ABSTRACT

The presence of an estrogen-regulated protein with 24,000 molecular weight has been studied in 47 patients with endometrial carcinomas and in 29 patients with cervical carcinomas in order to correlate its presence with that of estrogen receptors (ERs) and progesterone receptors (PgRs). In the cytosol tumor samples the M, 24,000 protein was detected by the Western blot technique using a monoclonal antibody (C11), while the presence of ER and PgR was studied by the one-point dextran-coated charcoal assay. In the tumor tissue sections immunohistochemistry was applied to detect M, 24,000 protein, ER, and PgR; in these cases monoclonal antireceptor antibodies (H222 and ml'KI) were used to localize the receptor proteins. In endometrial and endocervical adenocarcinomas the presence of M, 24,000 protein correlated significantly with that of ER (P ≤ 0.05) in the cytosol samples; when the evaluation was performed in the tumor sections, the presence of M, 24,000 protein correlated with that of ER (P ≤ 0.005) and PgR (P ≤ 0.05) as well. The study also showed that almost 70% of the well-differentiated adenocarcinomas had ER, PgR, and M, 24,000 protein. In 25% of the endometrial adenocarcinomas examined the tumors were associated with normal, proliferative, and hyperplastic endometrium; in these cases the presence of ER, PgR, and M, 24,000 protein was evaluated by immunohistochemistry in the malignant and nonmalignant endometrium.

On the other hand, there was a lack of correlation between M, 24,000 protein, ER, and PgR in the squamous carcinomas of the uterine cervix and in the endometrial adenocarcinomas with squamous cells. In most of these cases the tumors lacked ER and PgR although 80% of them contained the M, 24,000 protein to a variable degree. It is suggested that M, 24,000 protein is involved in growth and differentiation (the M, 24,000 protein is a heat shock protein) and that the gene coding of this protein is under hormonal control only in those tissues where growth and differentiation are strongly hormonally controlled (breast and endo-

INTRODUCTION

It is generally accepted that the presence of ER* in human breast cancer is useful for selecting patients for endocrine therapy and to predict the prognosis of the disease (1, 2). However, the interaction between the hormone and the receptor is one of the first steps in explaining the mechanism of estrogen action, and the presence of ER alone does not assure complete hormonal responsiveness of the tumor (3). There are many biochemical and physiological events after the binding of the hormone to its receptor, and for the exploration of these events it may be useful to know if the tumor remains responsive to the steroid at postreceptor level. One way to explore functional ER is achieved by measuring in the tumor the presence of PgR since they are generally up-regulated by estradiol (4), but PgR may also be constitutively present in human breast tumor cells (5). Another possibility is to measure the amount of PgR after a short period of tamoxifen treatment, since this antiestrogen may induce PgR synthesis (6). Recently, a nuclear binding assay has been introduced to assess biologically active receptors able to bind to nuclear acceptor sites (7). Additional markers of hormone action at postreceptor level are some estrogen-regulated proteins and a tamoxifen-induced protein (8, 9). One of the estrogen-regulated proteins under study is that found in the cytoplasm of the human breast cancer cell line MCF-7, characterized by its molecular weight of 24,000 (10). This protein has also been termed 28K in previous publications since this has been the reported molecular weight after its purification by monoclonal antibody affinity chromatography (11). The function of the M, 24,000 protein is unknown but it has been identified as a heat shock protein (12). Immunohistochemical localization of the M, 24,000 protein using monoclonal antibodies showed that it is mainly present in the female reproductive tract, and in breast tumor cell lines with ER and PgR (13). Elevated presence of M, 24,000 protein was found to correlate well with presence of ER and PgR in human breast tumor biopsy samples (14).

We have reported that in the human normal endometrium M, 24,000 estrogen-regulated protein undergoes quantitative differences in the various phases of the normal menstrual cycle (15). In women with abnormal endometrium, this protein appeared with maximum immunostaining in active forms of persistent proliferative endometria and in cystic glandular hyperplasia and decreased in adenomatous and atypical hyperplasia (16), and in malignant endometrium its presence was correlated with the degree of tumor differentiation (16, 17). In addition, the M, 24,000 protein has also been found in normal and abnormal samples from uterine cervix (18, 19). In the ectocervix the protein was detected in the parabasal and intermediate cell layers, with no prominent changes during the normal menstrual cycle, while in the ectocervix it was highly present during the process of indirect squamous metaplasia. In order to continue the analysis of the M, 24,000 estrogen-regulated protein, we have now performed a study correlating the presence of the protein with the presence of ER and PgR in patients with adenocarcinomas and squamous carcinomas of the uterus.

