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Letter to the Editor

Bulk Protein Secretion Induced by Estradiol in MCF-7 Cells*

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IN THE human breast cancer cell line MCF-7, estradiol-17β (E_2) is known to induce the synthesis and subsequent secretion of some proteins [1–4]. On the other hand, only preliminary data [2] were reported on the influence of E_2 on the secretion of preexisting proteins. This question was investigated here.

MCF-7 cells were removed from a confluent monolayer by trypsinization and plated in two T-75 flasks at 10^6 cells per flask (growth medium: Earle's based minimum essential medium supplemented with L-glutamine, antibiotics and 10% steroid-depleted fetal calf serum [5]). After 72 hr of culture the growth medium was replaced by a leucine and isoleucine medium containing these two amino acids in a tritiated form (500 μl of each amino acid/20 ml medium; [^{3}H]leucine, 113 Ci/mmol; [^{3}H]isoleucine, 130 Ci/mmol, Amersham). Twenty-four hours later, this labeled medium was removed and the cells washed three times. Subsequently, 3 ml of medium with E_2 (Sigma) at a final concentration of 10^{-8} M were put in one of the two flasks and 3 ml of E_2-free medium in the other (control). After 6 hr of further culture, each medium was replaced and the incubation pursued for 18 hr. Media were all collected, centrifuged at 800 *g* for 10 min and finally dialysed against 2 l of 10 mM Tris-HCL buffer, pH 7.5, containing 1.5 mM EDTA and 6 mM β-mercaptoethanol (TEM buffer) to eliminate free tritiated amino acids. Cells were detached by a Hanks-EDTA treatment (10 ml; 15 min) and centrifuged at 800 *g* for 10 min. After removal of adsorbed medium (two additional centrifugations in TEM buffer), cells were homogenized in TEM buffer by means of a Thomas homogenizer and centrifuged at 105,000 *g* for 60 min to obtain the cytosols. The

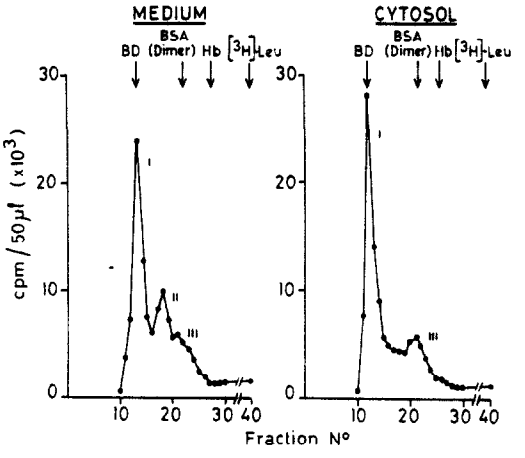

Fig. 1. Elution pattern of ^{3}H*-labeled proteins on Sephadex G-200 Superfine. Aliquots of* 500 μl *of medium or cytosol were sieved with TEM buffer (column* = 25 × 1.6 cm*). Forty fractions of* 0.900 ml *were collected. The radioactivity of* 50 μl *of these fractions was measured by liquid scintillation counting* (4 ml *Aqualuma Plus; Lumac). Blue dextran (BD), bovine serum albumin (BSA), human hemoglobin (Hb) and* [^{3}H]*leucine* ([^{3}H]*Leu) were sieved in an additional experiment for molecular weight calibration of the column.*

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Table 1. Influence of 10^{-8}M estradiol on the labeling of culture medium and cytosol proteins

		Estradiol (cpm)*	Control (cpm)*	Estradiol/control
Medium†	peak I	605,000	400,000	1.51
	peaks II + III	408,000	315,000	1.29
	total	1,013,000	715,000	1.42
Cytosol	peak I	490,000	500,000	0.98
	peak III	310,000	290,000	1.07
	total	800,000	790,000	1.01

*Chromatograms were adjusted to 1 mg of cytosol protein (measured concentration: E_2 = 0.15 mg/ml; control = 0.13 mg/ml).
†Medium: 6-24 hr after removal of labeled amino acids.

latter were dialysed against 2 l of TEM buffer. Cytosol proteins were measured by the Bio-Rad assay.

