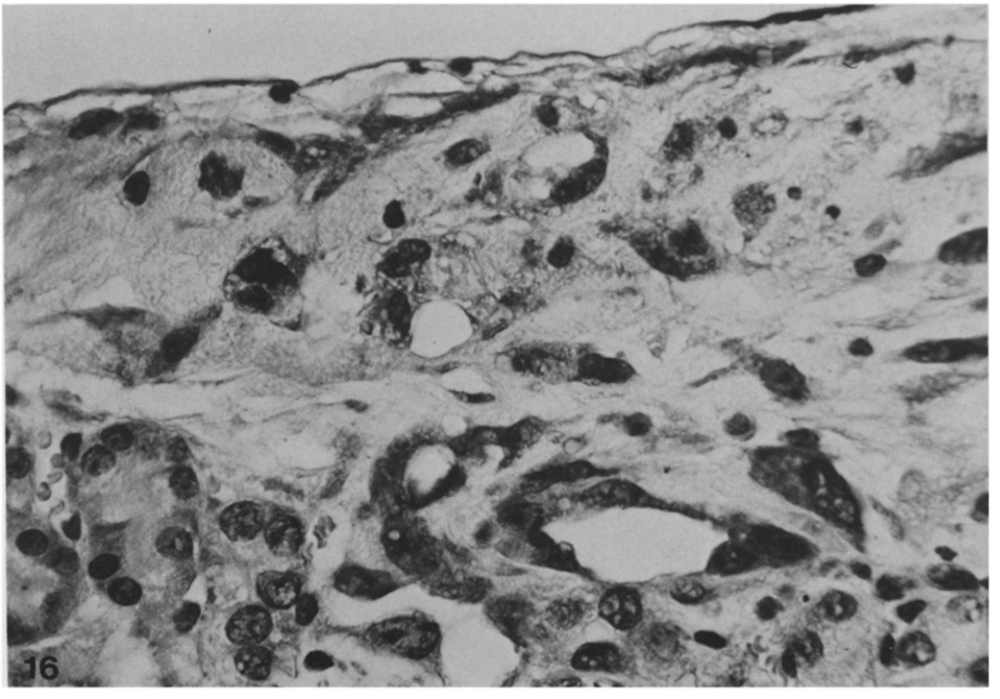
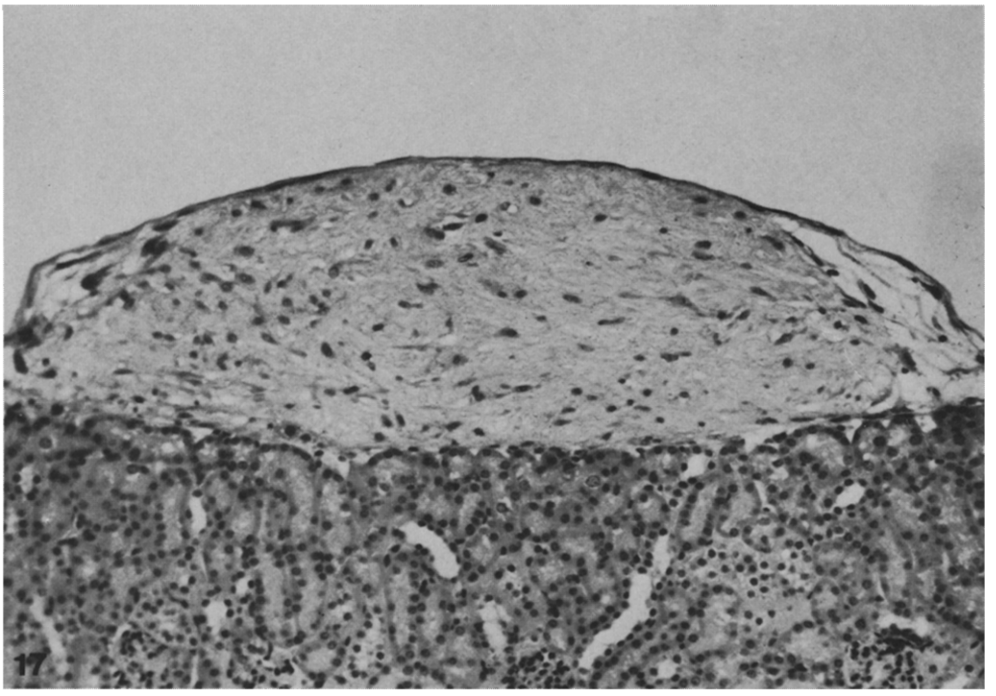


Fig. 15. *Ovarian adenocarcinoma in mouse SRCA treated with adriamycin, cyclophosphamide and cisplatin showing cellular disorganization, absence of nuclei and indistinct cell borders. HE,* × 750.

