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The Effects of Ionizing Irradiation on the Sedimentation Coefficient of Cytoplasmic Steroid Receptors in Rat Mammary Tumors*

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Abstract—*The effects of ionizing irradiation on the sedimentation coefficients of both estrogen receptor (ER) and progesterone receptor (PgR) have been examined in comparison to the effects of proteolysis. DMBA-induced rat mammary tumors were subjected to a treatment of* 20 Gy *and the ER and PgR concentrations were determined at different time intervals after irradiation. On a* 5–20% *sucrose gradient the ER sedimented as* 9–11 *and* 4–5 S *molecular forms, while PgR sedimented as a small* 8–9 S *peak and a major* 4–5 S *peak. Radiotherapy particularly reduced the* 4–5 S *sedimentation peaks of both receptors but did not initiate any new sedimentation forms. Although the* 4–5 S *ER receptor concentrations remained low, both progesterone receptor forms appeared to recover by* 60 days *after treatment. As these effects could be due to the release of proteolytic enzymes following irradiation of tumors, the receptors from untreated tumors were exposed to different concentrations of trypsin. The effects of trypsin were identical for ER and for PgR, and proved to be dependent on the trypsin concentration. Only concentrations of trypsin up to* 30 μg/ml *resulted in a reduction of* 9–11 S *ER or* 8–9 S *PgR forms which was accompanied by a simultaneous increase in the* 4–5 S *peaks, resulting in no change in total binding sites. Still higher trypsinization* (300–3000 μg/ml) *also reduced the* 4–5 S *ER and PgR fractions. In the presence or the absence of sodium molybdate, a stabilizer of the faster sedimenting forms of the receptor, no alterations were observed in the position of, or the total number of binding sites of, the sucrose gradient fractions from control or irradiated tumors. The irradiation effects appear to be due either to damage of the cytosolic ER receptor, thereby preventing its participation in the induction of* de novo *synthesis of ER and PgR, or to the non-specific damage of transcription and/or translation systems.*

INTRODUCTION

THE ANALYSIS of steroid receptors in malignant tissue is not only effective for the prediction of hormone dependency [1–3] but has also shown efficacy in forecasting prognosis [2, 4–8]. About 50% of all human breast cancers are hormone-dependent when estradiol receptor (ER) is present [3, 9], while in the absence of ER less than 10% respond to hormonal treatment. Improvement in the prediction rate can be achieved by the simultaneous and quantitative determination of estrogen [10] and progesterone receptors (PgR) in breast tumor biopsies [11, 12]. In addition, clinical and biochemical factors which influence the receptor concentrations should also be taken into consideration, e.g. age and menopausal state, which are both important clinical parameters correlated with receptor concentrations. Pre-operative irradiation also affects the number of receptor binding sites [13, 14]. This observation has been made in human breast cancers [13] and in DMBA-induced rat mammary tumors [14]. Both

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Abbreviations: DMBA, 7,12-dimethylbenz(a)anthracene; DCC, dextran-coated charcoal; TED buffer, Tris–EDTA–1,4-dithio-DL-threitol.

ER and PgR concentrations decrease progressively to 50% at day 5 and maximally at day 30 after irradiation.

The present report evaluates the influence of ionizing irradiation on the sedimentation coefficient of both ER and PgR as compared to the effect of proteolytic enzymes. In addition, the binding proteins are studied in the presence and absence of sodium molybdate, a known stabilizer of steroid receptors.

