Cystosarcoma Phylloides

A Steroid Receptor and Ultrastructure Analysis

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Six cases of cystosarcoma phylloides were evaluated by ultrastructure and steroid receptor analysis. Electron microscopy of the lesions supported previous reports of a heterogeneous tumor consisting of pleomorphic mesenchyme and normal or proliferative epithelium. In each case estrogen and progesterone receptor analysis indicated the presence of a nonsaturable estrogen and progesterone 4S binding protein rather than a specific steroid receptor as suggested by previous studies.

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CYSTOSARCOMA PHYLLOIDES is an uncommon fibroepithelial lesion which constitutes 0.5% of breast tumors.[12] While surgical extirpation is often effective therapy for this lesion,[16,17] local recurrence is not unusual. Effective treatment for metastasizing cystosarcoma phylloides has not yet been described. Cystosarcomas have been classified histologically as malignant in up to 27% of cases. In 12% of cystosarcomas, extensive invasion of adjacent tissues or metastasis are observed.[2]

Clinical treatment of multiple recurrent or metastasizing cystosarcoma has included surgical management, chemotherapy, radiotherapy, and hormonal manipulation.[6,8] While short-term remission has been described in one patient treated with Endoxan,[3] no other convincing reports of objective response to systemic therapy have been made. The suggestion of Rao[10] that selected cystosarcoma phylloides tumors might demonstrate hormonal responsiveness has prompted us to prospectively examine six cases of cystosarcoma for their steroid hormone receptor content.

Materials and Methods

Immediately upon tumor resection, the specimens were placed at 4° and representative areas were taken for electron microscopy and receptor analysis. The remaining tissue was sectioned at 3 mm intervals and fixed in 0.1M phosphate buffered 4% formalin (pH 7.2).

The EM specimens were minced into 0.5 mm^3 pieces and fixed in 4% glutaraldehyde, .1M cacodylate buffer (pH 7.4) for four hours. After postfixation in a 2% OsO_4, .1M collodine solution at 4° for 60 minutes, dehydration was accomplished in serial alcohols ending

TABLE 1. *Patient Characteristics*

	Patient	Age	Tumor Status	Tumor Location	Histologic Grade
1.	AS	53	Metastatic	R. breast, chest, lung	III
2.	ED	58	Primary	R. breast	I
3.	VP	67	Primary	R. breast	I
4.	KH	56	Recurrent	L. breast, L. axilla	II
5.	PY	58	Primary	L. breast	I
5a.*	PY	59	Metastatic	Lung	III
6.	MW	15	Primary	R. breast	III

* A recurrent lesion from patient #5.

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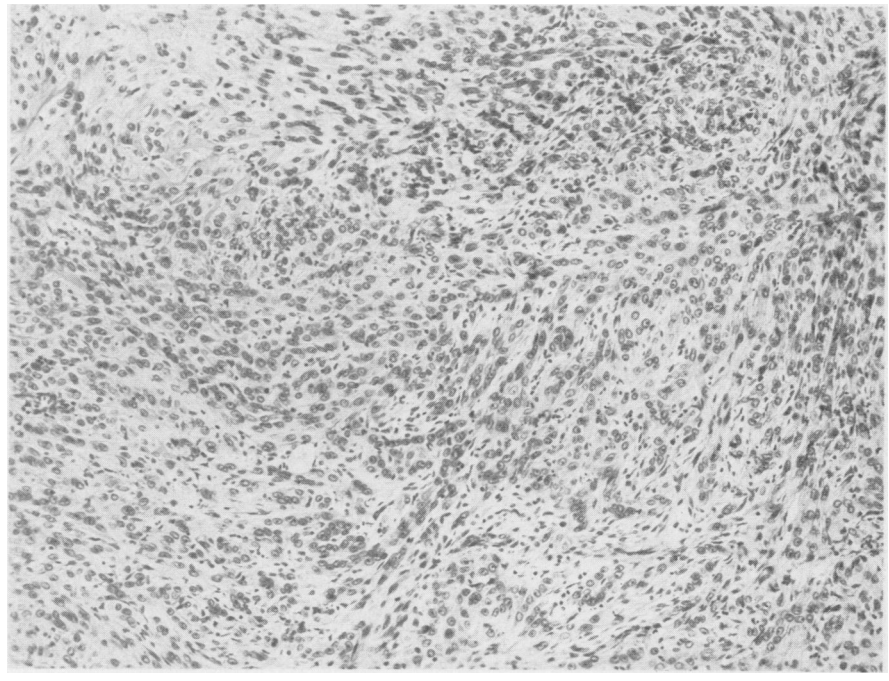

FIG. 1. Cystosarcoma phylloides histologic grade III. Markedly cellular mesenchymal tumor with relatively plump, pleomorphic, proliferative stromal elements is seen (×100).

