Dehydroepiandrosterone Sulfotransferase as a Possible Shunt for the Control of Steroid Metabolism in Human Mammary Carcinoma

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SUMMARY

Human primary mammary tumors were examined to determine what factors were of importance in deciding relative rates of sulfurylation of dehydroepiandrosterone and 17β-estradiol, such rates having been shown to correlate with the patient’s prognosis and response to adrenalectomy (T. L. Dao and P. R. Libby. Enzymic Synthesis of Steroid Sulfate by Mammary Cancer and Its Clinical Implications. Natl. Cancer Inst. Monographs, 34: 205-210, 1971). The sulfurylation of dehydroepiandrosterone and 17β-estradiol was studied in 41 tumors in vitro using tumor cytosol, adenosine triphosphate, [35S]SO₄²⁻, Mg²⁺, and added steroid. Six tumors showed no sulfurylating ability, 9 sulfurylated dehydroepiandrosterone at a rate greater than that for 17β-estradiol (ratio, >1), and 26 sulfurylated dehydroepiandrosterone at a rate lower than that for 17β-estradiol (ratio, <1). Evidence was obtained that low levels of dehydroepiandrosterone sulfotransferase were responsible for ratios of <1, in many instances. Adenosine 3'-phosphate 5'-phosphosulfate synthesis and steroid sulfotransferase activities were measured in 30 tumors. A significant correlation was found between synthesis of the former and levels of estrogen sulfotransferase, but this relationship did not hold for dehydroepiandrosterone sulfotransferase, again due to low levels of this enzyme in many tumors. It is suggested that dehydroepiandrosterone sulfate formation in the tumors is mainly controlled by the sulfotransferase, which acts as a shunt in regulating the level of free dehydroepiandrosterone, and related compounds, available for metabolism to steroids influencing the growth of mammary epithelial cells.

INTRODUCTION

Human mammary carcinoma tissue can sulfurylate steroids (3) and convert steroids into physiologically active metabolites (7). Of particular significance is the ability of some breast tumors to produce estrogens (1, 5, 7, 23). The significance of the sulfurylation process in steroid hormone metabolism remains unclarified. Transformations of the steroid moiety can occur with the sulfate group intact, but such pathways appear to be of minor significance (21). Dao and Libby (15, 16) have demonstrated that the sulfurylation of steroids by human mammary carcinoma tissue is highly correlated with prognosis and response to hormone- ablative procedures such as adrenalectomy. In patients undergoing adrenalectomy for late-stage breast cancer, the failure of tumor preparations to sulfurylate steroid hormones in vitro was related to a particularly grave prognosis and a complete lack of response to adrenalectomy. When DHEA and 17β-estradiol were compared as substrates for tumor sulfotransferases, then if the ratio of DHEA sulfate formation to 17β-estradiol sulfate formation was <1, this was also associated with a poor prognosis and response to adrenalectomy. The group with the best response and prognosis showed DHEA sulfate:17β-estradiol sulfate ratios of >1. In studies on primary tumors examined at the time of mastectomy, these ratios were also correlated with prognosis, as witnessed by the presence of metastases or by early recurrence (16).

Lowered ratios of DHEA sulfate to 17β-estradiol sulfate could be due to either decreased DHEA sulfurylation or increased 17β-estradiol sulfurylation. In this investigation, human mammary tumors were examined for their overall ability to form steroid sulfates from SO₄²⁻ and, in an associated study, for their ability to form the intermediate PAPS and catalyze subsequent transfer of the sulfate group from PAPS to added steroids. In this manner, the limiting steps in steroid sulfurylation were assessed.