MATERIALS AND METHODS

Induction of tumor and radiation treatment

Female Sprague–Dawley rats (Hannover strain × Hannover 91/D 3000; Zentral Institut für Verzuchtieren), 50–55 days of age, received an intragastric dose of 10 mg DMBA (Fluka AG, Buchs, Switzerland) dissolved in 0.5 ml of sesame oil twice with an interval of 24 hr [15, 16]. For 1 week the antibiotic Tylosin (Tylan-Eli Lilly and Co., Indianapolis, IN, U.S.A.) (1 g/l) was added to the drinking water to prevent pneumonia. The animals were fed Purina chow diet *ad libitum*. The first mammary tumors appeared 2–3 months after treatment. Only tumors measuring between 1 and 2.5 cm in diameter were used to avoid necrotic areas usually present in larger specimens. Radiotherapy on the tumors took place under general anesthesia with Nembutal (phenobarbital 60 mg/kg) and atropine (1 mg/kg). Atropine was added to prevent excessive bronchial secretion. The treatment consisted of 20 Gy (1 Gy = 100 rad) telecobalt photons from a Barazetti (Monza, Italy) cobalt unit. Only one session of 40–50 min was given and the dose was calculated at 4 cm depth with 1 cm Plexiglas as build-up zone. An area of 4 × 20 cm was irradiated. Four animals were placed in the irradiation beam with the tumors in the axis with care being taken to place the animals inside the penumbral area. A series of animals used as non-irradiated controls underwent the same anesthetic treatment as the irradiated rats. Ten controls and 10 treated animals were decapitated 5, 10, 30 and 60 days after irradiation. Another set of animals was used for trypsinization experiments. These were taken at random after induction with DMBA and were identical to the ones used for the radiation experiments. All tumors after removal were immediately placed in liquid nitrogen (−196°C).

Trypsinization experiments

One gram of DMBA tumor tissue from 3 or more tumors was cut into pieces and pulverized to powder in liquid nitrogen. After the addition of 2 ml of phosphate buffer [5 mM $Na_2H_2PO_4$ (Merck, Darmstadt, F.R.G.), 10% glycerol, 1 mM monothioglycerol (Carlo Erba)], pH 7.4, 4°C, the powder was homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle. After centrifugation at 105,000 *g* (International Ultracentrifuge B60, Model SB 405 swinging bucket) for 30 min, the pooled supernatant was used for the receptor studies as described before [14]. Disposable 10 × 75 mm glass culture tubes were utilized in these experiments. Fifty microliters of trypsin (Trypsin-TPCK, Worthington Biochemical Co., Freehold, NJ, U.S.A.) dissolved in phosphate buffer at room temperature were quickly added to the tubes containing 100 μl of the cold supernatant (4°C) and vigorously mixed. The concentrations of trypsin used in these experiments were 0, 3, 30, 300 and 3000 μg/ml. Incubation took place for 5 min at 22°C. The reaction was stopped when 50 μl of cold (4°C) soybean trypsin inhibitor (Merck) in phosphate buffer was added and thoroughly mixed. The tubes were immediately placed in ice. Finally 50 μl of 25 nM 17β-[2,4,6,7-^{3}H]-estradiol (sp. act. 115 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) or 17α-[methyl-^{3}H]-promegestone (R_{5020}; 17,21-dimethyl-19-nor-4,9-pregnadiene-3,10-dione-17α-[methyl-^{3}H]; sp. act. 87 Ci/mmol, New England Nuclear) in phosphate buffer was added at 4°C. These mixtures were incubated overnight (16 hr) at 4°C. A duplicate of each incubation contained in addition to the tracer a 100-fold excess of cold ligand.

Preparation of steroid–receptor complexes from cytosol

Non-irradiated control tumors and irradiated tumor tissue (1 g, representing 3 or more pooled tumors) were pulverized at −196°C. Two milliliters of phosphate buffer with and without 10 mM sodium molybdate (Sigma Chemical Co., St. Louis, MO, U.S.A.) were added and homogenization was again performed in a Potter–Elvehjem homogenizer with a Teflon pestle. After centrifugation at 105,000 *g*, 200 μl of the supernatant was added to 50 μl of tritiated tracer at 4°C with and without a 100-fold excess of cold ligand. The mixture was shaken and incubated overnight (16 hr) at 4°C in duplicate.

