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Potentiation of Estradiol Binding to Human Tissue Proteins by Unsaturated Nonesterified Fatty Acids*

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ABSTRACT. Nonesterified fatty acids (NEFAs) have been recently shown in the rat to be involved in steroid hormone expression, having effects on plasma transport and intracellular activity. This study examines the influence of saturated and unsaturated NEFAs on estradiol (E_2) binding to cytosol from human uterus, breast, and melanoma. Binding was analyzed after separation with dextran-coated charcoal or hydroxylapatite and by sucrose density gradient centrifugation. Unsaturated NEFAs induced a 2- to 10-fold increase ($P < 0.001$) in E_2 binding to cytosol from normal, fibromatous, and neoplastic uteri, while saturated NEFAs had a slight inhibitory effect ($P < 0.05$). Similar effects were seen with cytosol from metastatic melanoma lymph nodes and neoplastic breast tissues. By contrast, unsaturated NEFAs did not increase E_2 binding to serum from these patients. Density gradient centrifugation indicated that the increased binding was associated with the proteins present in the 2- to 4 S region. Analysis of E_2 metabolites in the presence of unsaturated NEFAs showed the formation of water-soluble derivatives. Seventy percent of these E_2 derivatives were trichloracetic acid precipitable, suggesting a covalent link between the steroid and a protein. The existence of such water-soluble metabolites could be erroneously interpreted as a true binding to soluble cytoplasmic receptors. (*Endocrinology* **118:** 1–7, 1986)

THERE IS now evidence to suggest that nonesterified fatty acids (NEFAs) are involved in the expression of steroid hormones, having effects both on their plasma transport and on their intracellular activity. Some authors have reported that NEFAs may influence cell growth and multiplication by actions such as inhibition of 17β-hydroxysteroid dehydrogenase activity (1, 2) and changes in membrane fluidity, which result in changes in both receptor and enzymic activities (3, 4). Our previous studies have shown that unsaturated NEFAs inhibit estrogen binding to murine serum α-fetoprotein (5–7) and modulate positively or negatively estrogen binding in the immature rat uterus (6, 8). It has also been demonstrated that free fatty acids are able to modulate the specific interaction of steroids with human serum sex steroid-binding protein (9).

We now report the effects of various classes of NEFAs on the estradiol (E_2) binding to cytosol preparations from human uterus, breast cancer, and melanoma tissue and their influence on estrogen metabolism within these organs.

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Materials and Methods

Reagents

[6,7-^{3}H]E_2 were purchased (46 Ci/mmol) and [2,4,6,7-^{3}H]E_2 (107 Ci/mmol) were purchased from Amersham International Ltd. Unlabeled steroids, E_2, and diethylstilbestrol (DES), were supplied by Roussel Uclaf Research Centre (Romainville, France). The following saturated and unsaturated fatty acids were purchased from Sigma Chemical Co. (St. Louis, MO): tetradecanoic acid (C14:0, myristic acid), hexadecanoic acid (C16:0, palmitic acid), octadecanoic acid (C18:0, stearic acid), *cis*-7-hexadecaenoic acid (C16:1, palmitoleic acid), *cis*-9-octadecaenoic acid (C18:1, oleic acid), 9,12-octadecadienoic acid (C18:2, linoleic acid), 6,9,12-octadecatrienoic acid (C18:3, linolenic acid), 5,8,11,14-eicosatetraenoic acid (C20:4, arachidonic acid), 4,7,10,13,16,19-docosahexaenoic acid (C22:6) and 7,10,13,16-docosatetraenoic acid (C22:4).

Tissue preparation

Postoperative tissues samples (uterus, breast, and metastatic melanoma lymph nodes) were obtained from the departments of Surgery and Anatomo Pathology, Hopital Xavier Bichat (Paris, France) and kept frozen in liquid nitrogen until processed. Tissues were suspended in TE buffer (10 mM Tris–HCl, 1.5 mM EDTA, 2 mM sodium molybdate, 10% glycerol, pH 7.4) and homogenized in an all-glass homogenizer (Kontes, Vineland, NJ). Homogenates were centrifuged at 25,000 × g for 30 min, and the resulting supernatants were recentrifuged at 105,000 × g for 1 h. All manipulations were done at 4 C. The final supernatant, referred to as cytosol, was used in the binding experiments.