MATERIALS AND METHODS

Tumor Tissue

Specimens of primary carcinoma tissue from patients undergoing mastectomy were received from the operating theater. They were placed in plastic containers packed in ice, transported to the laboratory, and stored at −20°. The time between receiving the specimen and freezing it did not exceed 1 hr in any instance. Subsequently, the tissue was allowed to thaw, fat and connective tissue were removed,

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The abbreviations used are: DHEA, dehydroepiandrosterone; PAPS, adenosine 3'-phosphate 5'-phosphosulfate; APS, adenosine 5'-phosphosulfate.
and a weighed sample (0.2 to 1.0 g) was cut into small pieces and frozen in the barrel of a stainless steel chamber precooled in dry ice. The frozen tissue was shattered by hammering a closely fitting stainless steel plunger into the chamber. (Details of this piece of equipment can be obtained from the authors.) The resulting fine powder was transferred to a small glass homogenizer, 4 volumes of 0.01 M sodium phosphate buffer (pH 7.4) in 0.9% NaCl solution were added, and the mixture was homogenized using a Teflon plunger. The cytosol fraction was collected after centrifugation at 100,000 × g for 1 hr.

In all cases histological diagnosis of carcinoma was confirmed from block sections.

Chemicals

DHEA, DHEA sulfate, 17β-estradiol, and APS were obtained from Sigma Chemical Co., St. Louis, Mo. Cholesterol was obtained from the British Drug Houses Ltd., London, England. Estrone 3-sulfate was purchased from Ikapharm, Ramat-Gan, Israel. 17β-Estradiol 3-sulfate was prepared from this by NaBH₄ treatment. Cholesteryl 3-sulfate, carrier-free, was purchased from the Radiochemical Centre, Amersham, England.

Protein

This was measured by the method of Lowry et al. (22).

Conditions of Incubation and Assay Procedures

1. Assay of Steroid Sulfotransferases. The incubation mixture contained the following ingredients in a total volume of 0.2 ml: 1 μmole ATP; 1.5 μmoles MgCl₂; 0.01 mmole Tris-HCl, pH 7.4; 0.04 μmole Na₂SO₄; 25 μCi carrier-free [³⁵S]SO₄²⁻; 0.1 ml cytosol fraction of tumor; and 0.01 μmole steroid added in 0.01 ml propylene glycol. Controls contained 0.01 ml propylene glycol. Incubation was for 2 hr at 37°. The steroid [³⁵S]sulfate formed was assayed by extraction into ethyl acetate (14). Briefly, 2 ml of 2 N NaOH, saturated with (NH₄)₂SO₄, were added, and the mixture was extracted with ethyl acetate (3 × 3 ml). The combined extracts were adjusted to 10 ml and back-extracted once with 0.5 ml of seven-eighths-saturated (NH₄)₂SO₄. An aliquot (3 ml) was evaporated in a vial, 10 ml of detergent-phosphor were added (3), and samples were counted by liquid scintillation. Sufficient counts were recorded to allow for an accuracy of 3%. After counts were subtracted in the control (all ingredients but no steroid), the net counts were expressed as pmoles of steroid sulfate per mg protein per 2 hr, from a knowledge of the specific activity of the [³⁵S]sulfate.

2. Assay of [³⁵S]PAPS. The incubation mixture was identical to that for the control in Paragraph 1, with the exception that an incubation time of 30 min was used. At the end of that time, a 0.01-ml aliquot was added to 0.05 ml of ethanol, and the solution was applied to a strip of Whatman No. 1 paper and subjected to electrophoresis for 3 hr in 0.05 M sodium phosphate buffer, pH 6.0. A potential of 10 V/cm was applied and the procedure was carried out in the cold room. After drying, the strips were scanned in a Nuclear Chicago Actigraph III instrument. The [³⁵S]PAPS and [³⁵S]APS peaks were identified by comparison with authentic compounds. Areas of these peaks were measured, and the activity of the PAPS-synthesizing system was expressed in arbitrary units defined as the sum of the areas of PAPS plus APS per mg of cytosol protein used in the incubation.

Assay of Acid Mucopolysaccharide Sulfotransferase.