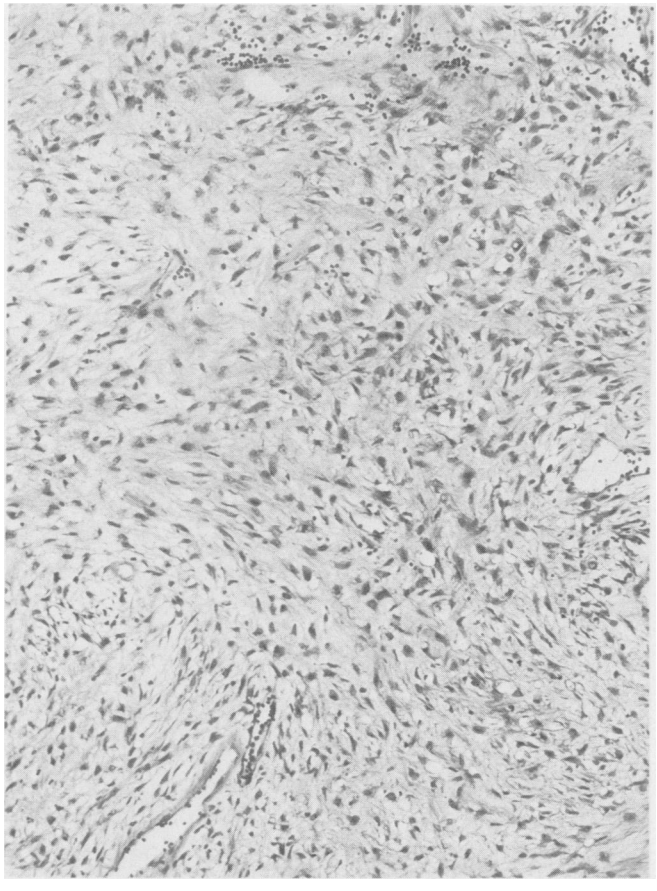

FIG. 2. Cystosarcoma phylloides histologic grade II. The proliferative mesenchyme assumes the more typical spindle configuration of the fibroblast (×100).

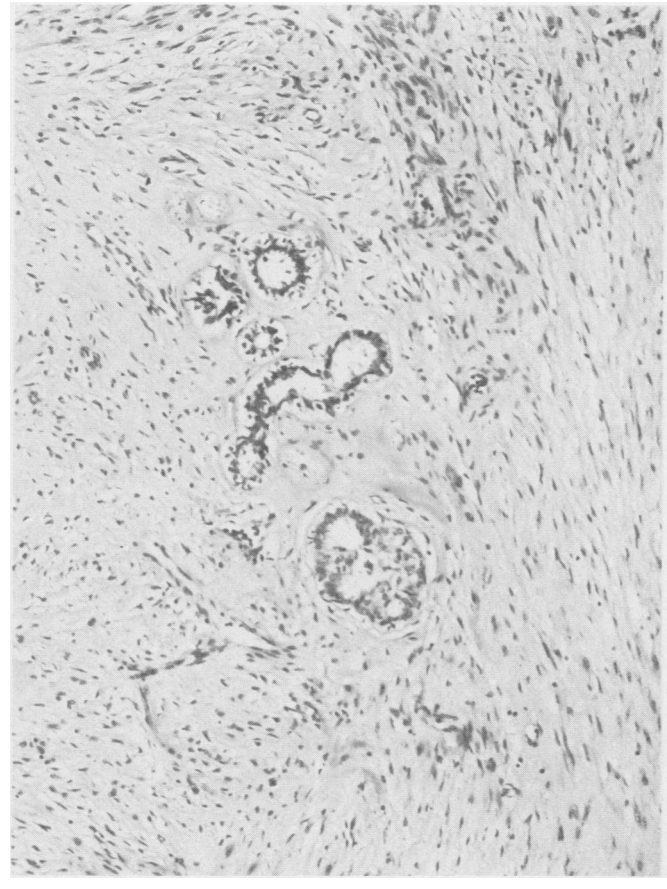

FIG. 3. Cystosarcoma phylloides histologic grade I. The lesion is noted to contain more regular appearing mesenchymal elements (×100).

with propylene oxide. The specimens (a minimum of 10 blocks/case) were embedded in Epon 812, sectioned at a thickness of one micron and stained with methylene blue. After initial study, selected blocks were sectioned at 200–250Å. The final sections were stained with lead citrate and uranyl acetate, carbon coated and examined with an Hitashi Hu-11E electron microscope at 75Kv.