Binding studies

Incubation. Cytosol preparations (0.5 ml, containing 1–1.5 mg protein) were incubated for 16 h at 4 C with a range of labeled E_2 concentrations (9–36 pmol/mg protein). The effects of each class of NEFA were tested by adding NEFA (25–50 μg/mg protein, *i.e.* 80–175 nmol/mg) to the cytosol-E_2 mixture. Nonspecific binding was determined using unlabeled DES (2.5 nmol/mg protein).

Fatty acid solubility was also checked. At the concentrations used, (≤100 μg/mg protein) the solubilities were 80% for saturated and 100% for unsaturated fatty acids. After incubation, the bound and free hormone levels were estimated after separation or the system was analysed by density gradient ultracentrifugation.

Dextran-coated charcoal method (DCC). Bound and free hormone fractions were separated by incubation with a suspension of DCC (0.5% charcoal, 0.05% dextran) for 2 h at 4 C followed by centrifugation at 3000 rpm for 10 min.

Aliquots of supernatant were counted in 4 ml ACS II scintillant (Amersham) in a Kontron S4000 scintillation counter (Kontron Electronics, Inc., Redwood City, CA). The system was checked to ensure that all free hormone was extracted by the DCC, even at the highest NEFA concentration.

Hydroxylapatite method (HAP). Bound hormone was removed by adding 200 μl HAP gel (Sigma), suspended in TE buffer, to the binding mixture and incubating at 4 C for 30 min, with agitation at 10-min intervals. The matrix containing the protein-bound hormone was collected by centrifugation at 800 × *g* for 5 min and the pellet washed twice with 2 ml Tris buffer containing 1% (vol/vol) Tween 80. The protein-bound steroid was then extracted from the pellet with 3 ml absolute ethanol and separated by centrifugation at 1600 × *g* for 10 min. The radioactivities in ethanolic supernatant and pellet were measured separately.

Density gradient ultracentrifugation. The incubation conditions were as previously described (8). Cytosol protein (3 mg) was incubated overnight at 4 C with 3 pmol [^{3}H]E_2/mg protein, either alone or in the presence of various nonradioactive fatty acids. Nonspecific binding was estimated by carrying out parallel centrifugations in the presence of excess DES (2.5 nmol/mg protein). Free estradiol was removed by DCC treatment, and the cytosol mixture was layered onto a 5–20% sucrose gradient. After centrifugation at 40,000 rpm for 15 h in a Beckman SW50 rotor (Beckman Instruments, Fullerton, CA), the tubes were removed and fractions collected from the top of the gradient using an Isco Inc. (Lincoln, NE) fraction collector. One-tenth milliliter of each fraction was added to scintillation fluid and the radioactivity measured. Density gradients were calibrated with horseradish peroxidase (3.6S) and *Aspergillus niger* glucose oxidase (7.9S) markers according to Martin and Ames (10)

Estrogen metabolite analysis

Bound and free steroids were separated with DCC and the aqueous bound fraction extracted with organic solvent (cyclohexane-ethylacetate, vol/vol), as described in Fig. 1.

Organic phase. The solution was evaporated to dryness and the metabolites separated by TLC in a benzene-methanol-acetic acid (96:4:1) system. Estrone and E_2 were used as standards. One-cm wide bands of gel were removed by scraping and their radioactivity measured in 5 ml ACS II.

Aqueous phase. Water-soluble metabolites were incubated overnight with *Helix pomatia* digestive juice at pH 5.3, and the steroid fraction was extracted with ether. The radioactivity in ether extract was measured. The remaining aqueous solution was made 10% in trichloroacetic acid (TCA) and protein precipitated by incubation for 1 h at 4 C. The precipitate was centrifuged, and the pellet was washed three times with 10% TCA, and dissolved in 0.5 ml 1 N NaOH. Aliquots of both the precipitate and the remaining aqueous supernatant were taken for radioactivity measurement.

Fatty acid analysis

Endogenous fatty acids were extracted from cytosol preparations with cyclohexane-ethyl acetate (1:1) and quantified by gas chromatography as previously described (11).

Protein measurement and statistical analysis

Proteins were measured by the method of Lowry *et al.* (12) and statistical analysis was performed using Student's *t* test.