The incubation mixture was identical to that for the control in Paragraph 1. After 2 hr of incubation at 37°, 10 ml of 0.2 M Na₂SO₄ and 1 ml of 1% (w/v) aqueous solution of sodium chondroitin sulfate (Sigma) were added. After mixing, 1 ml of 5% (w/v) aqueous solution of cetyl trimethylammonium bromide (Hopkin and Williams Ltd., Essex, England) was added, and the precipitate was allowed to stand for 1 hr or overnight if flocculation was delayed. The precipitate was centrifuged down and washed with 5 ml of 0.1 M Na₂SO₄. This was repeated 4 times, followed by 1 washing with water. Finally, the precipitate was centrifuged, the supernatant was discarded, and the tube was drained by inversion on tissue paper. The precipitate was dissolved in 2 ml of 10% (w/v) NaCl and sodium salts of acid mucopolysaccharides precipitated by addition of 1.5 volumes of ethanol. After standing for 2 hr at 0°, the material was centrifuged and the process of dissolution and precipitation was repeated. The material was dissolved in 1 ml of water, and 0.2 ml was removed for counting in 10 ml of detergent-phosphor (see Paragraph 1). Blanks carried out with bovine serum albumin in place of tumor cytosol were zero. The identification of the radioactive fraction isolated by this procedure as acid mucopolysaccharide was demonstrated as described previously (2).

Examination of Ester [³⁵S]Sulfates Formed from Endogenous Acceptors

Incubations of cytosol fractions of a number of tumors, in the absence of added steroid, were carried out as in Paragraph 1, but on 3 times the scale and in the presence of a total of 80 μCi of [³⁵S]sulfate. Reaction was stopped by addition of 1.5 volumes of ethanol and protein was removed by centrifugation. The supernatant was then chromatographed on paper.

Paper Chromatography Systems

System A: ethanol:1 M ammonium acetate, pH 7.5 (7.5:3).
System B: 0.4 μM sodium phosphate, pH 6.3. System C: diisopropyl ether:tert-butyl alcohol:concentrated ammonia:water (6:4:1:9). Steroid sulfates on chromatograms were cleaved to the free steroids by exposure to HCl vapor for 2 hr. Estrogens were then detected by Turnbull's reagent, and other steroids were detected by phosphotungstic acid.

RESULTS

Assessment of Method Used for Steroid Sulfotransferase Assay. Extracts from a number of tissues, when incu-
bated with ATP, Mg²⁺, and [³⁵S]sulfate of high specific activity, catalyze [³⁵S]sulfate activation to [³⁵S]PAPS and subsequent transfer of the sulfate group to endogenous acceptors present in the extracts (28, 29). When exogenous acceptors, e.g., steroids, are added to such incubation mixtures, then a new zone may appear on autoradiographs of chromatograms, indicating formation of exogenous acceptor sulfate. The presence of steroid sulfotransferase in human mammary carcinoma tissue was detected by this method (3).

Formation of total ester [³⁵S]sulfates, extractable into ethyl acetate, was linear with time for approximately 1 hr (Chart 1). When the ethyl acetate extracts, obtained in the normal assay procedure, were chromatographed in System C and scanned for radioactive components, the results shown in Chart 2 were obtained. In the control, without added steroid, 3 peaks were present. For the 1st tumor cytosol depicted, addition of DHEA did not increase the number of counts in the ethyl acetate extract or alter the pattern of zones obtained on chromatography (Chart 2A). Addition of 17β-estradiol, however, increased the counts in the ethyl acetate and resulted in the appearance of a new zone in the position of authentic 17β-estradiol 3-sulfate on the chromatogram. The other zones remained unchanged. Results obtained with another tumor are shown in Chart 2B. Increased counts upon addition of either DHEA or 17β-estradiol were recorded, and these were associated with new peaks on chromatograms corresponding to the appropriate sulfate esters of the steroids. Again, the size of the other peaks was unchanged upon addition of steroid (Chart 2B).