MATERIALS AND METHODS

Patients. The tumors were obtained from patients seen at Mendoza’s Hospitals by the Cooperative Group for the Study of Endocrine Dependent Tumors during the period of February 1986 to May 1988. These patients had operable endometrial or cervical carcinomas, and the diagnosis was done by preoperative endometrial curettage or cervical punch biopsy. Of the 86 cases entering into the study, 10 cases were excluded due to inadequate tissue for complete analysis or because no residual tumor was seen on histological examination; the 76 remaining cases comprised 47 primary endometrial carcinomas and 29 primary cervical carcinomas. Pre- and postmenopausal patients were included.
in the study; they had no clinical metastasis, had received no radiother-
apy before surgery, and only three patients with endometrial adenocar-
cinoma received endocrine therapy (medroxyprogesterone acetate) be-
after surgery.

Tissue Handling. The tumor samples were obtained immediately
after surgery, trying to avoid as much as possible the incorporation of
necrotic and hemorrhagic areas and of myometrium. In order to obtain
a homogenous representation of the tumor samples for the study, the
umor tissues were divided into small pieces which were mixed before
paration into three fractions: (a) for ER and PgR analysis by the
dextran-coated charcoal assay and for immunoblot study of the M,
24,000 protein; (b) for ER and PgR analysis by an immunohistochem-
ical procedure; and (c) for histological examination and immunohisto-
chemical study of the M, 24,000 protein. Fractions a and b were
immediately frozen and stored at —70°C until assays, while the tissue
of the fraction c for histological examination was fixed in a neutral-
buffered formalin and processed routinely for paraffin embedding.

Biochemical Studies. The frozen tissues were pulverized, thawed in
three volumes of homogenizing buffer (5 mM molybdic acid/1.5 mM
EDTA/500 mM dithiothreitol/10 mM Tris-HCl, pH 7.4), and homog-
emized in an Ultra-Turrax homogenizer at 4°C. Cytosol was prepared
from the homogenate by centrifugation at 140,000 x g for 50 min at
4°C. The protein concentration of the supernatant was determined by
the method of Lowry (20). The cytosols were diluted to 1.5-2 mg
protein/ml, and the content of ER and PgR was analyzed using a one-
point dextran-coated charcoal assay as described elsewhere (13). Ali-
quots of 200 μl of cytosol were incubated overnight at 4°C with 0.25
pmol of 'H-exelabled estradiol or with 1 pmol of 'H-R5020 (New
England Nuclear, Boston, MA). A 100-fold excess of diethylstilbestrol
(Sigma Chemical Co., MO) or of R5020 (NEN) was used in the cold
assay tubes of ER and PgR determinations, respectively. Endogenous
unbound steroid was removed by 0.25% (w/v) Norit A/0.0025% dextran
in 10 mM Tris, pH 8.0. Radioactivity was determined in a Kontron MR
300 liquid scintillation counter using 5 ml solution of 0.4% (w/v)
Omnifluor (NEN) in toluene.

SDS-PAGE analysis was performed on the cytosol samples by the
method of Laemmli (21). The samples were dissolved into 1.9% SDS
solution containing 9.5% glycerol/4.76% mercaptoethanol/10 mM
Tris, pH 6.8. A 100 μg of sample protein was loaded on the stacking
gel prepared with 3.2% of acrylamide and separated in the resolving
gel with 13.4% of acrylamide, under conditions of constant current and
running at 35 mA/gel (slab gels of 2.7 x 140 mm). In each gel, one
lane was loaded with molecular weight standards: ribonuclease A (M,
13,700), chymotrypsinogen A (M, 25,000), ovalbumin (M, 43,000), and
bovine serum albumin (M, 67,000) (Pharmacia Fine Chemicals, Piscata-
way, NJ). The proteins were then transferred from SDS-PAGE on
0.22-μm nitrocellulose membranes (Schleicher & Schuell, Keene, NH)
using the electroblotting technique (22). The electroblot buffer contained
150 mM glycine/20 mM Tris in methanol, pH 7.5-8.0. Following
transfer, the standard-containing nitrocellulose lanes were separated
and stained in 0.1% (w/v) Amido black/45% methanol, and 10% acetic
acid for 10 min, and destaining was effected in 45% methanol and 10%
acetic acid. The cytosol-containing nitrocellulose lanes were immuno-
stained with the C11 mouse monoclonal antibody against the M,
24,000 estrogen-regulated protein and treated with the ABC system to
detect the proteins immunoenzymatically as reported previously (23).
The dilution and time of incubation with the antisera were the same as
those for immunohistochemistry.