Sucrose density gradient analysis

The cytosol (250 μl) prepared as described above was added to a dextran-coated charcoal (DCC) pellet. This pellet, obtained by centrifugation of 500 μl of DCC suspension [TED buffer–0.25% activated charcoal–0.025% dextran (Grade C, BDH Biochemicals, Poole, U.K.)] at 3000 *g* for 10 min (International Centrifuge Size 2, Model K), was resuspended in the incubate and centrifuged immediately for another 10 min. Two hundred microliters of the resulting supernatant

were layered on a 5–20% sucrose gradient prepared in phosphate buffer, pH 7.4, at 4°C. For each run an identical sucrose gradient was layered with 200 μl of bovine serum albumin (methyl-^{14}C) (New England Nuclear) as an external standard. The gradients were centrifuged in a Beckman SW 50 Ti for 16 hr at 4°C at 190,000 *g*. Each gradient was fractionated into portions containing 4 drops (22–23 fractions per gradient) with a Beckman fraction recovery system. The gradient was replaced with paraffin oil (Merck, Darmstadt, Germany) and recovered by puncturing the bottom of the tube in a minicounting vial (Lumac Systems AG, Basel, Switzerland). The radioactivity was measured by adding 1 ml Aqua Luma scintillation liquid (Lumac Systems AG) and counting was performed in a liquid scintillation spectrometer (Rack Beta LKB, Wallac, OY, Turku, Finland). Protein concentrations were measured according to the Folin phenol method [17]. Care was taken to avoid differences in protein concentration in cytosol between gradients. In addition, the cytosol was well concentrated to obtain reproducible and significant peaks. In case of possible individual variations each experimental run was carried out at least 6 times.

RESULTS

Effect of ionizing irradiation on the steroid receptors

Treatment of the DMBA-induced mammary tumors with 20 Gy telecobalt photons in one session decreased the total amount of specific binding sites for both estradiol and progesterone. The ER sedimented as 9–11 and 4–5 S molecular forms on a 5–20% sucrose gradient. Although the area under the tritiated estrogen peaks differed somewhat from one experiment to another, no new peaks appeared following radiotherapy and the original two sedimentation peaks were still recognizable after irradiation. For all gradients performed the average cytosolic protein content amounted to 20.70 mg/ml ± 2.45 S.E.M. The ratio of the area beneath the two peaks, however, was affected by irradiation as the 4–5 S peak particularly became diminished in all pooled cytosols as the time after irradiation progressed (Fig. 1). Similarly, the PgR profiles on sucrose gradients as measured by tritiated R_{5020} remained relatively unchanged without the appearance of new peaks following irradiation. Throughout the period of 60 days following irradiation of the tumors, PgR sedimented as a small 8–9 S peak and a major 4–5 S peak (Fig. 2). Although the position and number of sedimentation peaks were unchanged by irradiation, both estradiol and progesterone 4–5 S receptor concentrations decreased progressively for 30 days as a result of such

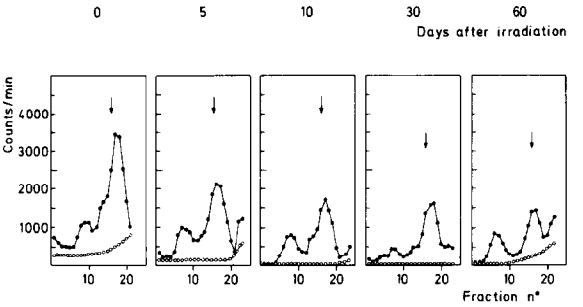

Fig. 1. Sucrose density gradient profiles of ER from DMBA tumors at various time intervals after 20 Gy *ionizing irradiation. (a) Day* 0 *(control); (b) day* 5; *(c) day* 10; *(d) day* 30; *(e) day* 60. ●——● *Total bound tritiated ligand;* O——O *non-specific binding; marker (arrow):* [^{14}C]-*BSA* (S = 4.6).

