

# A Correlation between Estrogen Sulfotransferase Levels and Estrogen Receptor Status in Human Primary Breast Carcinoma<sup>1</sup>

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## ABSTRACT

Estrogen sulfotransferase (EC 2.8.2.4) activity and estrogen receptor levels were measured in 32 human primary breast cancer cytosol preparations. Two types of tumors were identified: type 1, in which estrogen sulfotransferase levels were low (<40 pmol 17 $\beta$ -estradiol 3-sulfate formed per mg protein per 2 hr) and were independent of [<sup>35</sup>S]adenosine 3'-phosphate 5'-phosphosulfate production from [<sup>35</sup>S]sulfate and adenosine triphosphate, and type 2, in which estrogen sulfotransferase levels ranged from 50 to 200 pmol 17 $\beta$ -estradiol 3-sulfate per mg protein per 2 hr and were correlated with [<sup>35</sup>S]adenosine 3'-phosphate 5'-phosphosulfate formation ( $r = 0.70$ ;  $p < 0.005$ ). In type 1 tumors, 11 of 16 were estrogen receptor negative; in type 2 tumors, 2 of 16 were receptor negative. Estrogen sulfotransferase levels in receptor-negative tumors were significantly lower than the levels in receptor-positive tumors ( $p = 0.025$ ).

## INTRODUCTION

There is now very substantial and well-documented evidence that estrogen Rc<sup>3</sup> levels in human mammary tumors are related to subsequent response to hormone-ablative procedures. One other biochemical parameter measured in human mammary tumor preparations has also been shown to relate to prognosis and response to adrenalectomy in late-stage breast cancer and to time of recurrence in early breast cancer. This is the assessment of steroid sulfurylation (4). Therefore, Rc and steroid sulfurylation should show some correlation when measured in human mammary tumors. Despite the very large numbers of publications dealing with Rc levels and clinical response to hormone ablation, very little data have been presented on steroid sulfurylation in mammary carcinoma despite the obvious implications of gaining further understanding of control mechanisms governing hormone action in such tissue. In 1973, Leung *et al.* (6) demonstrated that a close correlation existed between steroid sulfurylation and the level of estrogen Rc in human primary breast cancer preparations. In 26 primary tumors, 13 were estrogen Rc positive, and these same 13 all sulfurylated 17 $\beta$ -estradiol and dehydroepiandrosterone. In 8 estrogen Rc-negative tumors, estrogen sulfotransferase was present in 3 and absent in 5; in 5 tumors borderline for estrogen Rc, estrogen sulfotransferase was present in 2 and absent in

3. These workers reported objective remission in 12 of 12 patients with estrogen Rc-positive tumors and lack of response in 8 patients with estrogen Rc-negative tumors. In their summary, they stated that "no correlation of steroid sulfurylation values to clinical response in these patients could be demonstrated." However, the sulfurylation values in the greater percentage of these patients were determined on metastases to tissues which themselves have high steroid-sulfurylating ability, such as adrenal and liver. Values for metastases to these sites were much higher than those obtained in primary breast tumors and would therefore be unsuitable for evaluation along with Rc. Indeed, this was presumed in the text of the paper (6).

One other study reported a lack of correlation between these same parameters (2). Unfortunately, in this instance, only ratios of the sulfurylation of dehydroepiandrosterone and 17 $\beta$ -estradiol, rather than the individual values, were compared to estradiol Rc. In addition, steroid sulfurylation was detected in only 51% of 41 primary tumors, a percentage which is unusually high compared to other reported values (1).

In 1972, studies were commenced in our laboratory aimed at confirming the original work of Dao and Libby (4) pertaining to steroid sulfurylation and time of recurrence in early breast cancer. Results of this study were published in 1977 (1), but insufficient numbers of patients had shown recurrence to fully evaluate the data, and follow-up has unfortunately not been possible in many cases. We now wish to report results of a separate study in which estrogen Rc levels, [<sup>35</sup>S]sulfate activation to [<sup>35</sup>S]PAPS, and subsequent formation of [<sup>35</sup>S]E<sub>2</sub>S have been assessed in human mammary tumor cytosol preparations. Two groups of tumors have been identified: one showing low estrogen sulfurylation and being predominantly Rc negative; and another group having high estrogen-sulfurylating ability and being almost exclusively Rc positive.

