vacuolar changes in liver cells were observed, however, these changes were noted in both test groups and did not correlate with the severity of clinical symptoms.

The finding that 2 conditions (one spontaneous and idiopathic, the other experimental) with manifestations of extrapyramidal motor dysfunction are associated with a deficiency of dopamine and serotonin in the caudate suggests that this deficiency may play a significant role in producing this dysfunction. In the present studies, the concentration of norepinephrine and serotonin in cerebrum and brain stem appeared essentially normal after MnO_2 treatment. Therefore, failure of the enzyme systems responsible for their formation and destruction in caudate was probably not the primary reason for depletion. Furthermore, it is important to recognize that clinical and biochemical abnormalities may appear before histopathological changes in brain can be demonstrated, and that the presence of histological changes in specific locations may not explain the clinical manifestations[12].

Table II. Concentrations of serotonin in caudate nucleus and cerebrum after MnO_2 treatment

	Caudate nucleus (μg/g)	Cerebrum (μg/g)
Control (2)	0.77 (0.66–0.88)[a]	0.19 (0.18–0.20)
Group B (6)	0.12 ± 0.05[b]	0.19 ± 0.03

Number of observations in brackets. [a] Mean (range). [b] Mean ± S.E.

Riassunto. Scimmie scoiattolo trattate cronicamente con MnO_2 presentano disturbi del sistema extrapiramidale ed una riduzione delle concentrazioni di serotonina e dopamina nel nucleo caudato. L'intensità dei disturbi extrapiramidali e la riduzione della concentrazione delle amine nel caudato appaiono correlate.

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Biosynthesis of Aflatoxins by Cell-Free Preparations from *Aspergillus flavus*

The aflatoxins are a group of hepatotoxic metabolites produced by *Aspergillus flavus* which infects foodstuffs, especially groundnuts[1]. Though a large number of reports have appeared on their occurrence in contaminated foodstuffs, their structure and toxicity, no major effort seems to have been made to investigate their biosynthesis. Mateles et al.[2,3] have studied the incorporation of some radioactive substrates into aflatoxins by the resting mycelium of *A. flavus* and Biollaz et al.[4] have determined the labelling pattern of aflatoxin B_1 so formed.

In the present investigation, the incorporation of some ^{14}C-labelled compounds into aflatoxins has been studied using a cell-free system prepared from *A. flavus* with a view to obtaining information on the mechanism of aflatoxin biosynthesis.

Materials and methods. Aspergillus flavus ATCC 15517 used in this study was maintained on a glucose peptone agar medium. Spores of 7-day growth on this medium were transferred to a 20% sucrose–2% yeast extract medium[5] in 500 ml Erlenmeyer flasks and incubated either on a rotary shaker for 5 days at 30 °C or without shaking for 15 days at 25 °C. The mycelial growth was filtered, washed with distilled water and ground up in a glass mortar with acid-washed sand and cold 0.05*M* phosphate buffer, pH 6.5. Subcellular fractionation was carried out in an International Refrigerated Centrifuge Model PR 2, by standard procedures[6]. The reconstituted homogenate was prepared by mixing equivalent amounts of nuclear and mitochondrial fractions with $^1/_{10}$ of the corresponding original volume of the supernatant.

As preliminary experiments indicated that the ability to incorporate acetate-1-^{14}C was found mostly in the mitochondrial fraction, either the reconstituted homogenate or the mitochondrial fraction was incubated in Erlenmeyer flasks for 6 h at 30 °C on a rotary shaker with the addition of suitable substrates and co-factors as given below: mitochondrial fraction or reconstituted homogenate in 0.05*M* phosphate buffer pH 6.5, 10 ml, glucose 60 mg, Difco yeast extract 40 mg, isocitrate 1 mg, ATP 1 mg, NADPH 0.5 mg and acetate-1-^{14}C (0.22 mc/m*M*) 10 μc, or DL-leucine-U-^{14}C (4.55 mc/m*M*) 8 μc or DL-mevalonic acid-2-^{14}C-lactone (3.35 mc/m*M*) 8 μc. At the end of 6 h, a carrier of a mixture of aflatoxins in 0.2 ml propylene glycol was added to the incubation mixture which was then extracted with chloroform and the extract was dried over anhydrous sodium sulphate, evaporated under reduced pressure and made up to 2 ml. An aliquot of this was subjected to thin-layer chromatography on Silica gel G (Merck) with 2% methanol in

[1] G. N. Wogan, Bact. Rev. *30*, 460 (1966).
[2] J. Adye and R. I. Mateles, Biochim. biophys. Acta *86*, 418 (1964).
[3] J. A. Donkersloot, D. P. H. Hsieh and R. I. Mateles, J. Am. chem. Soc. *90*, 5020 (1968).
[4] M. Biollaz, G. Buchi and G. Milne, J. Am. chem. Soc. *90*, 5017 (1968).
[5] N. D. Davis, U. L. Diener and D. W. Eldridge, Appl. Microbiol. *14*, 378 (1966).
[6] W. C. Schneider and C. Hogeboom, J. biol. Chem. *183*, 123 (1950).

chloroform. The aflatoxin bands were scraped out and eluted with methanol. The aflatoxins were determined by measuring the absorption at 363 nm and using the extinction coefficients reported by NABNEY and NESBITT[7]. The radioactivity was determined with a Tracerlab gas flow counter.

