mixture of 25 μ C of [3 H]uridine and 25 μ C of [3 H]guanosine. RNA was extracted separately from the uteri of the control and test groups.

In other experiments, by the dual-labelling procedure of Ellem (1967), the test animals received 1 μ g. of oestradiol 90 min. before being killed and the controls were given 0.1 ml. of 0.9% (w/v) NaCl. At the same time, the controls were given 100 μ C of [3 H]uridine and the test animals received 25 μ C of [14 C]uridine. The uteri from both groups were pooled before extraction of the RNA.

No significant differences were observed in the distribution of ultraviolet-absorbing material from the test and control animals. However, in the experiments with 3 H-labelled precursors the total radioactivity recovered from the MAK columns of RNA from treated animals was 72% greater than from the controls. The greatest change occurred in the Q1 RNA peak which showed an increase of 150% over the controls, whereas the ribosomal RNA peak increased by 67%. Smaller increases (30%) occurred in the Q2 RNA peak and in the components eluted with sodium dodecyl sulphate.

Somewhat similar results were obtained in the dual-labelling experiments. After allowing for the difference in specific radioactivities of the precursors, the total 14 C recovered was 63% greater than the total 3 H and the largest increase again occurred in the Q1 RNA peak.

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Urease in *Spirodela*

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Bollard (1966) found that the higher plant *Spirodela oligorrhiza* (a duckweed) could be grown vegetatively in sterile culture and under these conditions it used urea as sole source of nitrogen provided that the pH of the medium was not above 4.5. We have found that in urea-containing media of higher pH plants rapidly became nitrogen-deficient in appearance and made no further growth. Urease could not be detected in plants growing with ammonium sulphate as sole source of nitrogen, but in plants actively growing with urea as a sole source of nitrogen this enzyme was always present. Such an appearance of urease where it is normally absent suggests that, in this species, the enzyme may be induced by urea taken up from the medium. *Spirodela* also grew with the ureide allantoin as source of nitrogen and allantoin-grown plants contained urease. The metabolism of allantoin is likely to produce urea as an intermediate and such

urea produced endogenously might in turn induce urease production.

When *Spirodela* plants were placed in a medium lacking any source of nitrogen they rapidly became nitrogen-deficient in appearance and, after some days, urease activity appeared in such plants, reaching activities as high as in plants growing on urea-containing medium. Some redistribution of cell nitrogen must occur under the stress of nitrogen deficiency and it is likely that some normal catabolic reactions may produce urea endogenously.

It is clear that the activity of urease in plants is markedly affected by the pH of the medium. Plants in media at pH 7 took up urea but no urease appeared in the tissues and consequently such plants did not grow. Growth of plants at a high pH on a medium containing allantoin as a sole source of nitrogen or on a nitrogen-deficient medium lowered or prevented the development of urease activity. When the pH of the medium fell to 3.5 or lower the activity of urease rose to values up to ten times those present in plants growing on urea at about pH 4.3.

It thus appears that in this species urease is normally absent or present at a very low activity. Nonetheless it may be a normal catabolic enzyme that can increase markedly in activity when plants become nitrogen-deficient or when they are supplied with either allantoin or urea. Then what is normally a catabolic enzyme may serve as a primary assimilatory enzyme.

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Activation *in vitro* and Solubilization of Glucuronyltransferase (assayed with Bilirubin as Acceptor) with Digitonin

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Several microsomal enzymes have been activated by treatment with deoxycholate, Triton X-100 or other detergents (Ernster, Siekevitz & Palade, 1962; Dallner, 1963; Snoke & Nordlie, 1967). Recently glucuronyltransferase was shown to behave in a similar way (Lueders & Kuff, 1967; Van Roy & Heirwegh, 1968). With increasing detergent concentrations maximal activation was attained followed by gradual inhibition and loss of enzyme activity. Attempts to solubilize glucuronyltransferase with deoxycholate were unsuccessful (Leventer, Buchanan, Ross & Tapley, 1965; Tomlinson & Yaffe, 1966). In contrast, active non-sedimentable preparations were obtained with digitonin (Pogell & Leloir, 1961).

