seen are indicative of a hepatotoxic effect. Similar lysosomal and ultrastructural changes were observed in rats treated with 50 or 100 p.p.m. of coumarin for 2 weeks. These changes were accompanied by liver enlargement and a marked depression of microsomal mixed-function oxidase activity.

Thus it would appear that differences in the metabolic pattern of coumarin are not relevant to its hepatotoxicity.

Bar, F. & Griepentrog, F. (1967) Medizin Ernährung 8, 244–248

**Effects of Prolactin upon C_{19} Steroid Metabolism by Rat Mammary Carcinoma**

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It has been shown that mammary carcinomas induced in female Sprague-Dawley rats by the administration of the carcinogen dimethylbenzanthracene may metabolize steroid hormones (King et al., 1964, 1965). The aim of the present study was to determine the effects of increasing the circulating amounts of prolactin upon the metabolism of dehydroepiandrosterone and testosterone in these tumours.

Two groups of female Sprague-Dawley rats, in whom adenocarcinoma were induced by dimethylbenzanthracene (5 mg intravenously at 50 days of age), were investigated. One group received perphenazine (fentazin) in daily subcutaneous injections (5 mg/kg body wt.) from the age of 30 days to raise circulating prolactin amounts. The control group received only vehicle (0.2% citric acid). A comparison of tumours believed to be induced and grown in the presence of high and normal amounts of circulating prolactin was therefore possible.

The metabolism of dehydroepiandrosterone and testosterone in vitro was determined in ten adenocarcinomas from both groups of animals. A portion (1 g) of each tumour was finely sliced in 10 ml of Krebs-Ringer phosphate buffer. An NADPH-generating system (200 μmol of glucose 6-phosphate, 25 μg of NADP⁺ and 50 units of glucose 6-phosphate dehydrogenase) and 45 Ci of either [7-3H]dehydroepiandrosterone or [7-3H]testosterone were added. Incubation was immediately performed at 37°C for 2 h in O₂.

The steroid interconversions were determined by measuring the percentage incorporation of radioactive label into the individual metabolites after extraction and purification by t.l.c. Details of the methods of steroid purification and characterization have been described by Fahmy et al. (1968) and Jones et al. (1970).

The metabolism of [7-3H]dehydroepiandrosterone was not influenced by perphenazine treatment, no differences being found either in the amount of metabolism of dehydroepiandrosterone or the production of metabolites of dehydroepiandrosterone.

The results from the incubations with testosterone are presented in Table 1. The carcinomas from the perphenazine-treated animals displayed significantly greater metabolism of testosterone than those from control animals. The amount of metabolism varied considerably within each group, the variation being not only between individual animals but between tumours from different sites within the same animal. Of the metabolites of testosterone the mean production of 5α-dihydrotestosterone was higher (but not significantly so) in tumours from the perphenazine-treated animals. The conversion into 5α-androstanediol was, however, significantly increased in the perphenazine-treated rats.
Table 1. *Metabolism of testosterone by adenocarcinoma*

Results are given as means±s.e.m. for ten tumours, except for * mean for eight tumours and † mean for six tumours. % 5α-reduction is calculated as combined production of 5α-dihydrotestosterone and 5α-androstandiol.

<table>
<thead>
<tr>
<th></th>
<th>% Metabolized Testosterone</th>
<th>% 5α-Dihydrotestosterone Produced</th>
<th>% 5α-Androstandiol Produced</th>
<th>% 5α-Reduction</th>
<th>% Δ-4-Androstenedione Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethylbenzanthracene alone</td>
<td>34.06 ± 3.81</td>
<td>10.54 ± 2.42</td>
<td>4.00 ± 1.17</td>
<td>14.55 ± 2.91</td>
<td>0.42 ± 0.10*</td>
</tr>
<tr>
<td>Dimethylbenzanthracene + perphenazine</td>
<td>48.58 ± 4.33</td>
<td>15.58 ± 2.27</td>
<td>15.32 ± 2.43</td>
<td>31.90 ± 3.97</td>
<td>1.84 ± 0.37†</td>
</tr>
<tr>
<td>Significance</td>
<td>0.01</td>
<td>0.10</td>
<td>&lt;0.0005</td>
<td>&lt;0.0025</td>
<td>0.05</td>
</tr>
</tbody>
</table>
By combining the production of 5α-dihydrotestosterone and 5α-androstandiol and expressing the result as % 5α-reduction, an estimate of total 5α-reductase activity was obtained. This was significantly higher in the perphenazine-treated group as compared with the control group.

The conversion of testosterone into Δ4-androstenedione was also investigated. Although the mean amount of oxidation into Δ4-androstenedione was significantly higher in the perphenazine group, this was accounted for by two tumours, in which the amount of oxidation was markedly raised. These two tumours had the lowest 5α-reductase activity of the perphenazine group.

It is possible that the increased 5α-reductase activity demonstrated in tumours from perphenazine-treated animals is caused by differences in tumour cellularity. The findings of similar metabolism of another C-19 steroid precursor, dehydroepiandrosterone, and the similar conversion of testosterone into Δ4-androstenedione in both groups of tumours would, however, indicate that the effects on 5α-reduction of testosterone are relatively specific. Further evidence for this has come from preliminary studies in which ovine prolactin (50μg/ml) has been added to incubation mixtures of dimethylbenzanthracene adenocarcinomas in vitro with similar effects on testosterone metabolism.

Prolactin has been shown to influence the metabolism of testosterone in other steroid-metabolizing organs. For example, Boyns et al. (1972) have demonstrated that prolactin not only increased uptake of testosterone in rat prostatic cultures but changed the ratio of 5α-dihydrotestosterone to testosterone in favour of the non-reduced form. This effect upon 5α-reduction in the prostate is opposite to that demonstrated in the present study of rat mammary tissue.

King, R. J. B., Gordon, J. & Heffenstein, J. E. (1964) J. Endocrinol. 29, 103–110

Glutathione Metabolism in Sheep Erythrocytes with High and Low Concentrations of Reduced Glutathione
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Sheep with low levels of erythrocyte GSH were first found by Smith & Osburn (1967) and later by Tucker & Kilgour (1970). Animals with less than 55mg of GSH/100ml of cells (1.79mmol/litre of cells) have been termed low GSH and the rest high GSH. Tucker & Kilgour (1970) suggested that in Finnish Landrace sheep (Fins), GSH concentration is controlled by a single pair of autosomal alleles, the gene for high concentrations of GSH being dominant to that for low GSH. These animals do not appear to be affected by their low concentrations of erythrocyte GSH although they may be more susceptible to kale anaemia (Tucker & Kilgour, 1970). In contrast, in man, haemolytic disorders are generally associated with low concentrations of erythrocyte GSH (Beutler, 1972).

1974