The fragments of tissue submitted for receptor analysis were trimmed of fat and normal breast tissue, washed in 5 mM Tris HCl, 5 mM HEPES-HCl, 1.5 mM methylenediaminetetracetate. 0.5 mM dithiothreitol, pH 7.4 at 4° and quick frozen in liquid nitrogen. Cryostat sections for histologic evaluation were prepared from the specimens for confirmation of the presence of cystosarcoma phylloides in the tissue actually analyzed.

Receptor Analyses

Estrogen and progesterone receptor content of the tumors was analyzed using modifications of the methods previously described.[5] The difference between receptor levels of fresh tissues and tissues frozen in liquid nitrogen has been previously shown to be less than 5%. The frozen tissues were pulverized in liquid nitrogen using a Spex freezer mill at ¾ power with a stainless steel impeller, for 15 seconds (five 3 second cycles). The tissue powder was homogenized at a sample to buffer volume 1:4 at 4° using a Polytron (setting 3) for 60 seconds (four 15 second cycles with a 30 second cooling period). Cytosol was prepared from the homogenate by centrifugation at 1000 g × 10 minutes followed by 145,000 g × 1 hour (4°). Endogenous unbound steroid was removed by 0.75% w/v Norit A in 0.0025% Dextran. The supernatant protein content was analyzed by the coomassie dye method[1] and adjusted to 4–6 mg protein/ml. Optimal conditions of incubation for quantitation of estrogen and progesterone receptor by DCCA (dextran coated charcoal assay) and SDGA (sucrose density gradient analysis) were determined by methods as described by Schrader et al.[13] and were used in incubations as described below.