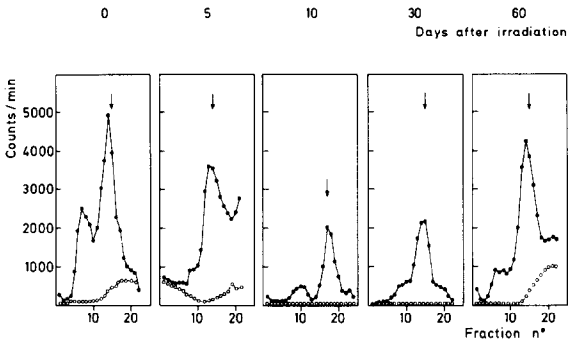

Fig. 2. Sucrose density gradient profiles of PgR from DMBA tumors at various time intervals after 20 Gy *ionizing irradiation. (a) Day* 0 *(control); (b) day* 5; *(c) day* 10; *(d) day* 30; *(e) day* 60. ●——● *Total bound tritiated ligand;* O——O *non-specific binding; marker (arrow):* [^{14}C]-*BSA* (S = 4.6).

treatment. While the 4–5 S ER receptor concentrations remained low, the progesterone receptors appeared to recover by 60 days after irradiation, an observation which suggests that at this time the remaining ER have become more effective in maintaining the level of PgR.

Effect of trypsin treatment

Since an expected result of ionizing radiation on these tumors might be the release of proteolytic enzymes, it was considered necessary to relate the possible effects of a peptidase on the sedimentation pattern of the two receptors. Trypsin had a similar effect on both estradiol and progesterone receptors from DMBA tumors, and this effect was shown to be dependent on the level of enzyme in the incubate.

Discrete trypsinization of the ER with up to 3 μg trypsin/ml incubate had no effect on the two molecular forms nor on the area under the two peaks. Mild trypsinization of this protein by 30 μg trypsin/ml affected the 9–11 S form, resulting in its reduction with a simultaneous increase in the 4–5 S peak. The overall receptor concentrations (area under both peaks), however, were nearly

identical to the control without trypsin. Stronger trypsinization (300–3000 μg trypsin) also affected the 4–5 S ER fraction by decreasing the area under this peak with an overall decrease in the receptor concentration. No shift in sedimentation coefficient was observed in these experiments since new peaks did not appear (Fig. 3).

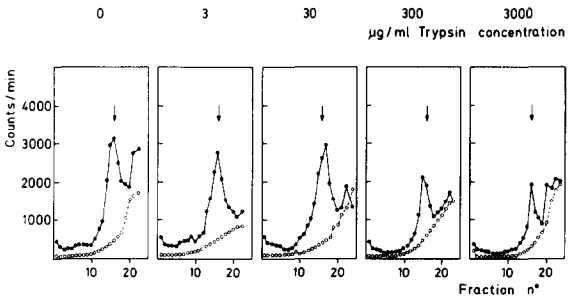

Fig. 3. Effect of trypsin on ER in DMBA cytosol of mammary tumors. Tumor cytosol (18 mg *protein*/ml) *was incubated with various levels of trypsin followed by the inhibition of enzyme activity and the equilibration of receptor with tritiated estradiol before analysis on density gradient (details in Materials and Methods).* ●——● *Total bound tritiated ligand;* O——O *non-specific binding; marker (arrow):* [^{14}C]-*BSA* (S = 4.6). 0 μg *trypsin: radioactivity under the* 10.5 S *peak:* 1566 dpm/mg *protein; under the* 4.6 S *peak:* 11694 dpm/mg *protein; total radioactivity:* 13260 dpm/mg *protein;* 3 μg *trypsin: radioactivity under the* 11.2 S *peak:* 2082 dpm/mg *protein; under the* 4.6 S *peak:* 11114 dpm/mg *protein; total radioactivity:* 13193 dpm/mg *protein;* 30 μg *trypsin: radioactivity up to fraction* 11: 379 dpm/mg *protein; under the* 4.6 S *peak:* 13668 dpm/mg *protein; total radioactivity:* 14047 dpm/mg *protein;* 300 μg *trypsin: radioactivity up to fraction* 11: 269 dpm/mg *protein; under the* 4.6 S *peak:* 4865 dpm/mg *protein; total radioactivity:* 5135 dpm/mg *protein;* 3000 μg *trypsin: radioactivity up to fraction* 11: 320 dpm/mg *protein; under the* 4.6 S *peak:* 3636 dpm/mg *protein; total radioactivity:* 3956 dpm/mg *protein.*