Results

NEFA effects on estradiol binding by cytosol proteins

Separation of bound and free steroid by DCC or HAP methods. There was a dramatic increase (2- to 10-fold, $P < 0.001$) of the binding of E_2 to cytosol from human normal, fibromatous, and neoplastic uteri on incubation with unsaturated NEFAs such as oelic (C18:1), arachidonic (C20:4) and docosahexaenoic (C22:6) acids, while saturated compounds such as stearic acid (C18:0) exerted a slight inhibitory action ($P < 0.05$). Results obtained using DCC separation are presented in Fig. 2. Similar results were obtained using HAP separation, *e.g.* when human fibroma cytosol was incubated with [^{3}H]E_2 alone, the binding levels were 540 fmol/mg protein after HAP extraction, and reached 842 fmol/mg protein in the presence of C18:1. It should be pointed out that the NEFA concentrations (≃100 nmol/mg protein) used to obtain these effects were 2- to 4-fold higher than endogenous NEFA levels in these tissues (Table 1).

A large increase in E_2 binding was also seen in other pathological tissues, such as the metastatic melanoma lymph nodes and neoplastic breast tissues. The E_2 binding levels in these tissues was similar to that of healthy uteri (≦30 fmol/mg protein), but the influence of NEFAs was considerably greater. In the presence of C22:6 acid, the E_2 binding level to metastatic melanoma lymph node cytosol reached 1724 ± 606 fmol/mg protein. Binding of E_2 to cytosol from neoplastic breast tissue increased to

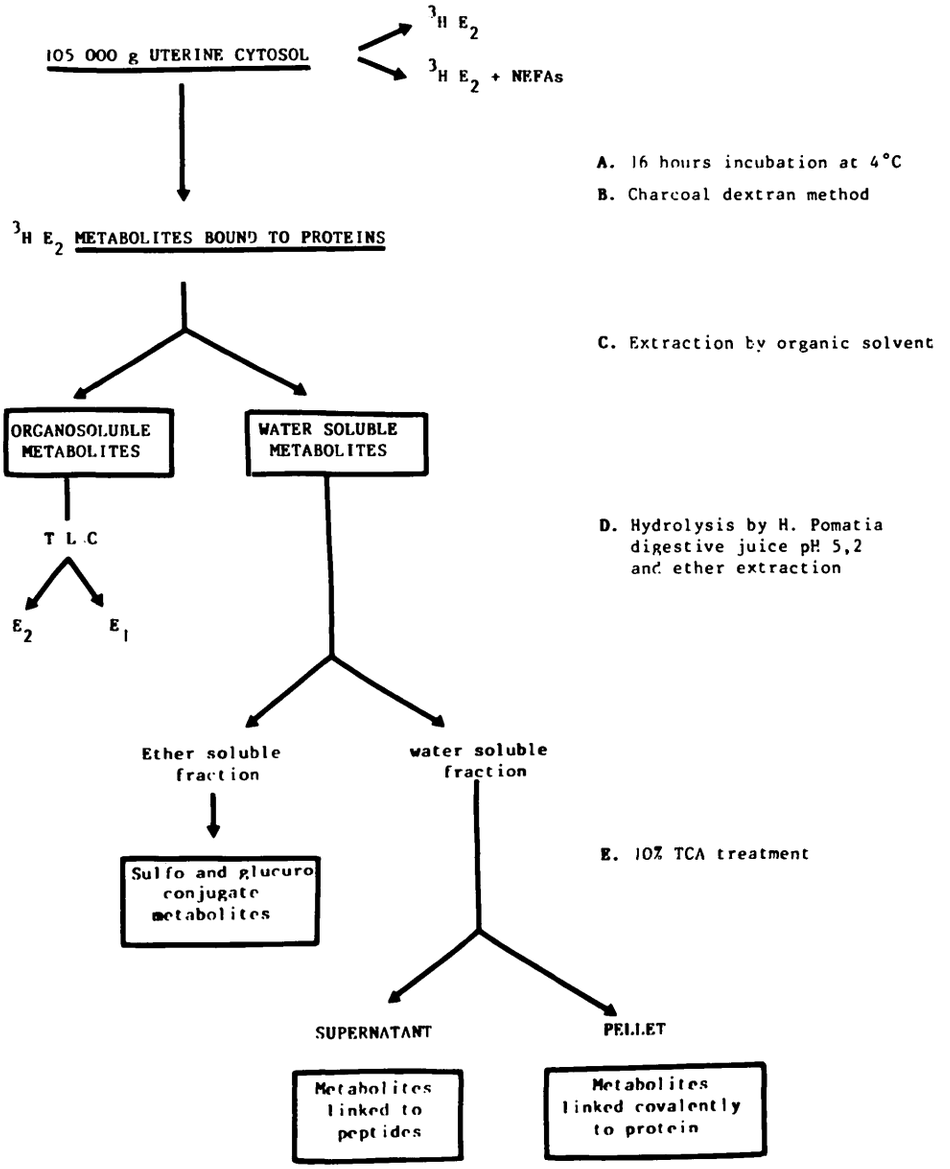

FIG. 1. Steps for analysis of estrogen metabolites.