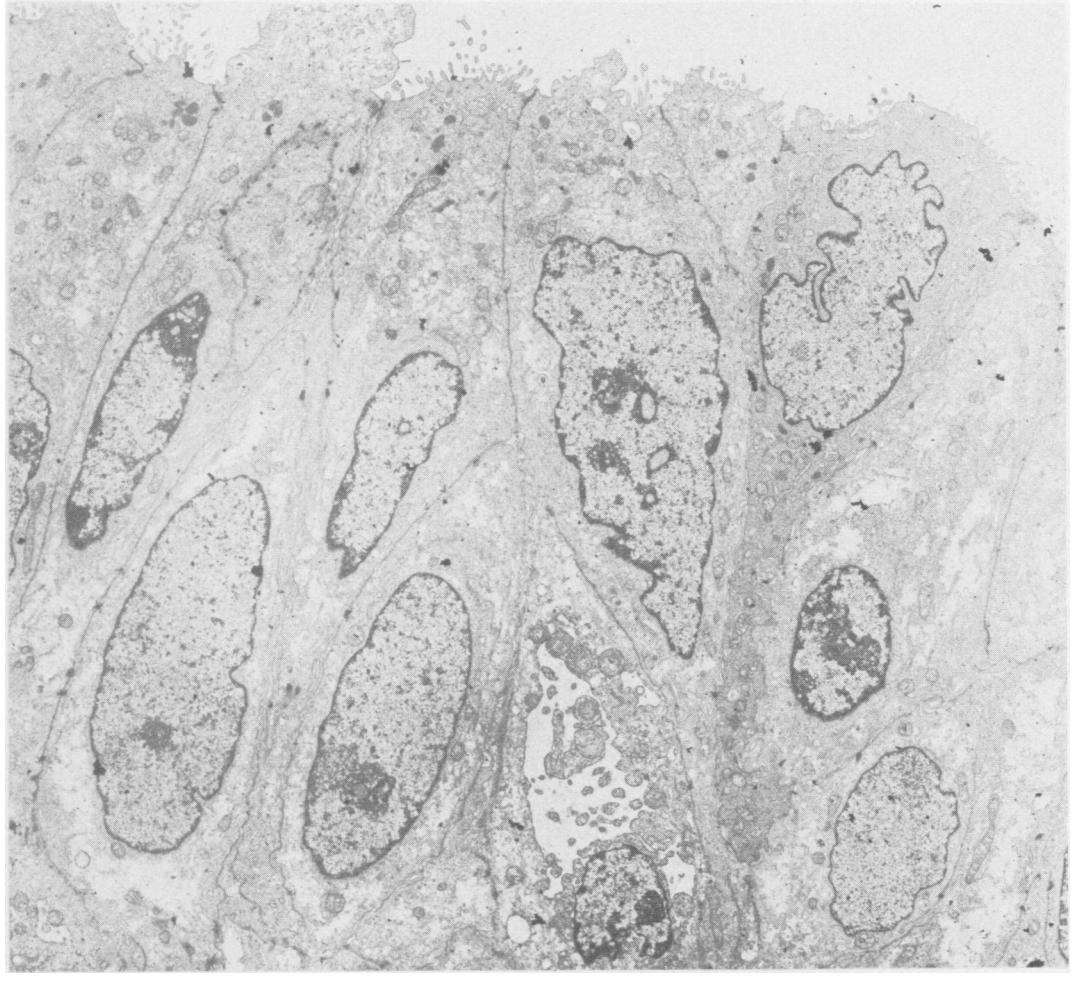

FIG. 4. Columnar cells containing dilated endoplasmic reticulum, glycogen, basilar nuclei, and junctional complexes consistent with proliferative mammary epithelium are observed (×10,500).

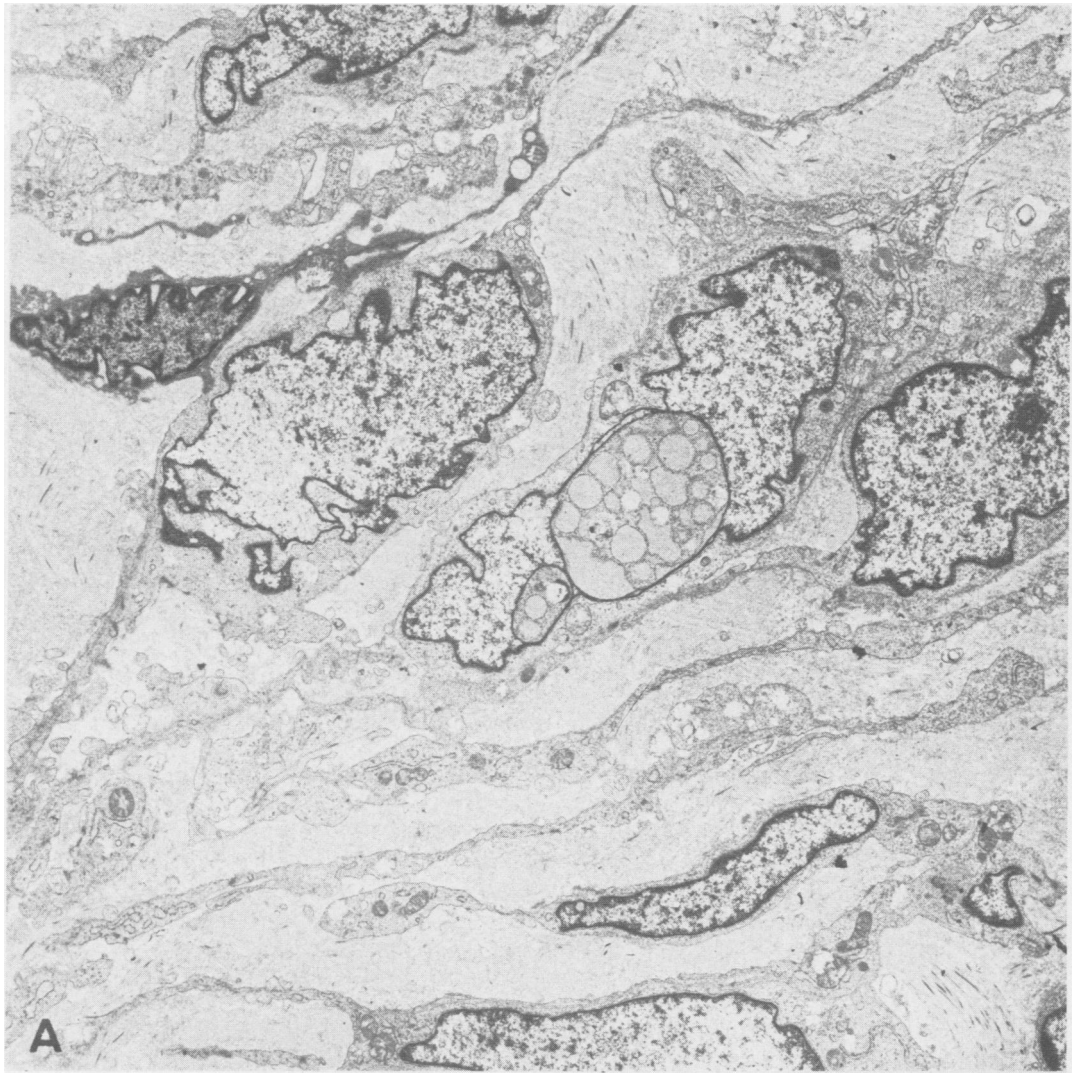

FIG. 5A. Ultrastructure of mesenchymal elements, cystosarcoma phylloides. The bizarre and varied forms of the mesenchymal cells are shown; note the nuclear pseudoinclusions (×21,000).