The progesterone receptor, identified by the specific binding with [^{3}H]-R_{5020}, also showed two peaks after gradient separation (Fig. 4). The most rapidly sedimentating receptor displayed an 8–9 S peak followed by a 4–5 S peak of radioactivity. Here again we found some variations between experiments but to a lesser extent than that observed with the ER. For R_{5020} binding, the 4–5 S peak always dominated the relatively small 8–9 S peak. Trypsinization with up to 3 μg trypsin/ml showed no significant differences from the control (0 μg trypsin). Mild trypsinization with 30 μg/ml resulted in a loss of the 8–9 S structure with a simultaneous enlargement of the 4–5 S peak. Utilizing up to 30 μg trypsin/ml brought about no loss in total receptor activity. Higher concentrations of trypsin also affected the 4–5 S PgR by decreasing its concentration. The overall receptor concentration decreased at the same time. Again, as with the trypsinization of ER, no new

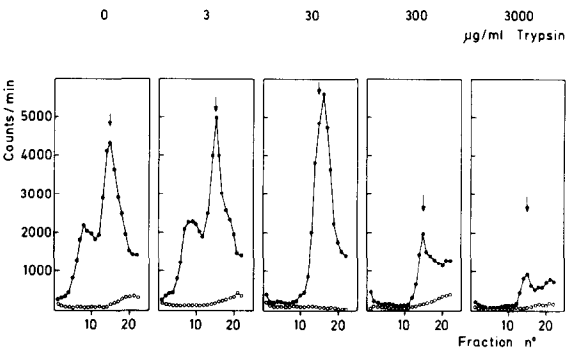

Fig. 4. Effect of trypsin on PgR in DMBA tumor cytosol. Tumor cytosol (23 mg *protein*/ml) *was incubated with various levels of trypsin followed by the inhibition of enzyme activity and the equilibration of receptor with tritiated promegestone before analysis on density gradient (details in Materials and Methods).* ●——● *Total bound tritiated ligand;* O——O *non-specific binding; marker (arrow):* [^{14}C]-*BSA* (S = 4.6). 0 μg *trypsin: radioactivity under the* 8.6 S *peak:* 7544 dpm/mg *protein; under the* 4.6 S *peak:* 18024 dpm/mg *protein; total radioactivity:* 25588 dpm/mg *protein;* 3 μg *trypsin: radioactivity under the* 8.6 S *peak:* 8104 dpm/mg *protein; under the* 4.6 S *peak:* 17956 dpm/mg *protein; total radioactivity:* 26061 dpm/mg *protein;* 30 μg *trypsin: radioactivity up to fraction* 11: 1077 dpm/mg *protein; under the* 4.6 S *peak:* 24489 dpm/mg *protein; total radioactivity:* 25567 dpm/ mg *protein;* 300 μg *trypsin: radioactivity up to fraction* 11: 620 dpm/mg *protein; under the* 4.6 S *peak:* 8090 dpm/mg *protein; total radioactivity:* 8711 dpm/mg *protein;* 3000 μg *trypsin: radioactivity up to fraction* 11: 383 dpm/mg *protein; under the* 4.6 S *peak:* 3950 dpm/mg *protein; total radioactivity:* 4333 dpm/mg *protein.*

peaks were observed following the treatment of PgR (Fig. 4).