335 ± 50 fmol/mg protein in the presence of the same unsaturated NEFA (13).

The degree of enhancement is clearly not constant from one tissue to another. The most positive responses were seen in melanoma tissue and in healthy or neoplastic uteri (Fig. 2). Furthermore, the binding potentiation was observed in presence of unsaturated NEFAs independent of the initial level of the E_2 binding (5–500 fmol/mg protein). This enhancement appeared to be tissue-specific, since the binding of E_2 by serum from these patients was not enhanced by unsaturated fatty acids (results not shown).

Density gradient analyses. The results of the analyses of E_2 binding to the cytosols from human uteri (healthy, fibromatous, and neoplastic) in the presence of NEFAs are shown in Fig. 3. The data shown are net counts per min, after subtraction of the apparent nonspecific binding values obtained by running parallel gradients in the presence of excess DES. The density gradient profiles of E_2 binding show that, for healthy (Fig. 3A) and cancerous uterine tissue (Fig. 3C), there were peaks in the 2–4S region only, while for the fibromatous tissue (Fig. 3B), there were two binding peaks, one at 4S and the other at 8S. In the region of 2–4S, there was a potentiation of E_2 binding by unsaturated NEFAs (C18:1, C22:6) in normal, fibromatous, and neoplastic uterine cytosol. In those tissues showing no 8S peak of E_2 binding (Fig. 3, A and C), there was a general increase in E_2 binding in the presence of unsaturated NEFAs but no clear peak. In contrast, the fibromatous tissue showed a reduction in E_2 binding in the 8S region (Fig. 3B).

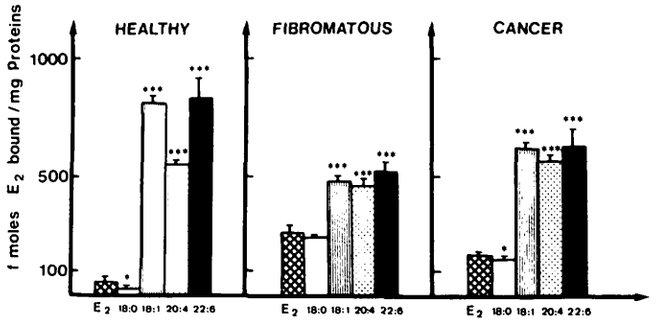

FIG. 2. Binding of E_2 to uterine cytosol. Cytosol containing 1.5 mg protein was incubated overnight at 4 C with 9.6 pmol [^{3}H]E_2/mg protein, either alone or in the presence of different NEFAs: C18:0, C18:1 (117 nmol/mg proteins), C20:4 (109 nmol/mg protein), C22:6 (101 nmol/mg proteins). Free E_2 was removed with DCC. The results are expressed as femtomole equivalents of [^{3}H]E_2 per mg of protein. Means ± SD of triplicate experiments for each pathological tissues. Significant difference from initial E_2 binding are indicated. *, $0.02 < P < 0.05$; ***, $P < 0.001$.

TABLE 1. Concentration and relative percentage of NEFAs in the cytosols of normal and pathological human uteri

	Uterine tissue		
	Healthy	Fibromatous	Cancerous
	Total NEFAs (nmol/mg protein)		
	40.5	26	24
Saturated	57[a]	38.5[a]	21[a]
Monounsaturated	15	19	38
Di-tri unsaturated	10	11.5	17
Polyunsaturated	18	31	24

[a] Values (% of NEFAs) are average of duplicate determinations on each tissue.

Effects of E_2/NEFA ratio on E_2 binding

The effect of E_2/NEFA ratio on E_2 binding to cytosol was tested by incubating fibroma cytosol with fixed NEFA concentrations (≃100 nmol/mg protein) and increasing concentrations of E_2 ($1–6 \times 10^{-8}$ M). The lowest concentration of E_2 was chosen to saturate the cytosol binding capacity. All the data shown are for specific binding, *i.e.* after subtraction of nonspecific binding. Figure 4 illustrates the importance of the E_2/NEFA ratio in controlling the degree of E_2 binding to cytosol. Binding of E_2 increased from 638 ± 57 fmol/mg protein at an E_2 concentration of 1×10^{-8} M (E_2/NEFA ratio = 3×10^{-5}) to 1981 ± 164 fmol/mg protein for an E_2 concentration of 6×10^{-8} M (E_2/NEFA ratio = 17×10^{-5}), when incubated in the presence of C18:1.