## MATERIALS AND METHODS

**Tumor Tissue.** Initially, only fresh tumor tissue obtained from the operating theater was used, and this was immediately processed. However, it became necessary to use frozen specimens in order to obtain a sufficient number of tumors for statistical evaluation of the data. These tumors were immediately frozen in dry ice, transported to the laboratory frozen, and subsequently stored in liquid N<sub>2</sub> or at -70°. Tissues were then processed within 1 week of storage. In order to evaluate the effect of freezing, a tumor specimen was freed of fat and connective tissue, cut into small pieces, and mixed thoroughly to provide a homogeneous sample. Portions were then assayed directly or after storage at -70° for periods up to 1 week. [<sup>35</sup>S]PAPS synthesis and [<sup>35</sup>S]E<sub>2</sub>S synthesis were not significantly altered when measured in the resulting cytosol preparations.

**<sup>35</sup>S-sulfurylated Nucleotides.** Formation and separation of

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<sup>3</sup> The abbreviations used are: Rc, cytosol receptor; PAPS, adenosine 3'-phosphate 5'-phosphosulfate; E<sub>2</sub>S, 17 $\beta$ -estradiol 3-sulfate; APS, adenosine 5'-phosphosulfate.

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[<sup>35</sup>S]APS and [<sup>35</sup>S]PAPS from ATP, Mg<sup>2+</sup>, and [<sup>35</sup>S]sulfate by tumor cytosol preparations were carried out as described previously (1). The labeled nucleotides were counted by liquid scintillation using a Packard 2650 instrument with external quench correction.

**Estrogen Sulfurylation.** Formation of [<sup>35</sup>S]E<sub>2</sub>S from ATP, [<sup>35</sup>S]sulfate, and 17β-estradiol was measured as described previously (1).<sup>4</sup>

**Estradiol Rc.** This was assayed by the European Organization for Research on Treatment of Cancer method (5) using at least 6 duplicate concentrations of 17β-[<sup>3</sup>H]estradiol in the presence and absence of a 100-fold excess of unlabeled 17β-estradiol. The data were analyzed by the method of Scatchard (10), following subtraction of the specific estrogen binding. Tumor cytosols with Rc less than 6 fmol/mg protein were judged as Rc negative. The sources of chemicals and all other procedures were as previously described (1).

**RESULTS**

All of the 32 primary tumor cytosol preparations examined showed formation of [<sup>35</sup>S]APS and [<sup>35</sup>S]PAPS from [<sup>35</sup>S]sulfate and ATP. Synthesis of PAPS and APS were significantly correlated (Chart 1, *r* = 0.69, *p* < 0.001). Ten of the extracts were made from unfrozen tissue, and the PAPS levels in these cases were evenly distributed throughout the values found using frozen tumor specimens. The time course of synthesis of the sulfurylated nucleotides was examined in 3 tumors. Very similar results were obtained, and one set of data is shown in Chart 2a. Formation of PAPS takes place in 2 stages; namely, ATP and SO<sub>4</sub><sup>2-</sup> react to form APS and pyrophosphate catalyzed by ATP sulfurylase (EC 2.7.7.4), and the APS is then converted to PAPS by APS kinase (EC 2.7.1.25) (7). In the tumor cytosol preparations (Chart 2), a build-up in concentration of APS occurred prior to formation of significant concentrations of PAPS, although some of the latter would have been utilized in the formation of sulfate esters of endogenous acceptors present in the extracts and acted on by various sulfotransferases (1). The attainment of a 1 μM concentration of APS after 30 min (Chart 2a), despite the unfavorable equilibrium for the reaction catalyzed by ATP sulfurylase (*K* = 10<sup>-8</sup>), would be possible due to the ubiquitous presence of pyrophosphatase (7). However, the tumor cytosols contain a 3'-nucleotidase which can convert PAPS to APS (1), and this reaction could contribute to the observed APS levels shown in Chart 2a. Addition of excess 3',5'-ADP, to act as an alternate substrate to the 3'-nucleotidase, did not greatly alter the concentration of APS attained over the time period studied (Chart 2b). This indicated that 3'-nucleotidase in the tumor cytosol played only a minor role in APS formation. Ester [<sup>35</sup>S]sulfate synthesis was completely blocked by addition of 1.25 mM 3',5'-ADP, and this would account for the accompanying rise in PAPS concentration (Chart 2b).