When increasing concentrations of DHEA were added, the size of the DHEA [³⁵S]sulfate peak on chromatograms increased up to a concentration of 0.05 mM DHEA and decreased at higher concentrations. The size of the other peaks remained approximately constant at all concentrations of DHEA. Measurement of DHEA sulfate formation was compared by 2 methods as shown in Chart 3. With increasing concentrations of DHEA, net counts, i.e., total counts less counts in the control, gave a curve similar to that obtained by measurement of DHEA sulfate peaks on chromatograms. Other tumors were examined similarly using DHEA and 17β-estradiol as substrates, and it was observed that net counts correlated well with the size of the respective steroid sulfate peaks. Thus, for routine estimations, it was justifiable to use net counts to determine steroid sulfotransferase activities. By contrast to the lack of effect of DHEA and 17β-estradiol on the formation of [³⁵S]sulfates from endogenous acceptors, addition of p-nitrophenol resulted in p-nitrophenyl [³⁵S]sulfate formation and the disappearance of ester [³⁵S]sulfates derived from endogenous acceptors.

Nature of the Endogenous Sulfate Acceptors. Boström (9) demonstrated that some of the ester sulfates present in urine probably represented aryl and steroid sulfates. This was based on their chromatographic properties in 2-dimensional systems. Endogenous steroids present in tissue extracts, which have been characterized as their sulfate esters, following incubation of the extracts with ATP, Mg²⁺, and [³⁵S]sulfate, are cholesterol (8) and estrone (4). Four primary human mammary tumors of varying steroid sulfurating ability were selected for experiments aimed at elucidating the nature of the endogenous sulfate acceptors. Incubation of cytosol fractions was carried out as for the

![Chart 1. Time course for formation of total ester [³⁵S]sulfates. Incubation was carried out with tumor cytosol fraction in the presence of ATP, [³⁵S]SO₄²⁻, Mg²⁺, and DHEA (0.05 mM) as described under "Materials and Methods," Paragraph 1.](chart1)

![Chart 2. Chromatography (System C) and scanning of the labeled components present in ethyl acetate extracts of tumor cytosols incubated with ATP, Mg²⁺, and [³⁵S]SO₄²⁻. Peaks present in the controls represent sulfate esters formed from endogenous acceptors. In A, addition of DHEA did not increase the total counts in the ethyl acetate and the pattern of peaks is unchanged. Addition of 17β-estradiol, however, increased the total counts and a new peak, in the position of authentic 17β-estradiol 3-sulfate, is evident. In B, using a separate tumor cytosol, addition of both DHEA and 17β-estradiol has resulted in increased counts and the appearance of new zones in positions corresponding to the authentic sulfate esters. Shaded areas, positions of authentic marker compounds run in parallel.](chart2)

![Chart 3. Effect of increasing concentrations of DHEA on formation of DHEA sulfate as measured by net counts (obtained after subtraction of counts in control) converted to pmoles DHEA sulfate based on the specific activity of the [³⁵S]SO₄²⁻ (•) and from measured areas under the appropriate peaks after chromatography, as in Chart 2 (O).](chart3)
routine steroid sulfotransferase assay procedure, but on a larger scale and with increased amounts of [35S]sulfate. Preliminary chromatography of the protein-free incubation mixture was carried out in System A and the broad estersulfate band eluted. Part of this eluate was chromatographed in System B, designed to separate the ester sulfates of the 3 classical estrogens (26). No peaks were present in the positions corresponding to the 3 estrogen sulfates or to DHEA sulfate. All 4 tumors showed 2 radioactive peaks with high RF values in System B. In addition, 2 of the tumors (Subjects D. C. and M. M.) showed a peak at the origin. In System C, this particular peak in both instances exhibited a high RF and moved to a position identical to that of cholesteryl sulfate, used as a marker. These radioactive areas corresponding to cholesteryl sulfate represented about one-half the total peak areas and were shown to be identical to this substance by means of reverse isotope dilution (Table 1).

The other radioactive components, common to all 4 tumors, possessed chromatographic properties in System B, which showed that they were more polar than were steroid sulfates. As mentioned earlier, such components, present in the normal incubation system used for assaying steroid sulfotransferase activity, were not formed when exogenous p-nitrophenol was sulfurylated. This indicated that the endogenous acceptors were probably phenolic, mass action effects dictating that endogenous phenols would not be sulfurylated at detectable rates in the presence of excess exogenous substrate. Estrogen sulfotransferase is specific for estrogens and will not sulfurylate simple phenols, although phenols possessing alkyl side chains are sulfurylated at low rates (6, 27).