Microscopical examination of the culture just before cell harvest did not reveal any symptoms of cell death. Moreover, measurement of the total radioactivity (cellular extract and secretion products) recovered from E_2-treated and control cultures gave similar values, indicating the same cellular incorporation during the 24 hr of labeling.

Media and cytosols were sieved on Sephadex G-200 Superfine (the elution pattern of labeled proteins from the control culture are shown in Fig. 1). In both cases a bulk of labeled proteins eluted with the blue dextran, indicating that they were excluded from the column (peak I; $M_r \geqslant 200{,}000$). In the medium a second protein peak (II) was located before the bovine serum albumin (BSA) dimer. This peak partially overlapped a third peak (III) eluting between the BSA dimer and hemoglobin. The latter peak was also found in the cytosol. Of note, no major difference was observed between the elution patterns of the medium taken 6 and 24 hr after removal of the ^{3}H-labeled amino acids. Moreover, addition of E_2 to the cultures did not modify the elution volumes of these three peaks, nor did the hormone produce the appearance of new peaks (data not shown).

A quantitative analysis of the data revealed that E_2 increased the amount of the labeled proteins in the medium taken at the end of the experiment (+42%; Table 1). The phenomenon affected to a larger extent the proteins of the first chromatographic peak (I = +51% vs II + III = +29%). This increase could not be ascribed to a secretion process of E_2-induced proteins in view of the fact that the pool of labeled amino acids was largely reduced over this period of time (6-24 hr after their removal). The absence of influence of E_2 on the labeling of the cytosol proteins also eliminated the possibility of such an interference. Additional fractionations by SDS-polyacrylamide gel electrophoresis of identical amounts of labeled proteins from E_2-treated and control cultures gave similar patterns (Fig. 2). However, as described in a previous study [2], there was a marginal increase in the radioactivity corresponding to a medium protein of 50,000 M_r under E_2 stimulation (arrow).

These results suggest that, besides its selective inductive effect [1-4], E_2 also favors a bulk secretion process. As a matter of fact, the marked increase in amount of labeled medium proteins cannot be accounted for by the slightly higher secretion of a few specific proteins such as the one of 50,000 M_r. In any event, the latter does not elute within the first chromatographic peak for which the labeling increase is maximal. This concept is supported by morphological data showing that MCF-7 cells exposed to E_2 contain secretory granules and are covered by numerous microvilli [6]. The physiological significance of this bulk secretion phenomenon is unknown. Its relevance to the estrogenic control of cell proliferation is under investigation. In this regard, it should be stressed that preliminary experiments revealed that the antiestrogen nafoxidine at the growth inhibitory concentration of 10^{-6} M [5] did not produce a similar secretion stimulation.

REFERENCES

1. Westley B, Rochefort H. A secreted glycoprotein induced by estrogen in human breast cancer cell lines. *Cell* 1980, **20**, 353-362.
2. Mairesse N, Devleeschouwer N, Leclercq G, Galand P. Estrogen-induced synthesis and secretion of proteins in the human breast cancer cell line MCF-7. *J Steroid Biochem* 1981, **15**, 375-381.