Immunohistochemical Studies. The presence of ER and PgR on the
tumors was detected by monoclonal antireceptor antibodies utilizing
frozen tissue sections. The H222 antibody (rat monoclonal origin)
against ER was kindly provided by Dr. E. De Sombre (University of
Leuven, Belgium), while the antibody mPRI against PgR (mouse monoclonal
origin) was purchased from Transbio SARL (Paris, France). The spec-
ificity and use of these antibodies in immunohistochemistry has been
previously reported (24-27). Briefly, frozen 6-μm thick tissue sections
were thaw-mounted onto 0.1% poly-L-lysine (w/v) (Sigma)-coated slides
and immediately fixed in a buffered picric acid-formaldehyde solution
(28) for 5 min at room temperature. After rinsing in phosphate buffer,
the sections were incubated with 3% normal goat or sheep serum for
30 min. Then, the sections were incubated overnight at 4°C with H222
(10 μg/ml), mPRI (10 μg/ml) or with normal rat and mouse serum in
a moist incubation chamber. Biotinylated anti-rat or anti-mouse anti-
odies (Amersham International, UK) diluted 1:50 were used as second
antibodies (incubated for 45 min at room temperature), while ABC
complex (Vector Laboratories, Burlingame, CA) diluted 1:100 was used
as the enzyme reagent ( incubated for 45 min). Hydrogen-peroxide and
diaminobenzidine tetrahydrochloride (Sigma) prepared as reported pre-
viously (29) was used for 15 min to give the brown color; after washing
the sections were dehydrated in serial alcohols to xylene and mounted
with Permount without counterstaining.

The paraffin-embedded sections (5-6 μm thickness) were mounted on
gelatinized slides and stained with hematoxylin & eosin or immuno-
stained. Briefly, the histological criteria used to diagnose endometrial
and endocervical carcinomas were the presence of glandular structures
closely packed together lined by a pseudostratified or stratified epithe-
lum, with frequent papillary projections. The epithelium was composed
by large nuclei, at times pleomorphic, with prominent nucleoli, and
spare cytoplasm. Mitosis were frequently seen (30-32). Well-differ-
entiated tumors had a glandular or papillary pattern, moderately differ-
entiated tumors had a glandular or papillary pattern mixed with solid
cores, and poorly differentiated tumors presented solid sheets with
absence of glandular or papillary formations. The term adeno-
canthomas was used for those adenocarcinomas with histologically
benign-appearing squamous elements, while the term mixed-adenos-
squamous carcinoma was used for those adenocarcinomas with histo-
logically malignant foci of squamous cells. The M, 24,000 protein
immunoreactivities in endometrial carcinomas according to the histo-
logical, mitotic, nuclear, and nucleolar grades have been presented
previously (17). On the other hand, invasive squamous cell carcinomas
of the cervix comprised large cell keratinizing carcinomas, large cell
nonkeratinizing carcinomas, and small cell nonkeratinizing carcino-
mas. Discrepancies in some interpretations were resolved by consensus.

The immunostaining of the paraffin sections was done as reported
elsewhere (29) using the ABC system (Vector). The M, 24,000 estrogen-
regulated protein was detected with the C11 mouse monoclonal anti-
body (16) kindly provided by Dr. W. L. McGuire (Texas). In some
tumors keratin was detected by a rabbit antibody raised against purified
human epidermal keratin (Polysciences Inc., Warrington, PA). The
antibody against the M, 24,000 protein was used at a concentration of
5 μg/ml, while the antibody against keratin was used at 1:100 dilution;
and the samples were incubated for 24 h in humidity chambers at
4°C. In the negative control slides the primary antibody was omitted and
sometimes we used the primary antibody preabsorbed. After immu-
nostaining, one of the slides from each sample was counterstained with
hematoxylin, and all of them were mounted with Permount.