Effect of NEFAs on the E_2 metabolism

Cytosols from melanoma and human uteri (healthy, fibromatous, and neoplastic) were incubated with a range of saturated and unsaturated NEFAs in the presence of E_2. The levels of organic solvent-soluble E_2 metabolites were similar in incubations with and without NEFAs (Fig. 5A). TLC analysis showed essentially unmetabolized E_2 (90%). The bound estrogens in aqueous phase were subsequently extracted and analyzed as outlined in Fig. 1. The increase in E_2 binding to cytosol proteins stimulated by unsaturated NEFAs appeared to be due to an increase in water-soluble derivatives (Fig. 5B).

When this fraction was analyzed in greater detail (Table 2), it was found that the increase was not due to the sulfate- or glucuronide conjugates as they accounted for only 5–10% of the aqueous phase. Most (60–70%) of these water-soluble derivatives were TCA-precipitable while 30–40% were not.

Discussion

We have shown that unsaturated NEFAs may enhance the binding of estradiol to cytosol preparations from a variety of normal and pathological human tissues. Saturated NEFAs, however, are slightly inhibitory. Furthermore, the degree of E_2 binding seems to be directly correlated with both steroid concentration and the E_2/unsaturated NEFA ratio.

The binding of E_2 to the serum of these same patients was not increased by unsaturated NEFAs. Other studies on the interaction between human serum sex hormone-binding proteins and sex steroids have shown that unsaturated NEFAs are very inhibitory, while saturated NEFAs are slightly stimulatory (9). Thus, this remarkable and important stimulatory effect of unsaturated NEFAs on E_2 binding seems to be specific to the intracellular compartment.

When the E_2 binding to uterine cytosol proteins was studied by sucrose gradient ultracentrifugation, the addition of unsaturated NEFAs induced a dramatic increase in the overall E_2 binding in the 2–4S region and appeared to shift the interaction away from the 8S steroid-protein complex when it is present toward a new group of water-soluble derivatives.

The majority of the new estradiol derivatives were TCA-precipitable, suggesting that they may be estradiol covalently linked to protein to form estroproteins. The remaining 30% of the nonprecipitable water-soluble derivatives could be formed from E_2 metabolites linked to small peptides such as glutathione (14, 15) or as a result of [^{3}H]H_2O production (16).

A similar increase in water-soluble metabolites of estrogens has been reported after incubation with rat liver microsomes (14, 15) and Syrian hamster embryo fibroblasts (17). It has been suggested that this compound results from the peroxidase activity of prostaglandin synthetase (PGS) (17), an enzyme which has recently been shown to be present in both uterine (18) and breast