Gradient Analyses (SDGA)

Aliquots of 200 μl of supernatant were incubated for four hours at 4° with 1.6 pmoles ^{3}H hexalabelled estradiol (New England Nuclear, Specific Activity 152 curies/mmole; >99% purity confirmed by thin layer silica gel chromatography; chloroform:acetone; 70:30 and formamide paper reverse phase chromatography, benzene:chloroform; 4:1 containing 100-fold excess of cold testosterone), or were incubated with 3.2 pmoles of the progesterone analogue ^{3}H R5020 (New England Nuclear, specific activity 86 Curies/mmole; purity verified by thin layer chromatography), with 100-fold excess of cold cortisol. The effectiveness of the progesterone analogue R5020 in demonstrating specific progesterone receptors has been previously shown.[9] Parallel control incubations also contained 250-fold excess of cold hormone (estradiol and progesterone respectively) in addition to the radioactive hormone. Two hundred microliters were layered on 10–28.5% isokinetic sucrose gradients and centrifuged to an ω^2t of 157,417 in an SW60 Beckman rotor at 4° (polyallomer tubes). Fractionation of gradients was by upward displacement with 75% glycerol into 40 fractions. Radioactivity was determined in an LS-4000 Intertechnique liquid scintillation counter set for tritium using 10 ml Biofluor (New England Nuclear). Counting efficiency was determined by external standardization used for computer calculations. Sedimentation coefficients were determined by the use of C^{14} labelled bovine serum albumin (4.6S) or γ globulin (7S) standards.

Dextran Coated Charcoal Analysis (DCCA)

The cytosol was diluted to 1–2 mg protein/ml and 200 μl aliquots incubated for 16 hours at 4° in eight Kahn tubes containing from 1.6–0.0125 pmoles estradiol or 3.2–0.025 pmoles R5020 containing 100 fold concentration of cold cortisol (for progesterone DCCA)