Fig. 16. *Preserved vascular structures in an ovarian adenocarcinoma in mouse SRCA treated with adriamycin, cyclophospamide and cisplatin, showing a scarcity of tumor tissue but distinct blood vessels. HE,* × 260.



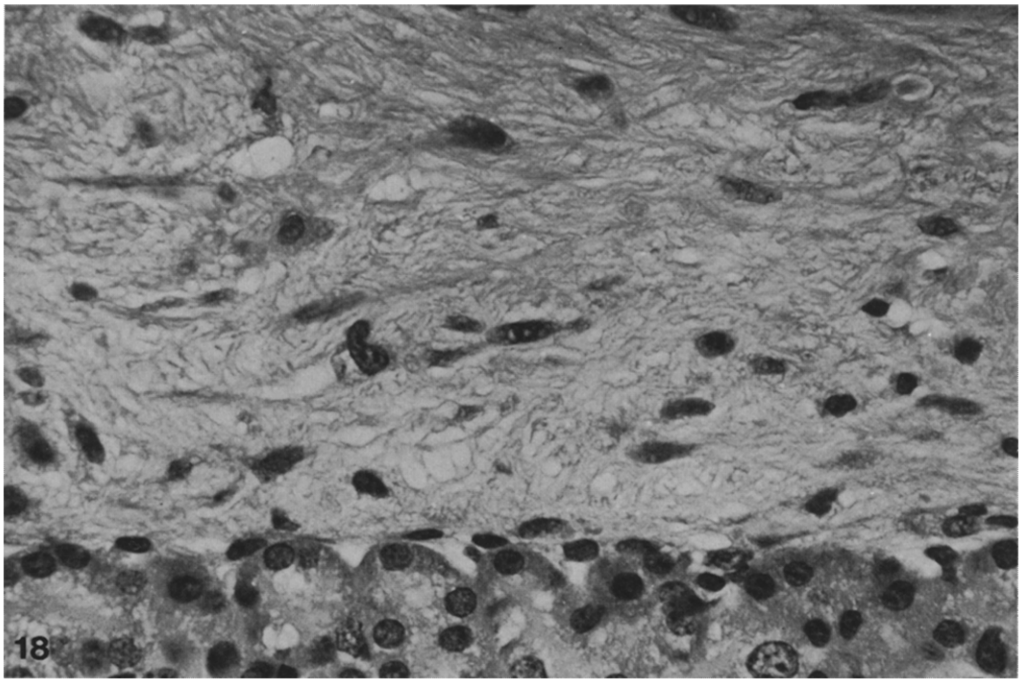


Fig. 17. *A fibrous scar as the only remnant of an ovarian carcinoma in mouse SRCA treated with adriamycin, cyclophosphamide amd cisplatin. HE,* × 180.

Fig. 18. *Minimal cellular activity in remnants of ovarian adenocarcinoma in mouse SRCA treated with adriamycin, cyclophosphamide and cisplatin. HE,* × 360.