It can be seen from Table 2 that the isolation of cholesteryl [35S]sulfate from tumors of Subjects D. C. and M. M. is related to the presence of relatively high DHEA sulfotransferase activities. This suggests that both DHEA and cholesterol are sulfurylated by the same enzyme. Cholesteryl [35S]sulfate was detected only when the specific activity of the [35S]sulfate was increased by addition of increased amounts of carrier-free [35S]sulfate. Additional experiments carried out with tumor tissue from Subject E. O., which possessed high estrogen sulfotransferase activity (Table 2), failed to detect sulfate esters of the 3 classical estrogens among the labeled ester sulfates derived from endogenous acceptors.

### Steroid and Acid Mucopolysaccharide Sulfotransferase Activities in Primary Tumors

A summary of the results obtained with 41 primary carcinomas is shown in Table 3. Generally, the overall transfer of sulfate to steroid was higher than that reported by Dao and Libby (15). Undoubtedly, the higher concentration of SO₄²⁻ used in the present procedure is responsible. In addition, the presence of higher concentrations of PAPS-degrading enzymes in the particulate fraction of the 12,000 x g supernatant, as used by Dao and Libby (15), would limit overall sulfation.

Six of the 41 tumors (15%) showed no sulfurylating ability, 9 sulfurylated DHEA at a greater rate than did 17β-estradiol (ratio, >1), and 26 sulfurylated DHEA at a lower rate than did 17β-estradiol (ratio, <1).

#### Correlation of Enzyme Activities

When endogenous acceptor sulfurylation was plotted in turn against DHEA, 17β-estradiol (Chart 4), and acid mucopolysaccharide sulfurylation (Chart 5), a correlation was established with the steroid sulfotransferases but not with acid mucopolysaccharide sulfotransferase. The 6 tumors that failed to demonstrate overall sulfuration are not included in the data presented in Charts 4 and 5.

#### Relationship between [35S]PAPS Synthesis and Sulfotransferase Activities

Plots of endogenous acceptor [35S]sulfate formation versus [35S]PAPS formation in 30 tumors showed a significant correlation (Chart 6A). A significant correlation was also obtained when estrogen sulfate formation was plotted against PAPS synthesis (Chart 6B), but this was not observed for DHEA sulfate (Chart 6C). The sum of APS plus PAPS was used in each instance, since it

<table>
<thead>
<tr>
<th>Subject</th>
<th>No steroid</th>
<th>DHEA added</th>
<th>Net DHEA sulfate</th>
<th>17β-Estradiol added</th>
<th>Net 17β-estradiol sulfate</th>
<th>Acid mucopolysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. S.</td>
<td>43</td>
<td>41</td>
<td>0</td>
<td>63</td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>D. C.</td>
<td>56</td>
<td>173</td>
<td>117</td>
<td>115</td>
<td>59</td>
<td>84</td>
</tr>
<tr>
<td>E. O.</td>
<td>34</td>
<td>54</td>
<td>20</td>
<td>432</td>
<td>398</td>
<td>98</td>
</tr>
<tr>
<td>M. M.</td>
<td>59</td>
<td>125</td>
<td>66</td>
<td>172</td>
<td>113</td>
<td>116</td>
</tr>
</tbody>
</table>

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**Table 1**

Crystallization data for the characterization of cholesteryl [35S]sulfate

Using [35S]SO₄²⁻ of high specific activity in incubations with tumor cytosols (see "Materials and Methods"), a labeled component with chromatographic properties corresponding to those of cholesteryl sulfate was formed in Subjects D. C. and M. M. (see Table 2). These areas of the chromatograms were eluted, mixed with 34 mg of cholesteryl sulfate, and crystallized from 70% ethanol.