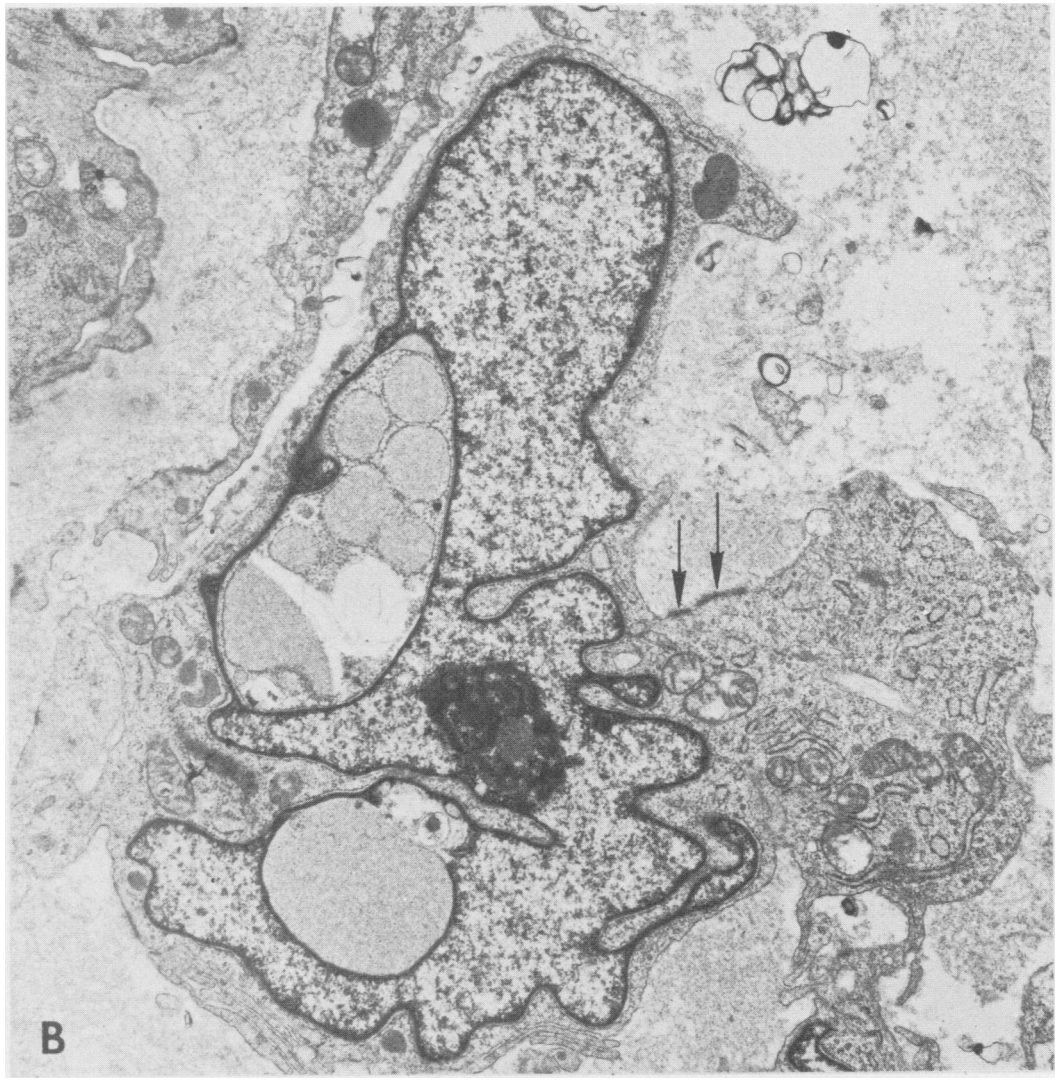

FIG. 5B. Mesenchymal cell displaying large pleomorphic nuclei with pseudoinclusions, deep invaginations, and prominent nucleoli; note also the stromal junction (arrow) (×21,000).

or dihydrotestosterone (for estrogen DCCA). Parallel control incubations at each concentration contained 250-fold excess of cold progesterone or diethylstilbestrol, added immediately prior to the radioactive steroid. Nonprotein bound free steroid was removed by 0.75% w/v Norit A (dextran treated) and bound radioactivity (not removed by the charcoal) determined. Calculations of K_d (dissociation constant) and bound receptor were made by the method of Woosley and Muldoon[18] and Scatchard[14] using an online LEM computer in an LS-4000 Intertechnique liquid scintillation counter.

Results

The study group consisted of six female patients ranging in age from 15 years to 67 years (Table 1). The mean age of the group was 51.2 years. There were four primary tumors, one was a locally recurrent tumor and one was a metastatic cystosarcoma. One patient's tumor was analyzed both as a primary and later as a metastatic lesion (Table 1). Using the histologic grading criteria of McDivitt,[7] three tumors were grade 3 (Fig. 1), one was grade 2 (Fig. 2), and three were grade 1 (Fig. 3).

The ultrastructural features of the various grades were consistent with those previously reported.[2,15] The glandular epithelium, when present, was characteristic of proliferative mammary epithelium (*e.g.*, typical junctional complexes, basilar nuclei with prominent nucleoli, microvilli, and apical changes consistent with normal apocrine epithelium) (Fig. 4). The stromal elements constituted the majority of the tumor tissue with marked mesenchymal pleomorphism. Many varied configurations of mesenchymal elements were seen within the same tumor specimen (Fig. 5A). Relatively normal fibroblasts with characteristic vesicular cytoplasm and typical nuclei were sometimes