Following these observations an examination of the effect of digitonin on the activity of glucuronyl-transferase with bilirubin as acceptor (Van Roy & Heirwegh, 1968) was made. Microsomal preparations (final protein concn. 1.2-1.6 mg./ml.) from rat liver were treated at 0° for 30 min. with various digitonin concentrations between 0 and 8.2 mg./ml., and were then assayed for glucuronyltransferase activity. By increasing the digitonin concentration it was possible to raise the enzyme activity to a maximal value which was 5 to 7 times that found with untreated microsomes. The preparations were fully activated at detergent-protein ratios between 2.2 and 6.4.

After standing for 30 min. at 0° the microsome-digitonin mixtures were separated into the corresponding sediment and supernatant fractions by centrifuging at 4° for 72 min. at 80000g_{av.}. The transferase activities of fully activated microsomes and of the derived sedimentable and non-sedimentable fractions were stable for at least 24 hr. at 4°. The sum of the activities of the latter two fractions amounted to 65-80% of the value found for the corresponding microsome-detergent mixtures. However, the initial activity could be restored by re-suspending the sediments in the corresponding supernatant solutions. In the solubilized fraction 20-30% of the activity was recovered.

Chromatographic analysis of the azopigments derived from conjugated bilirubin produced *in vitro* by enzymic incubation of microsomal preparations showed that azopigments A and G were formed in equal amounts (Van Roy & Heirwegh, 1968). Identical results were obtained with digitonin-treated microsomes and with the derived fractions.

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The Fate of 4,5- and 5,6-Dichloro-2-trifluoromethylbenzimidazole in the Rat and Rabbit

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Various derivatives of 2-trifluoromethylbenzimidazole possess antibacterial, antiviral, herbicidal or insecticidal activity (Bishop, Chelton & Jones, 1964; Fara & Cochran, 1963; Burton *et al.* 1965) and some are potent uncouplers of oxidative phosphorylation (Jones & Watson, 1967).

We have studied the fate of orally administered 4,5- and 5,6-dichloro-2-trifluoro[¹⁴C]methylbenzimidazole in the rat and rabbit. Both compounds were more toxic to rabbits than to rats. In the rat (dose, 50 mg./kg.), 82%, and in the rabbit (dose, 6 mg./kg.), 79%, of the ¹⁴C of the 4,5-isomer was excreted in the urine in 3 days. None of the compound was excreted unchanged. The urine of the first day was examined for metabolites by paper, thin-layer and gas chromatography and by comparison with synthetic reference compounds. The urines contained after acid hydrolysis, 6-hydroxy- (25, 11%), 7-hydroxy- (27, 8%) and 6,7-dioxo-4,5-dichloro-2-trifluoromethylbenzimidazole (28, 20%), the figures in parentheses being the % of the dose excreted as these metabolites by the rat and the rabbit respectively. These metabolites were excreted mainly as glucuronic acid or sulphate conjugates or both, and it appears that the 6,7-quinone is formed, during the hydrolysis, from the corresponding 6,7-diol.

The excretion pattern of the 5,6-isomer was different from that of the 4,5-isomer. In the rat (dose 33 mg./kg.), 33% of the ¹⁴C appeared in the urine and 28% in the faeces in 3 days, but in the rabbit (dose, 3.3 mg./kg.) in the same time only 10% appeared in the urine and none in the faeces. In 5 days after the dosing only 24% of the dose of ¹⁴C had been excreted by the rabbit and the remainder was found distributed evenly in the tissues. Only traces of the original compound were excreted by both species. The urine of the first 24 hr. after the dosing in the rat and of the first 5 days in the rabbit was examined for metabolites. There were identified as 5,6-dichloro-2-trifluoromethylbenzimidazole-1-glucuronide (6, 9%) and the glucuronic acid or sulphate conjugates (or both) of the 4-hydroxy (9, 2%) and 4,7-dihydroxy (4, 15%) derivatives of the 5,6-isomer (the figures in parentheses are the % of dose in the rat and the rabbit respectively). The 4,7-dihydroxy derivative was detected after acid hydrolysis as the corresponding 4,7-quinone.

Although both compounds are metabolized mainly by aromatic hydroxylation at the two unsubstituted carbon atoms there is a marked