<table>
<thead>
<tr>
<th>Subject D. C. (cpm/mg)</th>
<th>Subject M. M. (cpm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original 1270</td>
<td>875</td>
</tr>
<tr>
<td>1st crystallization</td>
<td>987</td>
</tr>
<tr>
<td>2nd crystallization</td>
<td>760</td>
</tr>
<tr>
<td>3rd crystallization</td>
<td>800</td>
</tr>
<tr>
<td>1st mother liquor</td>
<td>800</td>
</tr>
<tr>
<td>2nd mother liquor</td>
<td>808</td>
</tr>
<tr>
<td>3rd mother liquor</td>
<td>768</td>
</tr>
</tbody>
</table>

**Table 2**

Sulfotransferase activities of tumor cytosols used in investigation of the nature of endogenous sulfate acceptors

<table>
<thead>
<tr>
<th>Subject</th>
<th>No steroid</th>
<th>DHEA added</th>
<th>Net DHEA sulfate</th>
<th>17β-Estradiol added</th>
<th>Net 17β-estradiol sulfate</th>
<th>Acid mucopolysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. S.</td>
<td>43</td>
<td>41</td>
<td>0</td>
<td>63</td>
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<td>D. C.</td>
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<td>M. M.</td>
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<td>125</td>
<td>66</td>
<td>172</td>
<td>113</td>
<td>116</td>
</tr>
</tbody>
</table>
was judged that such a sum would be a better indication of the "PAPS potential." A high percentage of the tumors contained a 3'-nucleotidase that converted PAPS formed in situ, or introduced into the system, into APS. Degradation of PAPS, and the subcellular distribution of the degradative enzymes in human mammary tumors, will be the subject of a separate communication. When PAPS values alone were plotted against sulfotransferase activities, similar correlations to those found for the sum of APS and PAPS were obtained (see legend to Chart 6).

**DISCUSSION**

Estimations of the overall steroid sulfurylating capacity in such tissues as human liver were first made by Bostrom and

**Table 3**

<table>
<thead>
<tr>
<th>Sulfotransferase</th>
<th>Sulfate transferred (pmoles/mg protein/2 hr)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous acceptor</td>
<td>53 ± 37</td>
<td>2-132</td>
</tr>
<tr>
<td>DHEA</td>
<td>44 ± 70</td>
<td>0-374</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>80 ± 105</td>
<td>0-486</td>
</tr>
<tr>
<td>Acid mucopolysaccharide</td>
<td>25 ± 33</td>
<td>0.5-122</td>
</tr>
</tbody>
</table>

**Chart 6. Correlations between PAPS synthesis and sulfotransferase activities in 30 primary breast carcinoma cytosols.** Some of the PAPS formed is degraded to APS by a 3'-nucleotidase present in the cytosol. For this reason the sum of APS plus PAPS has been used. Similar correlations to those shown were obtained when PAPS alone was used. These were (A) $r = 0.54, p < 0.002$; (B) $r = 0.39, p < 0.05$; (C) $r = 0.11, p > 0.25$. NS, not significant ($p < 0.2$).

Wengle (10). For example, the amount of DHEA sulfate formed using cytosol preparations derived from human female liver was 385 ± 35 nmoles per g wet weight per hr. The amount of ester [35S]sulfate formed, in parallel incubations without added acceptor, was 20 to 30 nmoles per g wet weight per hr. When human primary breast carcinoma tissues were assayed in the present study, the amounts of steroid sulfates formed were much lower than the figures quoted for human liver, and the amounts of ester [35S]sulfates formed in controls without added steroid represented a significant part of total ester [35S]sulfates produced.

The steroid sulfotransferase activities summarized in Table 3 are somewhat higher than the values reported for primary breast tumors by Dao and Libby (14) and Braunsberg et al. (11). This is probably due to the quite low concentrations of sulfate used by these workers and could also explain why endogenous acceptor sulfurylation was not mentioned in these reports. Unfrozen tissue was also used by these workers, but it has been found that small samples of fibrous breast tumors defy homogenization by normal methods. The technique of powdering the tissue sample in the supercooled state proved very effective in these cases.