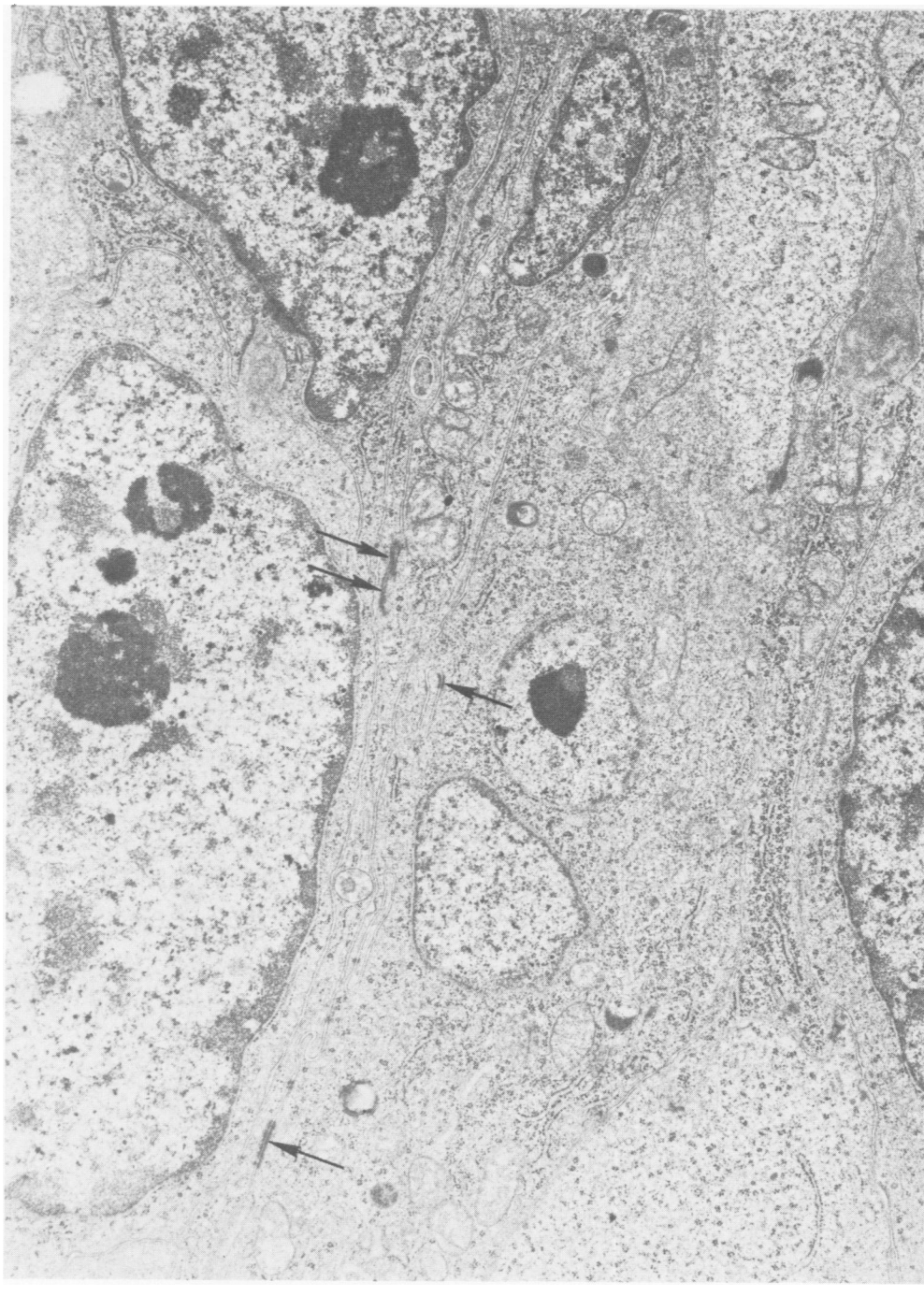

FIG. 6. Intercellular junctional complexes are observed between tumor cells (arrows) which resemble tight junctions (×21,000).

found adjacent to bizarre stromal cell types with high nuclear/cytoplasmic ratios, deeply invaginated nuclei with pseudoinclusions and prominent nucleoli (Fig. 5B). Intercellular junctions were observed connecting these anaplastic mesenchymal elements, resembling the tight junctions described by Fernandez (Fig. 6).[2] In this study, the primary lesions tended to contain the more bizarre stromal forms. The ultrastructure did not link the degree of cellular pleomorphism to more aggressive clinical behavior.

Significant levels of *nonsaturable* estrogen and progesterone binding were observed in all tumors analyzed by the multiconcentration saturation technique (DCCA) and gradient analysis. The binding protein sedimented as a 4S band on isokinetic sucrose density gradients. This is in accord with the binding protein reported by Rao.[10] Estrogen binding was observed with a mean binding of 31.07 fm/mg protein with a range of 6.60 fm/mg to 47.39 fm/mg (Table 2). The mean progesterone bound was 109.08 fm/mg pro-

TABLE 2. *Estrogen and Progesterone Binding of Tumor Cytosols*

Patient	Age	Estrogen Bound (fm/mg protein)	Inhibited × 100%† / Uninhibited	Prog. Bound (fm/mg protein)	Inhibited × 100%‡ / Uninhibited	E_2/P
1. AS	53	6.60	>99	59.62	>99	0.11
2. ED	58	47.39	95	114.79	>99	0.41
3. VP	67	14.09	>99	29.59	>99	0.48
4. KH	56	33.31	>99	79.63	>99	0.42
5. PY	58	46.16	>99	238.13	>99	0.19
5a.* PY	59	47.80	>99	252.00	98	0.19
6. MW	15	38.84	>99	132.74	>99	0.29
mean	51.17	31.07		109.08		0.32
range	15 → 67	6.6 → 47.39		29.59 → 238.13		0.11 → 0.48

* A recurrent lesion from patient #5; analyses conducted one year after analyses of primary. The data was omitted from computation of means and ranges.