In order to observe the pattern of the sedimentation peaks for ER and PgR in the presence of a stabilizer of non-activated steroid receptors, the cytosol from control and irradiated tumors were incubated with tritiated ligand in the presence and absence of 10 mM sodium molybdate. For both the estradiol and progesterone receptors slight changes in the area of the peaks were observed. Molybdate increased the area under the 8–9 S peak for [^{3}H]-R_{5020} (Fig. 5) and the 9–11 S peak for tritiated estradiol (Fig. 6). Simultaneously the area of the 4–5 S peak of both receptors decreased slightly. No position shifts of the sucrose gradient fractions were observed. The total amount of receptor was not influenced by the addition of molybdate. Nor was there any difference in the pattern changes observed between irradiated tumors (Figs 3 and 4) and controls (data not shown).

DISCUSSION

Ionizing irradiation reduces both ER and PgR in human [13] and rat DMBA mammary tumors [14]. This decrease is progressive and maximal at 20–30 days after irradiation as measured by the

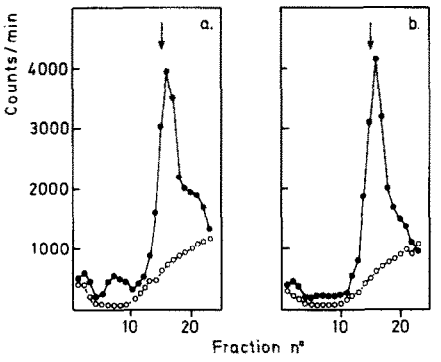

Fig. 5. Effect of molybdate on PgR in DMBA tumors. DMBA tumor cytosol (20.7 mg *protein*/ml) *was incubated with tritiated promegestone with* (O——O) *or without* (●——●) *an excess of cold ligand in the presence* (*a*) *and absence* (*b*) *of* 10 mM *sodium molybdate and analyzed by a sucrose density gradient.* (*a*) *Radioactivity under the* 8.6 S *peak:* 1721 dpm/mg *protein; under the* 4.6 S *peak:* 10357 dpm/mg *protein; total radioactivity:* 12811 dpm/mg *protein.* (*b*) *Radioactivity under the* 8.6 S *peak:* 529 dpm/mg *protein; under the* 4.6 S *peak:* 10227 dpm/mg *protein; total radioactivity:* 10763 dpm/mg *protein.*

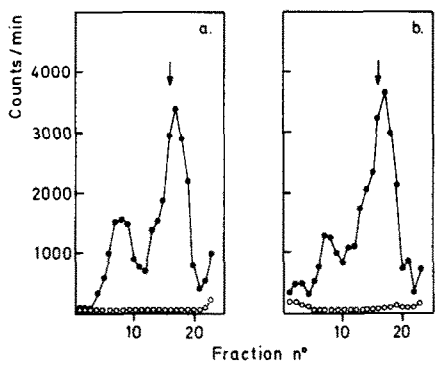

Fig. 6. Effect of molybdate on ER in DMBA tumors. DMBA tumor cytosol (21 mg *protein*/ml) *was incubated with tritiated estradiol with* (O——O) *or without* (●——●) *an excess of cold ligand in the presence* (*a*) *and absence* (*b*) *of* 10 mM *sodium molybdate and analyzed by a sucrose gradient.* (*a*) *Radioactivity under the* 9 S *peak:* 6331 dpm/mg *protein; under the* 4.6 S *peak:* 12850 dpm/mg *protein; total radioactivity:* 19182 dpm/mg *protein.* (*b*) *Radioactivity under the* 9 S *peak:* 5087 dpm/mg *protein; under the* 4.6 S *peak:* 16075 dpm/mg *protein; total radioactivity:* 21162 dpm/mg *protein.*

DCC assay. Comparable results are obtained after sucrose density gradient analysis when a gradual decrease of non-activated cytoplasmic ER and of PgR in pooled tumors is observed after treatment. Using pooled tumor samples from different animals throughout the experiments helps to eliminate possible interaction by endogenous hormone concentrations present in individual animals and to reduce characteristic effects of individual tumor structures. Scattering radiation and inadequate shielding make comparisons between irradiated and non-irradiated tumors from the same animal unreliable. The sedimentation coefficients for both receptors does not change significantly after radiotherapy. The 9–11 and 4–5 S receptor forms of ER and the 8–9 and 4–5 S forms of PgR remain intact. There is, however, a significant variability in the relative predominance of each of the two forms. Although freezing is known to reduce particularly the heavy forms of the receptor, as all tumors were kept in liquid nitrogen freezing does not seem to be responsible for the observed variations [11]. Since the 4–5 S structure of both steroid receptors is particularly affected by irradiation, one would suspect that the availability of this receptor for activation is considerably reduced. This is of major importance for the ER, which is also partially responsible for the regulation of PgR.

