rat urine was 3-hydroxypropylmercapturic acid and not one of two possible isomers of this mercapturic acid (see Table 1).

Acrolein is a reactive compound, which probably has a short life within the organism, thereby making it difficult to obtain direct evidence of its formation in an intact animal. Evidence is presented here, however, that administration of acrolein to rats leads to the excretion of 3-hydroxypropylmercapturic acid in the urine, and that in man and the rat excretion of this latter compound follows the administration of cyclophosphamide (see Scheme 1). Although they do not provide proof, these findings are consistent with a belief that acrolein is a metabolite of cyclophosphamide in man, and they lend added interest to the suggestion of Alarcon & Meienhofer (1971) that the therapeutic effects of cyclophosphamide may be related to the metabolic formation of acrolein.

We thank Professor G. Wetherley-Mein and Dr. P. J. Kingston for providing us with urine from patients under their care.

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Studies on the Metabolism and Hepatotoxicity of Coumarin in the Baboon
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Coumarin, a flavouring material, occurs naturally in many plants including the tonka bean, dates and a variety of fruits. Toxicity studies on this compound showed that coumarin was hepatotoxic in a number of species of animals, including the rat and dog (Hazleton et al., 1956; Hagan et al., 1967; Bär & Griepentrog, 1967), and that there was considerable difference in species sensitivity. As a consequence the use of coumarin as a food additive has been discontinued.

However, it is becoming increasingly recognized that in the extrapolation of animal toxicity data in terms of human hazard, account must be taken of the metabolism of the compound in the test species and in man.

Kaighen & Williams (1961) showed (Table 1) that the rabbit excreted in the urine about

Table 1. Metabolites of coumarin in the rat, rabbit and man as a percentage of dose administered

<table>
<thead>
<tr>
<th>Metabolites in urine</th>
<th>Rabbit (Kaighen &amp; Williams, 1961)</th>
<th>Rat (Shilling et al., 1969)</th>
<th>Man</th>
<th>7-Hydroxycoumarin</th>
<th>3-Hydroxycoumarin</th>
<th>o-Hydroxyphenylacetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolites in faeces</td>
<td>80.3–92.4</td>
<td>47.0–60.5</td>
<td>90–97</td>
<td>10.0–16</td>
<td>18.1–23.4</td>
<td>18.1–22.1</td>
</tr>
<tr>
<td></td>
<td>0.2–0.7</td>
<td>32.4–38.3</td>
<td>68–92</td>
<td>0.3–0.5</td>
<td>1.7–1.8</td>
<td>12.3–27.2</td>
</tr>
</tbody>
</table>

Main metabolites in urine

1974
Table 2. Percentage of orally administered coumarin (200mg/kg) excreted as 7-hydroxy-coumarin in urine in various species of animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Urinary excretion of 7-hydroxycoumarin (as % of administered coumarin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squirrel monkey</td>
<td>1.0</td>
</tr>
<tr>
<td>Ferret</td>
<td>1.0</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>1.0</td>
</tr>
<tr>
<td>Hamster</td>
<td>5.0</td>
</tr>
<tr>
<td>Mouse</td>
<td>3.0</td>
</tr>
<tr>
<td>Dog</td>
<td>3.0</td>
</tr>
<tr>
<td>Pig</td>
<td>12</td>
</tr>
<tr>
<td>Cat</td>
<td>19</td>
</tr>
<tr>
<td>Baboon</td>
<td>60</td>
</tr>
</tbody>
</table>

12% of a dose of coumarin as 7-hydroxycoumarin and about 20% as o-hydroxyphenylacetic acid. In the rat less than 1% of the dose was converted into 7-hydroxycoumarin and about 20% into o-hydroxyphenylacetic acid. Further, it was found that coumarin enters the entero-hepatic circulation in the rat. Shilling et al. (1969) investigated the metabolism of coumarin in man and found that about 80% of the orally administered coumarin was excreted in urine during 24h as 7-hydroxycoumarin and only 1–6% as o-hydroxyphenylacetic acid.

This marked difference in the metabolic pattern of coumarin between man, on the one hand, and the rat and rabbit, on the other, called into question the validity of the animal toxicity data and suggested the desirability of conducting studies on a species of animal that metabolized coumarin in a manner similar to that in man.

The metabolic conversion of coumarin into 7-hydroxycoumarin was investigated in nine species of animal (Table 2). The baboon was closest to man in its ability to convert coumarin into the 7-hydroxy derivative. This suggested investigation into the hepatotoxicity of coumarin in the baboon.

Coumarin was administered to one male and one female baboon at 50p.p.m. in the diet. The animals were kept on treatment for 3 weeks after which a bromsulphthalein excretion test was carried out and liver biopsy specimens were taken under anaesthesia. The animals were then allowed to recover from anaesthesia and maintained on a control coumarin-free diet for 3 months. They were then treated with 100p.p.m. of coumarin in the diet for 3 weeks, at the end of which a second bromsulphthalein excretion test was carried out and liver biopsy specimens taken. The animals were again allowed to recover from anaesthesia and placed on control diet for a further 3 months, after which a third bromsulphthalein excretion test and liver biopsy were performed. Bromsulphthalein excretion tests were carried out on four untreated baboons and pieces of liver were obtained at post mortem. Histochemical, electron-microscopic and histological examinations were carried out on the liver biopsy specimens.

Results showed that bromsulphthalein excretion was prolonged in the female but not in the male after treatment at both dosages. It returned to normal after 3 months. No histological abnormalities were observed. Histochemically the lysosomes were larger than normal and dispersed throughout the liver cells after the period of treatment at both dosages. In control animals, and after a 3 months recovery period, these organelles appeared small, discrete and limited to the pericanalicular area of the liver cells. Ultrastructurally numerous autophagic vacuoles were observed, and both smooth and rough endoplasmic reticulum were dilated in the treated animals at both dosages. These were absent in control animals and in the treated animals after a 3 months recovery period.

The results of the bromsulphthalein excretion tests and the lysosomal changes
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.

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Effects of Prolactin upon C19 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-androstandiol was, however, significantly increased in the perphenazine-treated rats.