Results. Under the experimental conditions described, labelled acetate, mevalonate and leucine were incorporated into aflatoxins by the mitochondrial fractions or reconstituted homogenates from *A. flavus* grown in either still or shake cultures (Table). With preparations from still cultures, the highest counts were given by mevalonate followed by leucine and then acetate. An incorporation of about 0.4% was achieved with mevalonate. In extracts from shake cultures, the differences were not so marked but mevalonate again gave slightly higher counts than leucine or acetate. The incorporation obtained from mitochondrial preparations generally varied from about 50% to more than 100% of that given by the reconstituted homogenates, suggesting that most of the enzymes involved in the biosynthesis of aflatoxins are localized in the mitochondrial fraction. With the reconstituted homogenate from shake cultures and ^{14}C-mevalonic acid lactone, specific activities obtained for aflatoxins B_1, B_2, G_1 and G_2 were 13,570, 252,600, 41,090 and 252,300 cpm/mg respectively.

Further studies are in progress to determine the properties of the cell-free enzyme system reported here, as well as the pathway by which the aflatoxins are synthetized[8].

Incorporation of labelled compounds into aflatoxins by cell-free extracts from *A. flavus*

Labelled compound	Fraction	Shake culture (cpm)		Stationary culture (cpm)	
		Aflatoxin B	Aflatoxin G	Aflatoxin B	Aflatoxin G
Acetate-1-^{14}C	Reconstituted homogenate	2216	2500	168	396
	Mitochrondrial	1624	1060	420	348
DL-mevalonic acid-2-^{14}C-lactone	Reconstituted homogenate	3048	2120	4712	872
	Mitochondrial	2308	1112	5828	2292
DL-leucine-U-^{14}C	Reconstituted homogenate	1848	1704	456	532
	Mitochondrial	1104	1496	396	696

The composition of the incubation mixture and assay procedures were as described under 'Materials and methods'. Reconstituted homogenate and mitochondrial fractions taken for incubation contained 75.0 mg and 41.3 mg of protein respectively in the case of preparations from stationary cultures and 42.0 and 20.0 mg of protein respectively in those from shake cultures.

Résumé. Incorporation d'acétate-1-^{14}C, de leucine-U-^{14}C et de mévalonate-2-^{14}C sous forme d'aflatoxine dans les extraits libres des cellules d'*Aspergillus flavus*. Dans le cas du mévalonate, l'incorporation de l'ordre de 0,4 pour 100 et les activités spécifiques sont 13000 à 40000 cpm/mg d'aflatoxine. La capacité d'incorporation des substrats en aflatoxine s'observe, en particulier, dans les mitochondries.

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[7] J. NABNEY and B. F. NESBITT, Analyst *90*, 155 (1965).

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Separation of Rat Liver Mitochondrial Amine Oxidases

Existence of a number of mitochondrial amine oxidases[1-3] rather than a single enzyme with broad substrate specificity ('monoamine oxidase', EC 1.4.3.4) was suggested. Partial separation[4] of amine oxidases deaminating *p*-nitrophenylethylamine[5,6] and m-nitro-*p*-hydroxybenzylamine[7] was a first indication of the possibility of physical separation of mitochondrial amine oxidases[4]. This was confirmed in other laboratories[8-10].

However, so far all the experiments[4,8-10] were carried out with mitochondria treated by non-ionic detergents. In view of an ability of the detergents to alter tertiary structure of proteins[11,12], the results obtained may not be considered as unequivocal evidence for 'multiplicity' of mitochondrial amine oxidases.

Sonication disintegrates mitochondrial membranes to particles having from about 50–200 Å in diameter[13] and separation of the particles by means of density gradient column electrophoresis[14] was undertaken.

Rat liver mitochondria[4] were sonicated in a 22-kc generator UZM-1.5 at maximal power output for 15 min in 0.025 *M* sodium borate (pH 9.2; concentration of protein 3 mg/ml). Samples (14 ml) were introduced into an LKB column electrophoresis apparatus using a device

[1] E. WERLE and F. ROEWER, Biochem. Z. *322*, 320 (1952).
[2] V. Z. GORKIN, N. V. KOMISAROVA, M. I. LERMAN and I. V. VERYOVKINA, Biochem. biophys. Res. Commun. *15*, 383 (1964).
[3] V. Z. GORKIN, Pharmac. Rev. *18*, 115 (1966).
[4] V. Z. GORKIN, Nature *200*, 77 (1963).
[5] E. A. ZELLER, H. R. BUERKI and T. ISHIMARU, Fedn Proc. *21*, 271 (1962).
[6] L. V. BRUSOVA, L. A. VYUGOVA and V. Z. GORKIN, Ukr. biokhem. Zh. *37*, 463 (1965).
[7] V. Z. GORKIN, N. A. KITROSSKY, L. B. KLYASHTORIN, N. V. KOMISAROVA, G. A. LEONTYEVA and V. A. POUCHKOV, Biokhimiya *29*, 88 (1964).
[8] G. G. S. COLLINS, M. B. H. YOUDIM and M. SANDLER, Fedn. Europ. biochem. Soc. Letters *1*, 215 (1968).
[9] H. C. KIM and A. D'IORIO, Can. J. Biochem. Physiol. *46*, 295 (1968).