In addition, these studies were designed to evaluate the possible proteolytic activity brought about by the effect of ionizing irradiation on these tumors. The effects of proteolytic enzymes on the steroid receptor forms in sucrose density gradient analysis were different from those observed by irradiation. Trypsin, a proteolytic enzyme, proved to have a pronounced influence on the molecular forms and on the number of binding sites for estradiol and progesterone. As controls and trypsinized samples are treated identically, a possible slight loss in total ER and PgR concentrations due to the incubation at 22°C for 5 min cannot affect the trypsinization results. A mild trypsinization (up to 30 μg/ml) practically abolished the 9–11 S molecular form of both the ER and PgR. However, the total amount of binding sites for estradiol and promegestone is not reduced since a simultaneous increase in the 4–5 S was noted. Similar observations were made in other experimental systems and with other receptors [18]. Beyond 30 μg trypsin/ml a gradual decrease of the solitary 4–5 S peak was seen, along with a gradual disappearance of the total amount of binding sites. These effects on ER are comparable with the effect on PgR.

The effect of molybdate on the molecular forms of cytoplasmic ER and PgR from DMBA tumors was not as pronounced as observed by others [9, 19, 20]. Homogenization and incubation of the cytosol in the presence of 10 mM molybdate gave a slight increase in the 8–9 S receptor form without significantly affecting the 4–5 S form of PgR. Molybdate, on the other hand, brought about a slight increase in the ER 9–11 S peak accompanied by a decrease in the 4–5 S peak. This effect was similar for control and irradiated tumors. Since molybdate protects the steroid receptors by inhibition of receptor transformation and by maintaining receptors in a 'native' 8–10 S form, irradiation does not seem to interfere with these stabilizing characteristics [8, 19, 21–23]. This was

clearly demonstrated by the inability of molybdate to change the sedimentation pattern of both the ER and PgR. More important is the different sedimentation behavior exhibited by both steroid receptors after irradiation as compared to the effect of a proteolytic enzyme (discussed above). The decreased steroid receptor concentrations after ionizing irradiation therefore appear not to be due to proteolytic activity in the cytosol [14]. Two possible explanations remain which might explain the diminution of ER: (1) irradiation-damaged cytosolic receptor may not be capable of inducing *de novo* synthesis of ER when complexed with estradiol; or (2) irradiation-damaged transcription or translation systems may not be totally functional. The latter would be expected to lead to a decrease in many cellular proteins, a fact supported by previous studies [24].