*A discrepancy appears in the concentration of sulfate (0.01 mM) as described in the original paper of Dao and Libby (14) and in details given subsequently (16) in which the concentration is quoted as 0.2 mM. The concentration of sulfate used in the present work was 0.2 mM.*
and was adopted as a general procedure. This, in turn, may account for the lower percentage of tumors that failed to show overall sulfonylation in this work, as compared to those of Dao and Libby (14) and Braunsberg et al. (11) (see below). Also, the cytosol fraction was used, rather than the 12,000 x g supernatant as used by Dao and Libby (14), since the sulfotransferases and PAPS-synthesizing enzymes are present in this cell fraction and the higher concentrations of PAPS-degradative enzymes, present in the microsomes, are avoided. Freezing of the cytosol fraction for 2 weeks did not alter steroid sulfotransferase activities appreciably; thus freezing the tissue would be unlikely to lead to a differential loss of one of these enzymes and thus alter the ratio of DHEA sulfate to 17β-estradiol sulfate. This is borne out by the fact that the same distribution of activities was found as was reported by Dao and Libby (15), namely, DHEA sulfate:17β-estradiol sulfate, >1 in one-third and <1 in two-thirds of the tumors.

The endogenous acceptors, detected in the tissue extracts under the conditions used in the routine assays, appeared to be phenolic. They were not substrates for estrogen sulfotransferase, since they were still formed in the presence of exogenous estrogen (Chart 2) but were eliminated upon addition of p-nitrophenol. It was previously shown that an endogenous acceptor-[35S]sulfate, formed upon incubation of a breast tumor extract with [35S]PAPS of high specific activity and having an RF identical to that of cholesteryl sulfate, was specifically eliminated when addition of DHEA was made to the incubations (3). Later, cholesterol was formally identified as an endogenous sulfate acceptor in breast tumors incubated under similar conditions (8). The identification of cholesteryl [35S]sulfate in 2 tumors, possessing relatively high levels of DHEA sulfotransferase (Table 2), would then support the previous data which suggested that both these steroids are sulfonylated by the same enzyme. Although estrogens were not detected among the endogenous acceptors, even in 1 tumor possessing a very high estrogen sulfotransferase activity (Table 2), steroids other than cholesterol have been detected. A primary breast carcinoma extract, upon incubation with [35S]PAPS of high specific activity, yielded a zone travelling in the area occupied by steroid sulfates in the 2-dimensional chromatographic system developed by Vestermark and Boström (29). Although this zone was not in the position occupied by authentic 17β-estradiol sulfate or DHEA sulfate, it alone among the numerous labeled ester sulfates present on the chromatograms was increased upon addition of either 17β-estradiol or DHEA to the incubations (3). At the time no explanation could be offered for this phenomenon, but it is significant that the [35S]PAPS used had such a high specific activity that only minute chemical amounts of steroid [35S]sulfates were produced. At these levels the chromatographic properties of the steroid [35S]sulfates were influenced by charged groups on the paper, and they travelled to different positions as compared with authentic unlabeled compounds. Overspotting with excess authentic steroid sulfate overcame this discrepancy (4).

Tumor specimens were judged as lacking overall steroid sulfotransferase activities when the formation of endogenous acceptor sulfate was less than 10 pmoles per mg protein per 2 hr and no marked change in total ester sulfate formation occurred in these tumor extracts in the presence of added steroids. Blanks carried out using bovine serum albumin, in place of cytosol, gave values of <1 pmole. The fact that acid mucopolysaccharide sulfotransferase activities were also generally low in these cases eliminated the possibility that high levels of this enzyme, by utilization of available PAPS, may have been responsible for the lack of demonstrable steroid sulfotransferase activity. This category comprised 15% of the tumors studied, a value considerably lower than the 32% of 92 primary tumors reported by Dao and Libby (15) or 49% of 41 primary tumors reported in the series of Braunsberg et al. (11). As mentioned previously, of those tumors that did show steroid sulfonylating ability, DHEA sulfate:17β-estradiol sulfate ratios of >1 occurred in 26% of cases and ratios of <1 occurred in 74% of cases. These figures were in excellent agreement with those reported by Dao and Libby (15), namely, 24 and 76%, respectively.