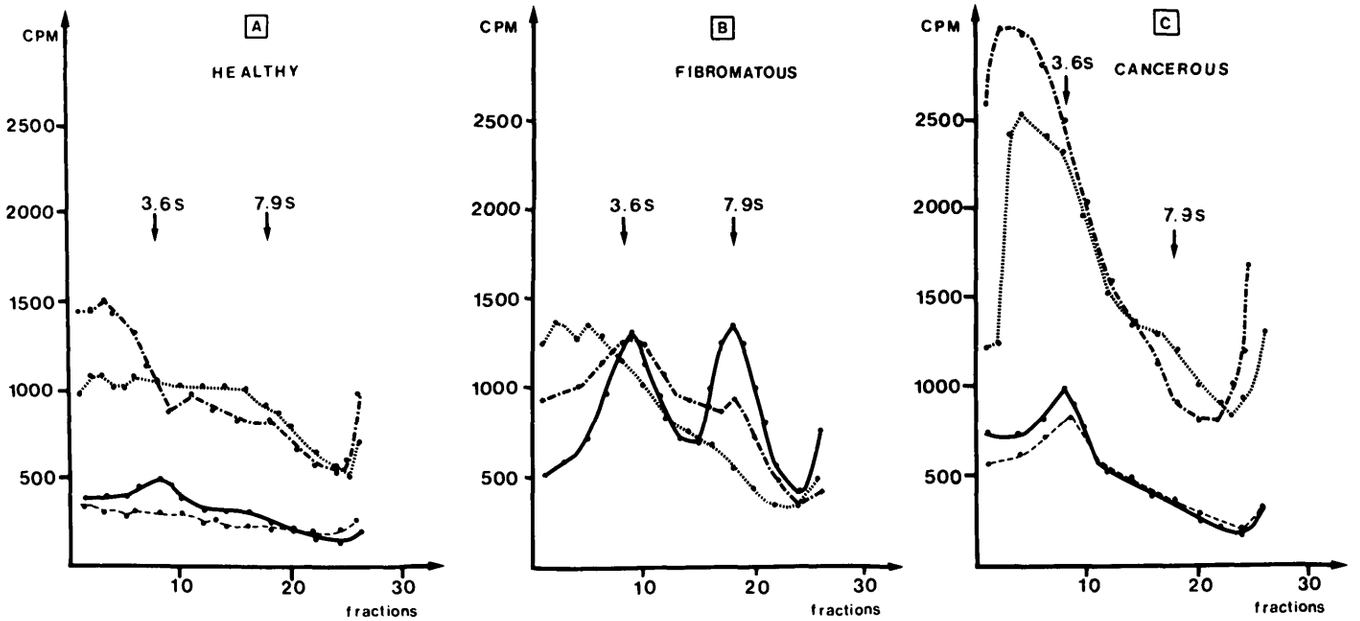

FIG. 3. Density gradient analysis of the effect of fatty acid on [^{3}H]E_2 binding to healthy (A), fibromatous (B), and cancerous (C) cytosol. An aliquot of 0.35 ml cytosol (3 mg protein) was incubated overnight at 4 C with 3 pmol [^{3}H]E_2/mg protein ▬ either alone or in the presence of 25 μg NEFA/mg protein *i.e.* C18:0 (88 nmol/mg - - -); C18:1 (89 nmol/mg ·····); C22:6 (76 nmol/mg ·—·—). Ultracentrifugation is performed at 40,000 rpm for 15 h in a Beckman SW-50 rotor. The data shown are net counts per min, after subtraction of nonspecific binding values.

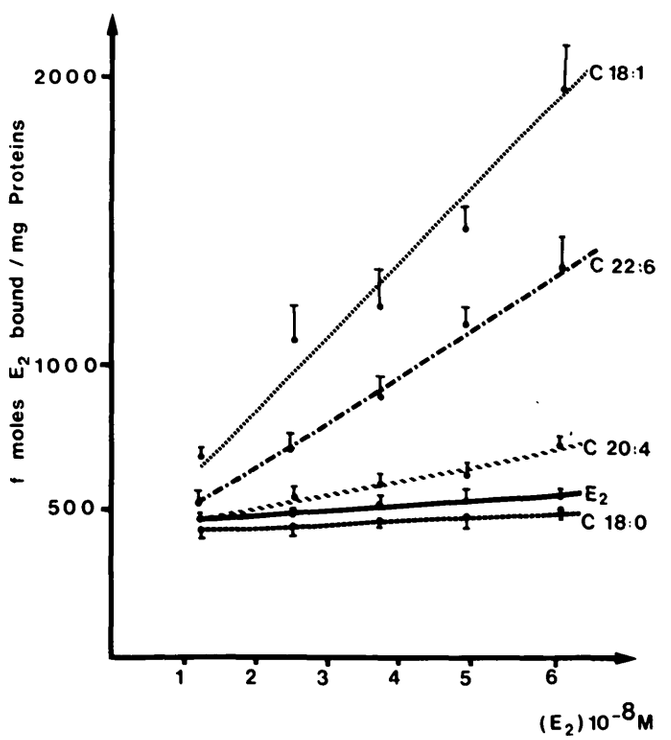

FIG. 4. Effect of ratio E_2/NEFAs on E_2 binding to cytosol. One-half milliliter of fibromatous cytosol (1 mg protein) was incubated overnight at 4 C with increasing [^{3}H]E_2 concentrations (9–36 pmol/mg protein) in the presence of 175 nmol C18:0/mg; 177 nmol C18:1/mg; 164.5 nmol C20:4/mg; 152 nmol C22:6/mg. Free hormone was removed with DCC.