REFERENCES

1. JENSEN EV, SMITH S, DESOMBRE ER. Hormone dependency in breast cancer. *J Steroid Biochem* 1976, **7**, 911–917.
2. MCGUIRE WL. Steroid hormone receptors in breast cancer: treatment strategy. *Recent Prog Horm Res* 1980, **36**, 135–156.
3. SINGHAKOWINTA A, MOHINDRA R, BROOKS SC, VAITKEVICIUS BK, BRENNAN MJ. Clinical correlation of endocrine therapy and estrogen receptor. In: MCGUIRE WL, CARBONE PP, VOLLMER EP, eds. *Estrogen Receptors in Human Breast Cancer*. New York, Raven Press, 1977, 131–149.
4. COOKE T, GEORGE D, SHIELDS R, MAYNARD P, GRIFFITHS K. Oestrogen receptors and prognosis in early breast cancer. *Lancet* 1979, **i**, 995–997.
5. HEUSON JC, LONGEVAL E, MATTHEIEM WH, DEBOEL MC, SYLVESTER RJ, LECLERCQ G. Significance of quantitative assessment of estrogen receptors for endocrine therapy in advanced breast cancer. *Cancer* 1977, **39**, 1971–1977.
6. KNIGHT WA, LIVINGSTON RB, GREGORY EJ, MCGUIRE WL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. *Cancer Res* 1977, **37**, 4669–4671.
7. LECLERCQ G, HEUSON JC. Therapeutic significance of sex-steroid hormone receptors in the treatment of breast cancer. *Eur J Cancer* 1977, **13**, 1205–1215.
8. PICHON MF, PALLUD C, BURNET J, MILGROM E. Relationship of presence of progesterone receptor to prognosis in early breast cancer. *Cancer Res* 1980, **40**, 3357–3360.
9. NIU EM, NEAL RM, PIERCE VK, SHERMAN MR. Structural similarity of molybdate-stabilized steroid receptors in human breast tumors, uteri and leucocytes. *J Steroid Biochem* 1981, **15**, 1–11.
10. JENSEN EV, DESOMBRE ER. The diagnostic implications of steroid binding in malignant tissues. *Adv Clin Chem* 1977, **19**, 57–89.
11. HORWITZ KB, MCGUIRE WL. Progesterone and progesterone receptors in experimental breast cancer. *Cancer Res* 1977, **37**, 1733–1738.
12. PICHON MF, MILGROM E. Characterization and assay of progesterone receptor in human mammary carcinoma. *Cancer Res* 1977, **37**, 464–471.
13. JANSSENS JP, BONTE J, DROCHMANS A *et al.* Effect of presurgical radiotherapy on the steroid receptor concentrations in primary breast cancer. *Eur J Cancer* 1981, **17**, 659–664.
14. JANSSENS JP, WITTEVRONGEL C, VAN DAM J, GODDEERIS P, LAUWERIJNS JM, DE LOECKER W. Effects of ionizing irradiation on the estradiol and progesterone receptors in rat mammary tumors. *Cancer Res* **41**, 703–707, 1981.
15. HUGGINS C, GRAND LC, BRILLANTES FP. Mammary cancer induced by a single feeding of polynuclear hydrocarbons and its suppression. *Nature* 1961, **189**, 204–207.
16. HUGGINS C, MOON RC, MORII S. Extinction of experimental mammary cancer. I. Estradiol-17β and progesterone. *Proc Natl Acad Sci USA* 1962, **48**, 379–386.
17. LOWRY OH, ROSEBROUGH MJ, FARR AL, RANDALL RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
18. WILSON EM, FRENCH ES. Effects of protease and protease inhibitors on the 4.5 S and 8 S androgen receptor. *J Biol Chem* 1979, **254**, 6310–6319.
19. HAZATO T, MURAYAMA A. Protection of steroid hormone receptors by protease inhibitors. *Biochem Biophys Res Commun* 1981, **98**, 488–493.
20. NISHIGORI H, TOFT D. Inhibition of progesterone receptor activation by sodium molybdate. *Biochemistry* 1980, **19**, 77–83.
21. ANDERSON KM, MAROGIL M, BONOMI PD, HENDRICKSON C, ECONANOU S.

Stabilisation of human breast cancer progesteron(R_{5020}) receptor by sodium molybdate. *Clin Chim Acta* 1980, **103**, 367–373.

22. GULLINO PM, LANZEROTTI RH. Mammary tumor regression. II. Autophagy of neoplastic cells. *JNCI* 1972, **49**, 1349–1356.
23. LANZEROTTI RH, GULLINO PM. Activities and quantities of lysosomal enzymes during mammary tumor regression. *Cancer Res* 1972, **32**, 2679–2685.
24. DE LOECKER W, DOMS D, STAS ML. The effects of ionizing radiation on protein metabolism in stored rat skin. *Radiat Res* 1975, **61**, 216–229.