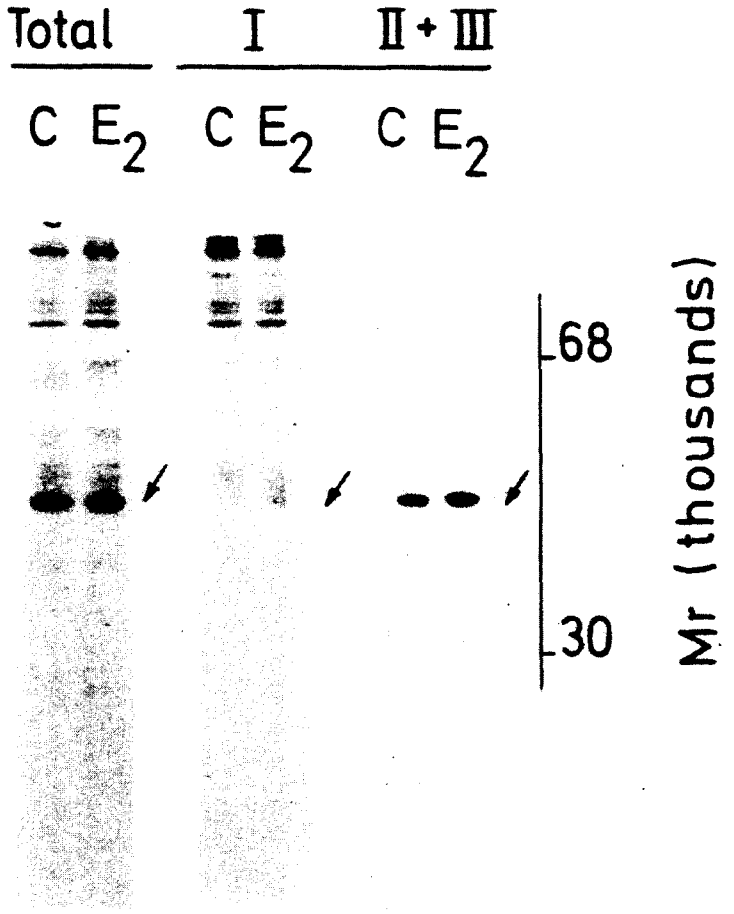

Fig. 2. Fluorogram of 7-20% acrylamide gradient gel electrophoresis of total and Sephadex G-200 Superfine fractionated medium. Aliquots were precipitated by 10% trichloracetic acid and washed with ethanol and diethylether. The precipitates were dissolved by boiling for 2 min in 50 µl SDS sample buffer. Aliquots of control and corresponding E_2-treated samples, containing identical amounts of radioactivity (~ 25×10^3 cpm), were then subjected to analytical SDS-PAGE according to the procedure of Laemmli [7] modified by Reiss and Kaye [8]. A calibration curve for molecular weight was drawn in parallel [(phosphorylase b (97,000), bovine albumin (68,000), carbonic anhydrase (30,000); New England Nuclear]. Gels were run overnight at 100 V and subsequently stained with Coomassie brillant blue and subjected to fluorography [9] using K-AR5 Kodak film. The arrows indicate the position of the 50,000 M_r protein.

3. Edwards DP, McGuire WL. Estrogen action in human breast cancer (Review). *Anticancer Res* 1982, **2**, 297-308.
4. Iacobelli S, Natoli V, Marchetti P. Stimulation by 17β-estradiol of a secreted glycoprotein in MCF-7 cells and in human breast cancer. *Recent Results Cancer Res* 1984, **91**, 295-300.
5. Leclercq G, Devleeschouwer N, Heuson JC. Guide-lines in the design of new antiestrogens and cytotoxic-linked estrogens for the treatment of breast cancer. *J Steroid Biochem* 1983, **19**, 75-85.
6. Vic P, Vignon F, Derocq D, Rochefort H. Effect of estradiol on the ultrastructure of the MCF-7 human breast cancer cells in culture. *Cancer Res* 1982, **42**, 667-673.
7. Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T_{41}. *Nature* 1971, **227**, 680-685.
8. Reiss N, Kaye AM. Identification of the major component of the estrogen induced protein of rat uterus as the BB isozyme of creatine kinase. *J Biol Chem* 1981, **256**, 5741-5749.
9. Bonner WH, Laskey PA. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Eur J Biochem* 1974, **46**, 83-88.