When estrogen sulfotransferase activities were plotted against PAPS synthesis, 2 types of tumors became evident (Chart 3). In type 1, estrogen sulfurylation was low (< 40 pmol E<sub>2</sub>S per mg protein per 2 hr) and remained low even when PAPS levels were high. These tumors were predominantly

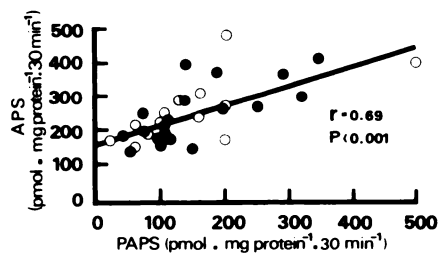


Chart 1. Synthesis of [<sup>35</sup>S]APS and [<sup>35</sup>S]PAPS from ATP, Mg<sup>2+</sup>, and [<sup>35</sup>S]sulfate in the cytosol fraction of human primary breast cancer specimens. ●, estrogen Rc-positive tumors; ○, estrogen Rc-negative tumors.

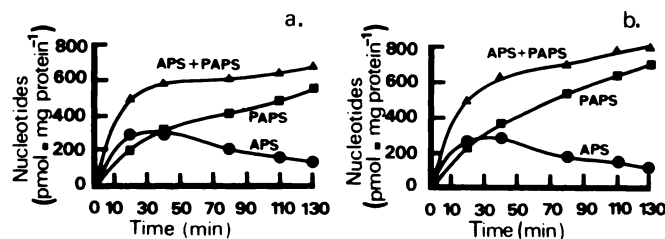


Chart 2. Rate of synthesis of [<sup>35</sup>S]APS and [<sup>35</sup>S]PAPS from ATP, Mg<sup>2+</sup> and [<sup>35</sup>S]sulfate in the cytosol fraction of a human breast tumor. a, in the absence of 3',5'-ADP; b, in the presence of 1.25 mM 3',5'-ADP.

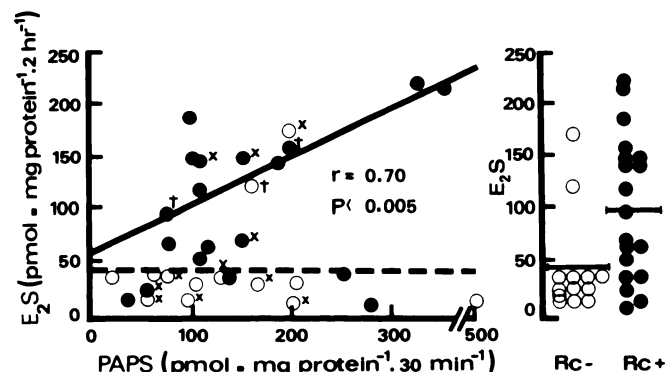


Chart 3. Left, relationship between estrogen sulfurylation and PAPS synthesis in human mammary tumor cytosol fractions. ----, arbitrary cut-off level of estrogen sulfurylation below which no correlation with PAPS synthesis was observed. Above this level, these parameters are significantly correlated. ●, estrogen Rc-positive tumors; ○, estrogen Rc-negative tumors; ×, premenopausal subjects; †, subjects in which the menopausal status could not be established. The remainder are postmenopausal. Right, data shown for estrogen Rc-negative and Rc-positive groups. Bars, mean values of estrogen sulfurylation, 44.8 ± 47.6 (S.D.) and 96.5 ± 70.8, respectively (*p* = 0.025).

estrogen Rc negative (11 of 16). In type 2, estrogen sulfotransferase activities ranged from 52 to 220 pmol E<sub>2</sub>S per mg protein per 2 hr, and the rate of sulfurylation was significantly correlated with PAPS synthesis (*r* = 0.70, *p* < 0.005). Such tumors were predominantly estrogen Rc positive (14 of 16). The average estrogen sulfotransferase activity in the Rc negative tumors was significantly lower than in the Rc-positive tumors (Chart 3, *p* = 0.025).