The tissue samples were observed and photographed with a Zeiss
IM35 microscope, with the use of different interference contrast
optics whenever required. A quantitative analysis of the immunon-
active cells was carried out by two observers who counted the percentage
of reacting nuclei (for ER and PgR immunostained cells) and the percent-
age of reacting cells containing nuclei (for M, 24,000 protein immuno-
reactive cells). Since in many of the tumors the distribution of the
immunostaining was heterogeneous we used a systematic random sam-
ping method similar to that employed in the selection of tissue blocks
for electron microscopy (33). In this way sample fields representative
of the different areas of the tumor were examined under the ×40
objective.

Test of incidence in contingency tables, two-way χ², was applied to
evaluate the biochemical data of ER versus M, 24,000 (Tables 1 and 3)
and PgR versus M, 24,000 protein (Tables 2 and 3); and for the
immunohistochemical data of ER versus M, 24,000 (Table 1) and
PgR versus M, 24,000 protein (Table 2). The two-way χ² test was also
used to evaluate the data on the presence of ER, PgR, and M, 24,000 protein
in carcinomas with squamous cells (Table 5). A regression analysis was
performed when the percentage of ER-positive cells was compared with
the percentage of M, 24,000-positive cells in endometrial adenocarcin-
omas (Fig. 2). In this case the straight line is the result of the equation:
log (%ERICA + 1) = log(a) + β(%ERICA); where log(a) = 0.52 and
β = 0.04.
RESULTS

Adenocarcinomas. The correlation between the presence of the M, 24,000 estrogen-regulated protein and ER in adenocarcinomas of the endometrium and endocervix is shown in Table 1. The biochemical studies of the cytosol tumor samples revealed that M, 24,000 protein and ER are not independent (P ≤ 0.05). When these two parameters were studied by immunohistochemistry, a higher significant positive correlation (P ≤ 0.005) was found. This was not unexpected since in the samples taken for the biochemical studies there were neoplastic cells mixed with stromal and sometimes with myometrial cells, cells that also contain ER but that have variable amount of M, 24,000 protein; therefore, the discrimination between the different cell populations was possible in the tissue sections evaluated by immunohistochemistry. Fig. 1, A–D, shows sections from a patient with endometrial adenocarcinoma reacting with the monoclonal antibodies against M, 24,000 protein, ER, and PgR. On the other hand, Fig. 1, E–H, shows the presence of M, 24,000 protein, ER, and PgR in a patient with an endocervical adenocarcinoma. In addition, the immunohistochemical study allowed us to evaluate the heterogeneity of the immunostaining. Fig. 2 shows the correlation obtained by counting the number of positive cells immunostained for M, 24,000 protein and for ER.

A significant positive correlation between M, 24,000 protein and PgR (P ≤ 0.05) was found in the adenocarcinomas studied by immunohistochemistry (Table 2), but not when these parameters were studied biochemically in the cytosol tumor samples. In the biochemical assay we decided to use a cutoff point of 190 fmol/mg cytosol protein for PgR because this has been the reported value for those endometrial adenocarcinomas that are

Table 1 Comparison between expression of M, 24,000 protein and ER in uterine adenocarcinomas

<table>
<thead>
<tr>
<th>M, 24,000 protein</th>
<th>M, 24,000 protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>immunoblot*</td>
<td>immunocytochemistry*</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>ER*</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21 (78)*</td>
</tr>
<tr>
<td>Negative</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Numbers in parentheses. percentage.</td>
<td></td>
</tr>
</tbody>
</table>

* Positive or negative according to the presence or not of the specific band.

# Positive, when M, 24,000 protein appeared in >10% of the tumor cell population.

# Positive, ER >10 fmol/mg cytosol protein.

# Positive, when ER appeared in >10% of the tumor cell population.

* Numbers in parentheses, percentage.
likely to be under the influence of estrogen (34). Even higher threshold values of PgR have been reported (35). As can be seen in Table 3, the presence of the M, 24,000 protein detected in the cytosol samples correlates significantly with the amount of ER (P ≤ 0.005) and with the amount of PgR (P ≤ 0.05).