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REFERENCES

1. Ovejera AA, Houchens DP. Human tumor xenografts in athymic nude mice as a preclinical screen for anticancer agents. *Semin Oncol* 1981, **8**, 386–393.
2. Giovanella BC, Stehlin JS Jr, Shephard RC, Williams LJ Jr. Correlation between response to chemotherapy of human tumors in patients and in nude mice. *Cancer* 1983, **52**, 1146–1152.
3. Giovanella BC., Stehlin JS Jr, Williams LJ Jr, Lee S, Shepard RC. Heterotransplantation of human cancers into nude mice. *Cancer* 1978, **42**, 2269–2281.
4. Ichihashi H, Kondo T, Sakakibara S, Akiyama S, Watanabe T. Application of radioactive precursors for the evaluation of sensitivity of cancer cells to anticancer drugs. *Oncology* 1984, **41**, 88.
5. Salmon SE, ed. *Cloning of Human Tumor Stem Cells*. New York, Alan R. Liss, 1980.
6. Weisenthal LM, Dill PL, Kurnick NB, Lippman ME. Comparison of dye exclusion assays with a clonogenic assay in the determination of drug-induced cytotoxicity. *Cancer Res* 1983, **43**, 258–264.
7. Kangas L, Grönroos M, Nieminen A.-L. Bioluminescence of cellular ATP: a new method for evaluating cytotoxic agents in vitro. *Med Biol* 1984, **62**, 338–343.
8. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumour to "nude" mice. *Acta Pathol Microbiol Scand* 1969, **77**, 758–760.
9. Sharkey FE, Fogh JM, Hadju SI, Fitzgerald PJ, Fogh J. Experience in surgical pathology with human tumor growth in the nude mouse. In: Fogh J, Giovanella BC, eds. *The Nude Mouse in Experimental and Clinical Research*. New York, Academic Press, 1978, 187–214.
10. Stanbridge EJ. Use and validity of tumorigenicity assays in immune-deficient animals. In *Immune-Deficient Animals. 4th International Workshop on Immune-deficient Animals in Experimental Research, Chexbres*, 1982. Basel, Karger, 1984, 196–208.
11. Shimosato Y, Kameya T, Hirohashi S. Growth, morphology and function of xenotransplanted human tumors. In: Sommers SC, Rosen PP, eds. *Pathology Annual*. New York, Appleton-Century-Crofts, 1979, Vol. 14, Part 2, 215–257.
12. Reid LM, Holland J, Jones C. *et al.* Some of the variables affecting the success of transplantation of human tumors into the athymic nude mice. In: Houchens DP, Ovejera AA, eds. *Proceedings of the Symposium on the Use of Athymic (Nude) Mice in Cancer Research*. New York, Gustav Fischer, 1978, 107–121.
13. Auerbach R, Morrissey LW, Sidky YA: Regional differences in the incidence and growth of mouse tumors following intradermal or subcutaneous inoculation. *Cancer Res* 1978, **38**, 1739–1744.
14. Hajdu SI, Lemos LB, Kozakewich H, Helson L, Beattie EJ. Growth pattern and differentiation of human soft tissue sarcomas in nude mice. *Cancer* 1981, **47**, 90–98.
15. Bogden AE, Haskell PM, LePage DJ, Kelton DE, Cobb WR, Esber HJ. Growth of human tumor xenografts implanted under the renal capsule of normal immunocomponent mice. *Exp Cell Biol* 1979, **47**, 281–293.
16. Bogden AE, Cobb WR, LePage DJ *et al.* Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse: sixday subrenal capsule assay. *Cancer* 1981, **48**, 10–20.
17. Marquet RL, Westbroek DL, Jeekel J. Interferon treatment of a transplantable rat colon adenocarcinoma: importance of tumor site. *Int J Cancer* 1984, **33**, 689–692.
18. Aamdal S, Fodstad O, Pihl A. Human tumor xenografts transplanted under the renal capsule of conventional mice. Growth rates and host immune response. *Int J Cancer* 1984, **34**, 725–730.
19. Aamdal S, Fodstad O, Kaalhus O, Pihl A. Reduced antineoplastic activity in mice of cisplatin administered with high salt concentration in the vehicle. *JNCI* 1984, **73**, 743–752.
20. Aamdal S, Fodstad O, Nesland JM, Pihl A. Characteristics of human tumour xenografts transplanted under the renal capsule of immunocomponent mice. *Br J Cancer* 1985, **51**, 347–356.
21. Dumont P, Van Der Esch, EP, Jabri M, Lejeune F, Atassi G. Chemosensitivity of human melanoma xenografts in immunocomponent mice and its histological evaluation. *Int J Cancer* 1984, **33**, 447–451.
22. Grönroos M, Mäenpää J, Söderström K-O, Kangas L, Leppänen M, Cantell K. Effects of alpha and gamma interferons and cytostatics on ovarian cancer in the subrenal capsule assay. *Med Biol* 1983, **61**, 275–279.
23. Stenbäck F, Wasenius V.-M, Kangas L. Morphology of transplanted tumors and drug induced regression in the subrenal capsule of mice and rats. *Ann Chir Gynaecol* 1985, **74**, (Suppl. 199), 31–37.
24. McDowell EM, Trump BF. Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Pathol Lab Med* 1976, **100**, 405–414.