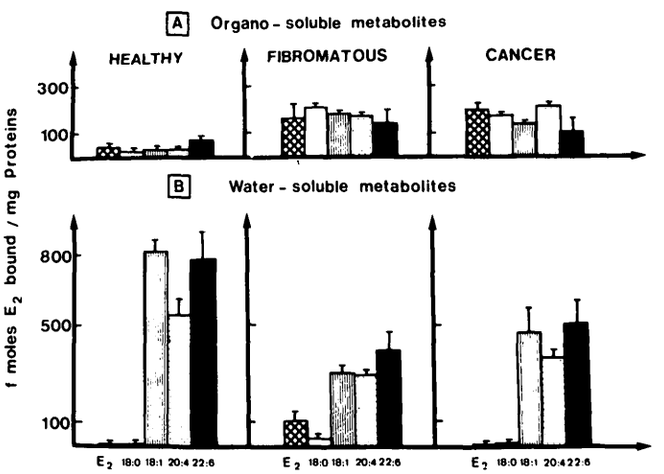

FIG. 5. Analysis of E_2 metabolites bound to normal, fibromatous, and cancerous uterine cytosol proteins. The experimental conditions of incubation were the same as described in Fig. 2. Separation of organic solvent-soluble and water-soluble fractions is outlined in Fig. 1. The results are expressed as femtomole equivalents of [^{3}H]E_2 per mg of protein. Means ± SD of triplicate experiments for each pathological tissues are shown.

(19) tissues.

These observations may not, however, be sufficient to fully explain our results. These studies have been carried out with cytosol and not on microsomal preparations. Furthermore we observed these stimulations in the presence of the major PGS substrate, *i.e.* arachidonic acid

TABLE 2. Analysis of water-soluble E_2 metabolites in normal and pathological uterine cytosol

	E_2 (fmol) bound/mg uterine proteins					
	Healthy		Fibromatous		Cancerous	
	Precipitable TCA	Nonprecipitable TCA	Precipitable TCA	Nonprecipitable TCA	Precipitable TCA	Nonprecipitable TCA
Control [^{3}H]E_2	0	5 ± 3	14 ± 5	95 ± 40	0	2 ± 1
NEFAs + [^{3}H]E_2						
18:0	5 ± 2	0	9 ± 4	17 ± 7	10 ± 5	2 ± 1
18:1	630 ± 25	178 ± 27	254 ± 26	48 ± 5	350 ± 52	128 ± 46
20:4	410 ± 16	136 ± 9	207 ± 14	91 ± 6	255 ± 15	106 ± 10
22:6	489 ± 68	174 ± 35	281 ± 58	105 ± 19	385 ± 80	110 ± 25

The results are means of triplicate experiments for each analyzed pathological uteri.

(n–6) but also with other fatty acids such as oleic (n–9) and docosahexaenoic (n–3) acids, which are not PGS substrates.

Preliminary studies performed with fibroma cytosol in the presence of indomethacin (0.5 mM) indicate that this PGS inhibitor does not inhibit the formation of these water-soluble metabolites. It may be that the unsaturated NEFAs have an effect on estrogen metabolism as a result of their action on other oxydative systems, such as the lipoxygenase system or the peroxidase activity of eosinophils recently described in the uterus (20).

The presence of these water-soluble metabolites may be erroneously interpreted as the existence of a true positive target tissue for estrogens. This may explain why a percentage of estrogen receptor-positive tumors do not respond to hormone therapy.

The demonstration of the presence of inducible water-soluble estrogen derivatives within the cytosol of both target (uterus, breast) and nontarget (melanoma) (21) tissues raises many questions as to their nature and function. Before their biological importance can be clearly evaluated, it will be necessary to elucidate their structure and metabolism. It will be of particular interest to evaluate their effect on the growth of estrogen-dependent tissues or tumors and their overall influence on estrogen potency.