On the other hand, when the degree of tumor differentiation was evaluated, we found that 69% of the well differentiated adenocarcinomas were positive for M, 24,000 protein, for ER, and for PgR, while most of the moderately and poorly differentiated tumors were positive only for ER (40%), or lacked ER, PgR, and M, 24,000 protein (20%) (Fig. 3). It is important to mention here that we excluded from the group of adenocarcinomas those presenting squamous elements (36), because in the six cases examined there was M, 24,000 protein immunodetection while ER was detected in only two cases and PgR in one (Fig. 4, A and B). Although not shown here, the presence of the squamous cells was corroborated by immunostaining with the antikeratin antibody.

In 25% of the endometrial adenocarcinomas examined we found varying amounts of normal, proliferative, and hyperplastic endometrium. In these cases we were able to compare, by immunohistochemistry, the expression of ER, M, 24,000 protein, and PgR in the malignant endometrium and in the associated nonmalignant endometrium (Table 4). In these postmenopausal patients normal endometrium was rich in ER, while the content of PgR was moderate, and M, 24,000 protein content was low to absent. Proliferative endometrium showed from 50% to more than 90% of the epithelial cells with moderate to strong immunostaining intensity for ER; M, 24,000 protein was also high in these samples, while PgR was negative in one case and high in the others. Cystic and adenomatous hyperplasias showed variable degree of ER, M, 24,000 protein, and PgR; some of them were rich in the three parameters studied while some others showed low content of ER, PgR, and almost absence of M, 24,000 protein. It is interesting to note the patient (Case 4, Table 4) with an adenocarcinoma negative for ER, M, 24,000 protein, and PgR but whose associated proliferative endometrium and cystic hyperplasia showed considerable amount of ER, M, 24,000 protein, and PgR.

Squamous Carcinomas and Adenocarcinomas with Squamous Cells. The presence of ER, PgR, and M, 24,000 protein phenotype in the squamous carcinomas of the uterine cervix and in the adenoacanthomas and mixed adenosquamous carcinomas of the corpus uteri is shown in Table 5. The statistical study revealed a lack of correlation among the three parameters evaluated; in most of the cases these tumors lacked ER and PgR. However, 82% of these receptor-negative tumors expressed the M, 24,000 protein in variable degree. Fig. 4, C and D, shows a squamous carcinoma of the uterine cervix with immunoreactive M, 24,000 protein but lacking ER. The identity of the protein was corroborated in the immunoblot studies, where it was compared with the M, 24,000 protein present in endometrial adenocarcinomas and from a positive control patient with a breast carcinoma (Fig. 5).

**DISCUSSION**

The results obtained show that in endometrial and endocervical adenocarcinomas the presence of M, 24,000 protein is correlated with that of ER and PgR. We have used two different immunodetection systems for M, 24,000 protein, Western blot and immunohistochemistry, and when the evaluation was done in the tumor cytosols the presence of M, 24,000 protein correlated significantly with that of ER. However, when the evaluation was performed in the tumor sections by immunohistochemistry the presence of M, 24,000 protein correlated significantly with that of ER and PgR as well. In a previous study

Fig. 2. Correlation between percentage of M, 24,000 estrogen-regulated protein-positive cells in immunocytochemistry (EPICA) and percentage of ER-positive cells in immunocytochemistry (ERICA) in 43 patients with endometrial adenocarcinomas. For statistical analysis see "Materials and Methods" (n = 44, a = 1.66, b = 0.04, R² = 0.73).

**Fig. 3.** Relation between percentage of incidence of ER, PgR, and M, 24,000 protein in well differentiated versus moderately and poorly differentiated endometrial adenocarcinomas.

Table 2 Comparison between expression of M, 24,000 protein and PgR in uterine adenocarcinomas

<table>
<thead>
<tr>
<th>M, 24,000 protein immunoblot</th>
<th>M, 24,000 protein immunocytochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>PgR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 (46)</td>
</tr>
<tr>
<td>Negative</td>
<td>11 (42)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Positive or negative according to the presence or not of the specific band.

Table 3 Presence of M, 24,000 protein by immunoblot (Western blot) according to the amount of ER and PgR in uterine adenocarcinomas

<table>
<thead>
<tr>
<th>M, 24,000 protein</th>
<th>ER&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percentage</th>
<th>M, 24,000 protein</th>
<th>PgR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>n</td>
<td>Percentage</td>
<td>Positive</td>
<td>n</td>
<td>Percentage</td>
</tr>
<tr>
<td>ER&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;10</td>
<td>4</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10–100</td>
<td>3</td>
<td>9.3</td>
<td>16</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>1</td>
<td>3.1</td>
<td>8</td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, percentage.