25. Risteli J, Bächinger HP, Engel J, Furthmayr H, Timpl R. 17-S collagen: characterization of an unusual basement membrane structure. *Eur J Biochem* 1980, **108**, 239–250.
26. Risteli L, Timpl R. Isolation and characterization of pepsin fragments of laminin from human placental and renal basement membranes. *Biochemistry* 1981, **193,** 749–755.
27. Hsu S, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin–biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* 1981, **75**, 734–738.
28. Hunter RE, Reich SD, Griffin TW, Bogden AE. Responsiveness of gynecologic tumors to chemotherapeutic agents in the 6-day subrenal capsule assay. *Gynecol Oncol* 1982, **14**, 298–306.
29. McCormick KJ, Panje WR, Seltzer S, Merrick RH. Single agent chemotherapy for head and neck cancers. The murine subrenal capsule assay. *Arch Otolaryngol* 1983, **109**, 715–718.
30. Mäenpää J, Grönroos M, Leppänen M, Kangas L. The subrenal capsule assay in mice. Prediction of rat tumor sensitivity to chemotherapy. *Cancer* 1984, **54**, 1530–1534.
31. Peehl DM, Stanbridge EJ. The role of differentiation in the control of tumorigenic expression in human cell hybrids. *Int J Cancer* 1982, **30**, 113–120.
32. Weizsäcker M, Nagamune A, Rathmer K, Wechsler W. Brain tumor growth and response to chemotherapy in the subrenal capsule assay. *J Cancer Res Clin Oncol* 1983, **106**, 229–233.
33. Fingert HJ, Treiman A, Pardee AB. Transplantation of human or rodent tumors into cyclosporine-treated mice: a feasible model for studies of tumor biology and chemotherapy. *Proc Natl Acad Sci USA* 1984, **81**, 7927–7931.
34. Levi FA, Blum JP, Lemaigre G, Bourut C, Reinberg A, Mathé G. A four-day subrenal capsule assay for testing the effectiveness of anticancer drugs against human tumors. *Cancer Res* 1984, **44**, 2660–2667.
35. Edelstein MB, Fiebig HH, Smink T, van Putten LM, Schuchhardt C. Comparison between macroscopic and microscopic evaluation of tumour responsiveness using the subrenal capsule assay. *Eur J Cancer Clin Oncol* 1983, **19**, 995–1009.
36. Dexter DL, Matook GM, Meitner PA *et al.* Establishment and characterization of two human pancreatic cancer cell lines tumorigenic in athymic mice. *Cancer Res* 1982, **42**, 2705–2714.
37. Thompson DMP, Neville AM, Phelan K, Scanzano RS, Vandevoorde JP. Human cancers transplanted in nude mice retain the expression of their organ-specific neoantigens. *Eur J Cancer Clin Oncol* 1981, **17**, 1191–1197.
38. Gold DV, Shochat D, Primus FJ, Dexter DL, Calabresi P, Goldenberg DM. Differential expression of tumor-associated antigens in human colon carcinomas xenografted into nude mice. *JNCI* 1983, **7**, 117–124.
39. Povlsen CO, Rygaard J. Growth of human tumors in the nude mouse. In: Bloom BB, David JR, eds. In Vitro *Methods in Cell-mediated and Tumor Immunity*. New York, Academic Press, 1976, 701–711.
40. Povlsen C, Rygaard J, Fogh J. Long-term growth of human tumors in nude mice: Evaluation of stability. In: Fogh J, Giovanella BC, eds. *The Nude Mouse in Experimental and Clinical Research*. New York, Academic Press, 1982, Vol. 2, 79–93.
41. Sharkey FE, Fogh J. Considerations in the use of nude mice for cancer research. *Cancer Metast Rev* 1984, **3**, 341–360.
42. Horten BC, Basler GA, Shapiro WR. Xenograft of human malignant glial tumors into brains of nude mice: a histological study. *J Neuropathol Exp Neurol* 1981, **40**, 493–511.
43. Houchens DP, Ovejera AA, eds. *Proceedings of the Symposium on the Use of Athymic (Nude) Mice in Cancer Research*. New York, Gustav Fischer, 1978.
44. Kyriazis AA, Kyriazis AP, Kereiakes JG, Soloway MS, McCombs WB. Histopathologic evaluation of response to treatment of human tumors grown in the nude mouse. *Exp Cell Biol* 1983, **51**, 83–95.
45. Söderström K-O, Mäenpää J, Cantell K, Kangas L, Grönroos M. Ultrastructural effects of alpha and gamma interferons and cytostatics on ovarian cancer cells in the subrenal capsule assay. *Ann Chir Gynaecol* 1985, **74**, (Suppl. 199), 38–43.