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References

1. Wahawlsan R, Gorelli TA 1980 Steroidal control of rat uterine 17β-hydroxysteroid deshydrogenase activity. Steroids 36:115
2. Blomquist CH, Kotts CE, Habanson E 1980 Phospholipase A2 inactivation of microsomal 17β-hydroxysteroid oxidoreductase: rates of phospholipides hydrolysis and enzyme inactivation, effects of hydrolysis products and properties of the phospholipase A2-treated enzyme. Steroids 36:97
3. Burns CP, Luttenegger DG, Dudley DT, Buettner GR, Spectror AA 1979 Effect of modification of plasma membrane fatty acid composition on fluidity and methotrexate transport in I 1.210 murine leukemia cells. Cancer Res 39:1726
4. Pascaud DM 1980 Acides gras essentiels et développement animal. Ann Nutr Aliment 34:275
5. Benassayag C, Vallette G, Delorme J, Savu L, Nunez EA 1980 High affinity of non esterified polyunsaturated fatty acids for rat alpha-fetoprotein (AFP). Oncodevel Biol Med 1:27
6. Nunez EA, Benassayag C, Vallette G, Savu L, Clerc-Hofmann F, Delorme J, Christeff N 1983 The role of non esterified fatty acids and alpha-fetoprotein in the estrogen-dependent endocrine system. Ann NY Acad Sci USA 417:137
7. Savu L, Benassayag C, Vallette G, Christeff N, Nunez EA 1981 Mouse alpha-fetoprotein and albumin. A comparative study of their binding properties with estrogen and fatty acid ligands. J Biol Chem 256:9414
8. Clerc-Hofmann F, Vallette G, Secco-Millet C, Christeff N, Benassayag C, Nunez EA 1983 Inhibition de la fixation utérine des oestrogènes par les acides gras insaturés chez la ratte immature. C R Seances Acad Sci [III] 296:53
9. Martin ME, Vranckx R, Benassayag C, Nunez EA, Modulating effects of NEFAs on the interactions between sexual steroid and human sex binding protein (SBP). Abstracts of The Seventh International Congress of Endocrinology, Québec City, Quebec, Canada, 1984, p 934 (Abstract)
10. Martin RG, Ames BN 1962 A method for determining the sedimentation behavior of enzymes: application to protein mixtures. J Biol Chem 236:1372
11. Delorme J, Benassayag C, Christeff N, Vallette G, Savu L, Nunez EA 1984 Age-dependent responses of the serum non esterified fatty acids to adrenalectomy and ovariectomy in developing rats. Biochim Biophys Acta 792:6
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265
13. Benassayag C, Hassid J, Vallette G, Raymond JP, Nunez EA, Unsaturated nonesterified fatty acids (NEFAs) highly potentialize the estradiol binding to human tissular proteins: true estrogen binding receptor or estradiol metabolites? Abstracts of The Seventh International Congress of Endocrinology. Quebec City, Quebec, Canada, 1984, p 343 (Abstract)
14. Marks F, Hecker E 1969 Metabolism and mechanism of action of estrogens. XII. Structure and mechanism of formation of water-soluble and protein-bound metabolites of estrone in rat liver microsomes *in vitro* and *in vivo*. Biochim. Biophys Acta 187:250
15. Jellinck PH, Lewis J, Boston F 1967 Further evidence for the formation of an estrogen-peptide conjugate by rat liver *in vitro*. Steroids 10:329
16. Zava DT, Goldhirsch DT 1983 Estrogen receptor in malignant melanoma: fact or artefact. Eur J Cancer Clin Oncol 19:1151
17. Degen GH, Wong A, Eling TE, Barett JC, McLachlan JA 1983 Involvement of prostaglandin synthetase in the peroxidative metabolism of diethylstilbestrol in Syrian hamster embryo fibroblast

cell culture. Cancer Res 43:992
18. Kelly RW 1981 Prostaglandin synthetase in the male and female reproductive tract. J Reprod Fertil 62:293
19. Rolland PH, Martin PL, Jaquemier J, Rolland AM, Toja M 1980 Prostaglandin production and metabolism in human breast cancer. Adv Prostaglandin Thromboxane Res 6:575
20. Lyttle CR, Medlock KL, Sheehan DM 1984 Eosinophils as the source of uterine nuclear type II estrogen binding sites. J Biol Chem 259:2697
21. Carsten R, Pedersen L, Henning T 1983 Antiandrogen and antiestrogen in the treatment of advanced malignant melanoma. J Steroid Biochem [Suppl]19:1255 (Abstract)

International Symposium on Recent Advances in Adrenal Regulation and Function

An International Symposium on Recent Advances in Adrenal Regulation and Function will be held in Madrid September 19 and 20, 1986, in conjunction with the VII International congress on Hormonal Steroids. The meeting is sponsored by Serono Symposia. Topics include: Regulation of ACTH secretion, Intraadrenal Regulation Mechanisms of Steroid Secretion, Regulation of Adrenal Androgen Secretion and Function, Cellular and Molecular Mechanisms of Glucocorticoid Action, and Clinical Aspects (Hypothalamic-pituitary-adrenal function in endocrine and psychiatric disorders, molecular pathophysiology of congenital adrenal hyperplasia, actions and applications of antiglucocorticoids, physiology of surgical stress).

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