† Per cent of binding observed after preincubation with non-radioactive diethylstilbestrol indicating non-saturability.

‡ Per cent of binding observed after preincubation with non-radioactive R5020 indicating non-saturability.

tein with a range of 29.59 fm/mg to 238.13 fm/mg (Table 2). A ratio of bound estrogen to bound progesterone was calculated for each case and yielded a mean value of .32 and a range of 0.11–0.48 (Table 2). In no case was *specific inhibitable* estrogen or progesterone receptor protein observed.

Discussion

Despite the report of a specific progesterone receptor in a single case of recurrent cystosarcoma phylloides,[10] clinical evidence of hormonal responsiveness is lacking.[6,8] The absence of any reported examples of successful hormonal therapy and the report of Lester and Stout of recurrent malignant nodules remaining unaltered through at least one full term pregnancy,[4] contradict an hypothesis of specific steroid hormonal reactivity.

Assays for estrogen and progesterone binding capacity performed on specimens from six patients with cystosarcoma phylloides gave similar results. In each case, *nonsaturable* 4S estrogen binding (Fig. 7A) was observed despite the presence of excess unlabelled dihydrotestosterone to inhibit testosterone–estrogen binding globulin contribution to total binding. When parallel analysis of the tumor cytosol was carried out utilizing radiolabeled R5020 (or progesterone) in the presence of excess cortisol a 4S band was also observed (Fig. 7B). The patterns of binding were similar to the estrogen binding assays with the exception of an amplified net binding of this progesterone analogue. The similar slopes of the inhibited and uninhibited gradients confirm the data obtained by the multiconcentration saturation analysis which indicated that the binding protein is *nonsaturable*. These observations suggest that the observed binding is due to a nonspecific, nonsaturable steroid binding protein rather than a specific progesterone (or estrogen) receptor as previously suggested.[10,11] Electron microscopy of the lesions studied indicate the predominant proliferative element to be mesenchyme derived cells as has been reported by Fernandez[2] and Toker.[15] These data taken together suggest a low probability of this tumor being responsive to estrogen or progesterone manipulation, in agreement with clinical observations of cystosarcoma phylloides.[6,8]

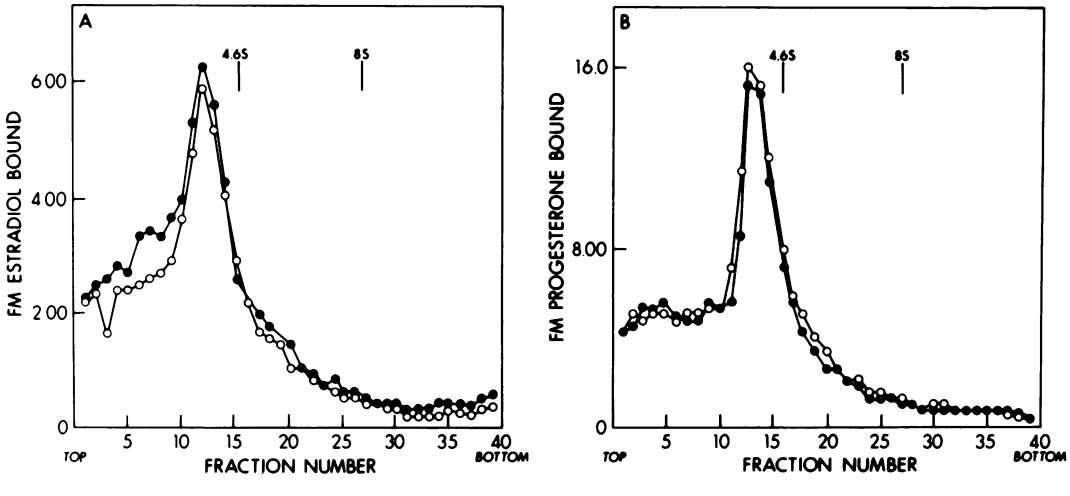

FIG. 7A and B. Estrogen and progesterone receptor analysis by sucrose density gradient technique. This pattern of estrogen and progesterone binding (Case #5) was typical of all six cases. There is a distinct 4S binding (open circles) which is not inhibited by 250-fold excess of cold hormone (closed circles). Note the relatively higher net binding of progesterone. No evidence of saturable steroid binding is seen.

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