of the *cyclo*propane ring, and these await further elucidation.

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Biliary Excretion of Aflatoxin in the Rat after a Single Dose

WHEN aflatoxin damages the liver[1] the cells most affected are those which are concentrated around the portal vein and bile duct. There seemed to be no published data on the quantitative pattern of biliary excretion of this toxin and the nature and partition of its metabolites in bile, and so we have investigated the problem.

Our experiments were made possible by the availability of aflatoxins *B* and *G*, labelled with carbon-14 in the uridine, and synthesized by a procedure developed here. The method involved the growing of spores of *Aspergillus flavus* in a Czapeck medium for 60 h before the addition to the culture of sodium acetate-2-^{14}C. The product has a specific activity of 30 mμc./mmole, and the method thus differs from the resting cell technique of Adye and Mateles[2,3]. A male rat weighing 350 g was lightly anaesthetized with ether before the establishment of a biliary fistula as described by Boyland *et al.*[4]. Bile was collected in a saddle-shaped container attached to the back of the rat by 'Elastoplast'[5]. In order to obtain samples of bile at regular intervals, a polythene tube was introduced into the chamber, and through this the bile was removed by suction. A dose of 50 μg of labelled aflatoxin B_1 in saline with a specific activity of 30 mμc./mmole, was given by intraperitoneal injection to the rat and bile was collected every 15 min. A sample of bile (0·1 ml.) was transferred to a planchet, dried on a hot plate, and counted at infinite thinness in an end-window Geiger–Müller counter. The rate of excretion of labelled metabolites of aflatoxin B_1 in the bile is shown in Fig. 1.

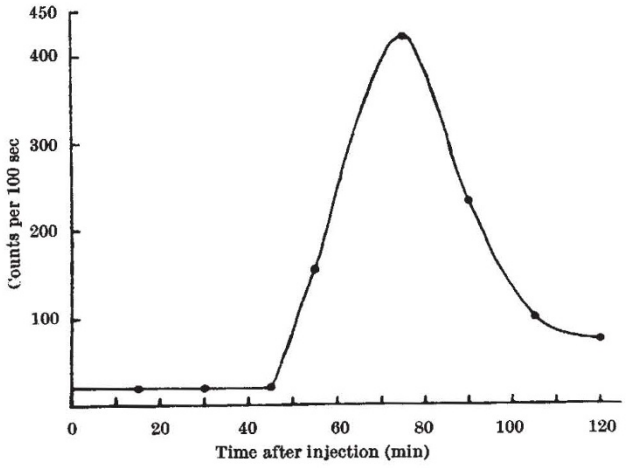

Fig. 1. Rate of excretion of metabolites of aflatoxin B_1 in rat bile.

There was a latent period of about 60 min between the time of injection and the first appearance of these metabolites in the bile samples collected. This was partly a result of the slow rate of flow of bile in the anaesthetized rat. It was, however, clear from the graph that aflatoxin was rapidly excreted through the bile, as was indicated by the observation of Falk *et al.*[6]. Aliquots of the 6 h collections of bile were shaken with equal volumes of chloroform for 20 min. The amounts of radioactivity in the chloroform layer were estimated as described earlier. The distribution of the counts in the various fractions is shown in Table 1.

Table 1. PARTITION OF LABELLED METABOLITES IN THE BILE SAMPLES OBTAINED FROM THE TREATED RAT

Time of collection	Total counts in bile samples (*C–B*)	Recovery of initial counts (per cent) Total bile	$CHCl_3$ extract	Conjugates
0– 6 h	13,800	30	10	20
6–12 h	770	1·7	0·7	1·0

C–B, counts in sample minus background counts.

Aliquots of the chloroform extracts were run on thin-layer chromatograms of silica gel *G*, using a mixture of 1 ml. of acetic anhydride; 2 ml. of methanol and 97 ml. of chloroform as the solvent. Each of these extracts contained a mixture of aflatoxin B_1 and aflatoxin M_1, with similar properties to those described by other authors[7–9]. The principal constituent was a conjugate compound which gave a positive ninhydrin reaction on paper chromatography and was alkali labile. On alkaline hydrolysis of this conjugate, it yielded two fractions, one of which was identifiable with taurocholate on thin-layer chromatography, while the other had a slightly lower R_F value than aflatoxin B_1 and gave a blue fluorescence in ultra-violet light. This last compound may have been a degradation product of aflatoxin B_1. Work is in process with the aim of elucidating the nature of the aflatoxin bonding in the conjugate. Analysis of urine samples from the treated rat showed that approximately 26 per cent of the dose count was excreted as a glucuronide conjugate in the first 6 h after the injection of aflatoxin.

Our experiments with bile and urine suggest the existence of various pathways of aflatoxin B_1 metabolism in the rat, including that of demethylation proposed by Shank and Wogan[10], who recovered 14–69·8% per cent of labelled aflatoxin in the faeces of four experimental rats, and Wogan[11] concluded from this that most of an aflatoxin dose was excreted through the bile. Our results show excretion of a glucuronide in the urine and of a taurocholate conjugate in the bile to be of great importance.

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