Very few subjects in this series have shown recurrence, so that at this stage it is not possible to attempt to relate steroid sulfotransferase ratios with prognosis. In Dao and Libby’s series of 92 primary carcinomas, some 40 cases that showed recurrence within 3 years of mastectomy all fell into the group exhibiting either no sulfonylation or that in which the DHEA sulfate:17β-estradiol sulfate ratio was <1 (16). If we momentarily leave the question of the possible significance of a complete lack of steroid sulfonylation as being associated with a poor prognosis, the other group having a poor prognosis was, as stated, that in which the DHEA sulfate:17β-estradiol sulfate ratio was <1.8 This can be due to either depressed DHEA sulfate formation or elevated 17β-estradiol sulfate formation. Comparison of such activities with a 3rd sulfotransferase, preferably not a steroid alcohol sulfotransferase due to lack of knowledge on their substrate specificities, might then suggest an answer to these alternatives. Endogenous acceptor sulfonylation should serve this purpose. From Chart 4 it can be seen that both 17β-estradiol and DHEA sulfonylation correlate with the sulfonylation of endogenous acceptor. However, for DHEA sulfotransferase, the scatter diagram reveals a bunching of points near the ordinate. This is not evident for 17β-estradiol sulfotransferase; thus the bunching of the points cannot be due to lack of available PAPS. Rather, a diminished level of DHEA sulfonylation is suggested in these cases. This conclusion is supported by the data in Chart 6, which show that the significant correlation between PAPS synthesis and 17β-estradiol sulfate formation does not hold in the parallel case involving DHEA sulfate formation, due evidently to low DHEA sulfotransferase activities in many of the tumors. Although the ability to form PAPS is of obvious importance in the overall synthesis of 17β-estradiol sulfate (Chart 6B), in this instance also the level of estrogen sulfotransferase

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8 One other explanation for the lack of sulfonylation, or altered steroid sulfotransferase activities; namely, one which possibly involved steroid sulfotransferases, would appear to be ruled out. These enzymes have been studied in human mammary carcinomas and their levels were found to be unrelated to ratios of the concentrations of DHEA and 17β-estradiol sulfates formed in incubations (13).
plays a role, as seen from the wider scatter of points when comparison is made with endogenous acceptor sulfurylation (Chart 6A).

These results implicate a lowered DHEA sulfotransferase as being responsible for depressed ratios of DHEA sulfate to 17β-estradiol sulfate in many cases. Such a conclusion is not in agreement with that reached by Dao and Libby (14), who favor an increased 17β-estradiol sulfotransferase to explain such ratios. It is difficult to reconcile an elevated sulfotransferase, namely, 17β-estradiol sulfotransferase, on one hand and the lack of sulfotransferase activity on the other as being 2 parameters correlated with a poor prognosis. Lowered or immeasurable levels of sulfotransferase, in this instance DHEA sulfotransferase, are reconcilable, however.

As mentioned previously, steroidogenic pathways involving steroid sulfates as obligatory intermediates are usually of minor significance (21). The sulfurylation step could then serve as a control shunt to limit the availability of free steroids, in particular 3β-hydroxy-Δ5-steroids, for conversion to metabolites influencing growth or homeostasis of mammary tissue. DHEA can serve as a precursor for androstenedione and testosterone in mammary carcinoma tissue (18). 5α-Androstane-3α,17β-diol is an active androgen which is capable of maintaining prostatic weight in castrated rats (17).

Estrogen sulfotransferase could also function in the control of the concentration of free, i.e., physiologically active, estrogen. It has been shown that estrogen sulfotransferase, in both porcine (25) and human (12) uteri, is under hormonal control and serves, in conjunction with 17β-estradiol dehydrogenase, to regulate the concentration of free 17β-estradiol in the uterus during the estrus and menstrual cycles.

An imbalance in the levels of the 2 types of sulfotransferases in the tumor, along the lines suggested, could disturb the hormone milieu and increase the growth rate of the tumor. The extremely poor prognosis associated in late-stage breast cancer with those tumors that exhibit no sulfurylating ability (16) is also compatible with an hypothesis implicating steroid sulfurylation as a control shunt.

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