**DISCUSSION**

The data presented in Chart 3 are in agreement with the results on primary breast cancer reported by Leung *et al.* (6) and point to a relationship between estrogen sulfurylation and estrogen Rc status. A simplistic interpretation of this relationship would be that estrogen Rc values are negative due to the

<sup>4</sup> A complete assessment of the method used in measuring estrogen and dehydroepiandrosterone sulfurylation is given in this reference.

presence of a sufficiently high concentration of estrogen (or perhaps some other steroid) to cause Rc depletion. Thus, estrogen sulfotransferase, by converting  $17\beta$ -estradiol to  $E_2S$ , which does not combine with Rc, could then act to limit the concentration of free  $17\beta$ -estradiol and hence lead to higher Rc values. If this were correct, one would expect to find estrogen receptor in the nuclei of estrogen Rc-negative tumors. However, this is not the case, since McGuire *et al.* (8) have failed to find charged, or uncharged, estrogen receptors in the nuclei of Rc negative tumors while they are present in Rc positive tumors. However, it is of interest that in the human breast cancer cell line MCF-7 75% of the total population of estrogen-binding sites of the cell are in the nuclei and are not charged with estrogen (11). Whatever the explanation may be for the deletion of receptor in mammary tumors, it could be closely tied to depleted levels of estrogen sulfotransferase.

Recently, estrogen sulfotransferase has been found to be involved in the control of estrogen levels *in situ* in pig and human endometrium. In a penetrating analysis of estrogen action in the gilt uterus, Pack *et al.* (9) have shown that, at the time of the estrous cycle when charged estrogen receptor is at a maximum within the nucleus (Day 1), estrogen sulfotransferase in the cytosol is at a minimum. Nuclear estrogen receptor levels then decrease rather rapidly and both estrogen Rc and estrogen sulfotransferase begin to rise in the cytosol. Since there was always uncharged cytoplasmic receptor present throughout the cycle to bind  $17\beta$ -estradiol and, furthermore, since the latter remained at significant levels in the plasma throughout the cycle, Pack *et al.* have suggested that estrogen metabolism via  $17\beta$ -estradiol dehydrogenase, but more particularly via sulfurylation, would explain the lack of nuclear migration. Buirchell and Hähnel (3) have also demonstrated a role for estrogen sulfotransferase and  $17\beta$ -estradiol dehydrogenase in controlling the level of  $17\beta$ -estradiol in the human endometrium. In this instance, these enzymes appear to be induced by progesterone at the end of the proliferative phase of the cycle. Estrogen sulfotransferase in estrogen Rc-positive tumors may perhaps then function in a manner parallel to that proposed for the endometrium. Alternatively, the enzyme may in some way be concerned in the exit of estrogen from the nucleus, and thence the cell itself, after the charged Rc has elicited its signal at the genome.

Dao and Libby have claimed that diminished PAPS synthesis was the reason for low steroid sulfurylation observed in a percentage of human breast tumors (4). This was based on

indirect evidence, namely, that addition of partially purified ATP sulfurylase and APS kinase from yeast to tumor preparations lacking significant steroid sulfurylating ability led to measurable amounts of steroid alcohol and estrogen sulfotransferase activities. PAPS formation from ATP and sulfate by the tumor preparations was not assayed. However, in all 32 tumors studied here (Chart 1) and in virtually all of 41 tumors studied previously (1), PAPS synthesis was demonstrated. It is also apparent from Chart 3 that quite high concentrations of PAPS can be associated with type 1 (low estrogen sulfotransferase) tumors. This discrepancy between the present results and those of Dao and Libby could be explained by the use of  $12,000 \times g$  supernatants by these workers which may not allow sufficient concentrations of PAPS to be formed in certain cases, due to the presence of degradative enzymes in the particulate components of such preparations.

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