<sup>b</sup> Positive, PgR >100 fmol/mg cytosol protein.

<sup>c</sup> Positive, when PgR appeared in >10% of the tumor cell population.

<sup>d</sup> Positive, when M, 24,000 protein appeared in >10% of the tumor cell population.

<sup>e</sup> Numbers in parentheses, percentage.

<sup>f</sup> Positive, PgR >100 fmol/mg cytosol protein.

<sup>g</sup> Positive, when PgR appeared in >10% of the tumor cell population.

<sup>h</sup> Numbers in parentheses, percentage.

<sup>i</sup> Positive, M, 24,000 protein expressed in >10% of the tumor cell population.

<sup>j</sup> Numbers in parentheses, percentage.

<sup>k</sup> Positive, PgR >100 fmol/mg cytosol protein.

<sup>l</sup> Positive, when PgR appeared in >10% of the tumor cell population.
performed in human breast tumors, it was also found that elevated expression of \( M, 24,000 \) protein correlated well with presence of both ER and PgR, with the correlation coming primarily through ER (14). We believe that the immunohistochemical study is better than the biochemical study because with the former we were able to analyze the presence and distribution of ER, PgR, and \( M, 24,000 \) protein exclusively in the tumor cells, avoiding stroma, myometrium, and nontumorous areas which are sometimes present in the homogenate. This is consistent with the heterogeneity of ER and PgR in human endometrial adenocarcinomas reported previously (25, 37).

The present study also shows that almost 70% of the well-differentiated adenocarcinomas had ER, PgR, and \( M, 24,000 \) protein, and most authors agree that well-differentiated endometrial adenocarcinomas frequently have ER and PgR (38–40). However, we cannot predict the hormone dependence by morphological examination, it is necessary to study the receptor status of these tumors (40, 41). Today the immunohistochemical study of ER and PgR is increasingly more accepted because of its advantages (lower cost, exploration of tumor heterogeneity). Nevertheless, at present the monoclonal antireceptor antibodies are recommended for use in frozen sections, and our study shows that it is possible as an alternative and/or complementary method to explore the presence of the \( M, 24,000 \) protein in tumors fixed and processed for routine paraffin sections. We are now following these patients to analyze if the presence of \( M, 24,000 \) protein is useful in predicting their...
If estrogens in circulation are low, it is reasonable to our study allows us to draw some observations. In these post-cancer patients with associated nonmalignant endometrium, carcinoma; and 5, patient with squamous carcinoma of the ectocervix. Standard control from a patient with breast carcinoma; 3, patient with endometrial adenocarcinoma; and 4, patient with endocervical adenocarcinoma. M, 24,000 protein is a 24,000 dalton protein that is a member of the oncoprotein family. It is involved in differentiation processes was found in the present administration (50, 51). More evidence that M, 24,000 protein may show considerable biochemical alterations (45); that the presence of ER and PgR in cervical carcinomas is being evaluated as prognostic parameters (46); and that patients with cervical cancer are generally refractory to hormonal therapy (47). In addition, M, 24,000 protein has also been found in normal skin biopsy samples from males and females, in normal merocrine sweat glands, and in skin and bladder carcinomas (13). Therefore, it is reasonable to suggest that M, 24,000 protein is under hormonal control only in those tissues where growth and differentiation are strongly hormonally controlled (breast and endometrium). We can compare this situation with another model involved in growth processes, the c-myc oncogene whose expression is stimulated in fibroblasts by platelet-derived growth factor, in T-lymphocytes by concanavalin A, while in ER-positive human breast cells by estrogen administration (50, 51). More evidence that M, 24,000 protein is involved in differentiation processes was found in the present study, since the protein was always found in squamous cells in patients with adenocanthomas and with mixed adenosquamous carcinomas. This is consistent with the finding of M, 24,000 protein in patients with cervical intraepithelial neoplasias rising from indirect squamous metaplasia.7

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Study of Estrogen Receptor, Progesterone Receptor, and the Estrogen-regulated $M_r$ 24,000 Protein in Patients with Carcinomas of the Endometrium and Cervix

Daniel R. Ciocca, Libertad A. Puy and Liliana C. Fasoli