

Influence of Germ-Free Status on the Excretion of Simple Phenols of Possible Significance in Tumour Promotion

Local application of phenol, *p*-cresol and 2-ethylphenol promotes the development of skin tumours in mice after a single initiating application of dimethylbenzanthracene¹. The former 2 compounds and the *para* isomer of ethylphenol are the major urinary simple phenols when natural plant-containing diets are fed to rats², and it has been shown that the intestinal microflora plays a major role in the formation of these substances³. It was therefore deemed of interest to study the excretion of simple phenols in germ-free and conventional rats.

Germ-free rats of the CDF-strain were raised and reared as described elsewhere⁴. Control animals were of the same strain and were from the 4th generation after conventionalization. 4 rats aged 110–130 days and weighing 150–200 g were used in each group. 10 days before and during the study a commercial pellet diet (Felleskjøpet, Oslo) described previously² was fed. The diet was autoclaved at 120 °C for 20 min. The animals were allowed free access to food and water. The rats were placed in metabolic cages and the individual 48-h urines were obtained by the use of separators which disposed of the faeces. Control urines from the conventional rats were collected in containers placed in solid carbon dioxide whereas the urines from the germ-free rats were collected at room temperature and removed from the isolators every 24 h. The urines were stored at –20 °C and thawed, filtered and diluted to 20 ml immediately prior to analysis. Samples of 10 ml of the diluted urines were hydrolyzed with β -glucuronidase (type H2 containing sulphatase, Sigma Chemical Co.), extracted and analyzed by gas chromatography as described previously^{2,5}.

Except for traces of phenol (<0.005 mg/24 h), simple phenols were not detected in the hydrolyzed urine of the germ-free rats. The excretion of the major urinary phenols in the conventional rats was as follows (mg/24 h, mean with range in parentheses): phenol 0.09 (0.06–0.16), *p*-cresol 0.28 (0.08–0.73), and 4-ethylphenol 0.38 (0.31–0.47). Smaller amounts of catechol, 4-methylcatechol and resorcinol and traces of guaiacol were also identified in these urines. The almost complete absence of urinary simple phenols is a previously unreported biochemical feature of germ-free animals. This finding demonstrates that with the exception of phenol which appears to arise to a very small extent in the tissues the normal microflora is essential for the formation of these compounds.

The process of continuous absorption of small amounts of simple phenols produced in the intestinal lumen is quite different from the experimental conditions used in the study of tumour promotion. Nevertheless, the fact that tumour-promoting activity is a property of some volatile simple phenols^{1,6} should not be disregarded although the

significance of the normal production of these compounds has not been investigated.

TANNENBAUM and SILVERSTONE⁷ found that the feeding of a low protein diet reduced the incidence of spontaneous hepatomas in C3H mice and this suggests that protein or protein metabolites may be involved in the formation of these tumours. The urinary excretion of phenol and *p*-cresol in rats also decreases when the dietary protein intake is reduced⁸. Recently, GRANT and ROE⁹ reported that germfree C3H mice are more resistant to hepatoma induction by dimethylbenzanthracene than mice of the same strain removed from the isolators and kept under 'minimal disease' conditions. They discussed whether immunological factors, virus etiology or microbial destruction of a protecting agent may explain this observation. In view of the information now available this difference in susceptibility might also be related to tumour promotion by some simple phenols produced through the action of the intestinal microflora on precursors normally present in the diet.

Zusammenfassung. In keimfrei aufgezogenen Mäusen werden fast keine einfachen Phenole mit dem Urin ausgeschieden, was auf den Mangel an Darmbakterien zurückzuführen ist.

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Calcein Blue as a Fluorescent Label in Bone

Some fluorescent compounds which are fixed in newly formed calcified tissues may be used to label bone deposition. Such labels help to determine the time sequence of bone growth, repair and adaptation to functional demand. The use of alizarin red S^{1,2}, tetracyclines³, porphyrines⁴ and the fluoresceins Calcein⁵ and DCAF^{5,6} has been reported. These labels may be given singly or sequentially, in the latter case to enhance the effectiveness of the method in monitoring modelling⁷ and remodelling. A

double label is obtained by combining 2 different tetracyclines⁸ or by combining a single tetracycline with alizarin red S⁹ or haematoporphyrine⁴. To provide a trichrome label, alizarin red S⁶ or haematoporphyrine¹⁰ may be used with DCAF and tetracycline. Four different tetracyclines have also been administered¹¹. There are disadvantages in the use of some of these substances. Alizarin red S has been claimed to inhibit further bone growth⁹ and it is hardly detectable in the presence of