whichever enzyme was used. It is most unlikely that the reductions of $1\alpha,2\alpha$ -epoxy- 17β -hydroxy- 5α -androstan-3-one involved any change other than reduction of the 3-oxo group. The $1\alpha,2\alpha$ -methylene compound was an inhibitor. The $1\alpha,2\alpha$ -epoxy and $1\alpha,2\alpha$ -methylene compounds may be expected to be closely similar in shape (ring A half boat), though both probably differ somewhat from the shape of the 1α -methyl and 2α -methyl compounds (ring A chair).

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The Effect of Thiols on Oestradiol Binding by Components of Human Malignant Breast Tumours

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The method of Feherty *et al.* (1971) has been used to study the effect of β -mercaptoethanol, dithiothreitol and L-cysteine on the concentration of oestrogen-binding sites in supernatant preparations (800g) from human breast tumours. Eight out of 12 tumours with measurable concentrations of binding sites (0.5-10.8 fmol/mg wet wt.) showed increased binding (1.2-fold to more than 6-fold) after treatment of the supernatant with mercaptoethanol (1-100 mM) for 10 min at 0°C. In only one out of 14 preparations that did not bind initially could binding be induced by mercaptoethanol. Pretreatment with dithiothreitol (0.1-10 mM) appears to have a greater effect (on an equimolar basis) than mercaptoethanol. In contrast, L-cysteine can decrease the ion-concentration of binding sites and increase K_d . Pretreatment of supernatants with a thiol-blocking agent (1 mM-N-ethylmaleimide) abolished subsequent binding of oestradiol as estimated by this technique.

These results confirm the involvement of thiol

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groups in oestradiol binding by components of human breast tumours (Hähnel & Twaddle, 1971). The enhancement of oestradiol-binding activity by some thiols may be associated with reduction of disulphide bridges or protection of thiol groups in the high-affinity binding component(s). The effect of L-cysteine may be competitive and may or may not involve its thiol group.

In some organisms the onset of cell division may be controlled by specific 'division' proteins and sulphur-containing macromolecules (Morris, 1965). The evidence for the involvement of thiol groups in growth has been reviewed by Harington (1967), and it is noteworthy that such groups appear to be involved in the binding of oestradiol by breast cancer preparations. However, Harington (1967) points out the difficulty of assigning significance to interaction with so highly reactive a group. Whether oestrogen binding is related to breast-tumour growth in human subjects is not known at the present time.

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Functional Groups in the Catalysis and Regulation of *Escherichia coli* Citrate Synthase

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The key position of citrate synthase in the tricarboxylic acid cycle favours it as a site for metabolic regulation, and allosteric feedback inhibition by both NADH and α -oxoglutarate has been demonstrated (Weitzman, 1966a,b; Wright *et al.*, 1967). We have subjected the purified enzyme to photo-oxidation and specific chemical modification in order to identify amino acids participating in the catalytic and regulatory processes.

Photo-oxidation with Methylene Blue demonstrates the involvement of three photo-oxidizable amino acids in catalytic activity and two in regulation (one in NADH inhibition and one in α -oxoglutarate inhibition). The dependences of the rates of photo-oxidation on pH, the characteristics of photo-oxidation with Rose Bengal and the results of amino acid analyses suggest that the susceptible residues involved in activity are two of histidine and one of methionine or tryptophan, and that those associated with regulation are cysteine (NADH inhibition) and histidine (α -oxoglutarate inhibition).

Supporting evidence is derived from specific modi-