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.

H. G. RAJ, L. VISWANATHAN, H. S. R. MURTHY and T. A. VENKITASUBRAMANIAN

Department of Biochemistry,
Vallabhbhai Patel Chest Institute,
University of Delhi,
Delhi-7 (India), 24 June 1969.

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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

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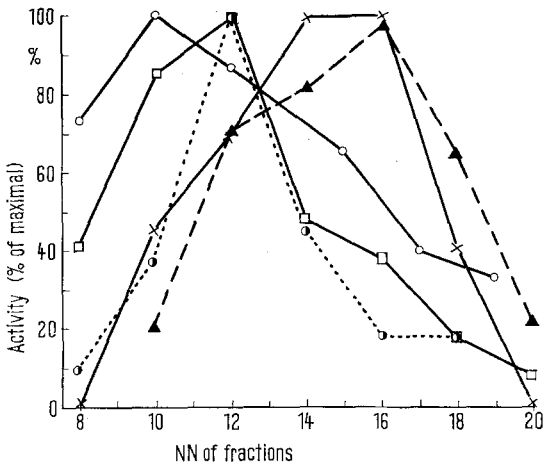
for automatic sample injection with simultaneous establishing of sucrose density gradient in borate buffer system (ref.[14], pp. 216 and 224). Electrophoresis was carried out at 200 V (12 mA) for 63 h. In 5 ml fraction rates of deamination of monoamines were determined using following final concentrations of the substrates: kynuramine.HBr 1 mM; pH 7.4 (ref.[15]), *p*-nitrophenylethylamine.HCl 0.83 mM and *m*-nitro-*p*-hydroxybenzylamine.HCl 0.53 mM, both at pH 7.0 (ref.[4]), tyramine.HCl 3.2 mM and 5-hydroxytryptamine. cratininesulphate 5 mM, both at pH 7.4 (ref.[16]).



Separation by means of density gradient electrophoresis of amine oxidases from rat liver mitochondria disintegrated by sonication in absence of detergents. Conditions of the experiment see text. ○—○, 5-hydroxytryptamine; □—□, tyramine; ◐---◐, *p*-nitrophenylethylamine; ×—×, kynuramine; △---△, m-nitro-*p*-hydroxybenzylamine. Activity in fractions in per cent of the highest activity.

A typical example of the results obtained (Figure 1) shows that the amine oxidases of rat liver mitochondria may be more or less distinctly separated. Similar results are observed in experiments with longer (up to 85 h) but not shorter (24–48 h) electrophoretic runs[17]. After the treatment with urea of sonicated rat liver mitochondria, separation of amine oxidases by density gradient electrophoresis was observed even within 43 h runs[18].

Выводы. Аминоксидазы озвученных митохондрий печени крысы могут быть разделены в отсутствие детергентов путем электрофореза в градиенте плотности.

V. Z. Gorkin

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow G117 (USSR), 6 June 1969

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The Identification of Quantitation of Extremely Polar, Free Corticosteroids in Liquor amnii

During the course of work on the steroid content of liquor amnii, it has become apparent that a number of free polar corticosteroids are present in substantial amounts.

6β, 11β, 21α, 21 tetrahydroxypregn-4-ene-3, 20-dione (6β hydroxycortisol), 6β, 11β, 17α, 20α, 21 pentahydroxypregn-4-ene-3-one (20α dihydro 6β-OH-F) 6β, 11β, 17α, 20β, 21 pentahydroxypregn-4-ene-3-one (20β dihydro 6β-OH-F) as well as 11β, 17α, 21 trihydroxypregn-4-ene-3-20 dione (cortisol) and 11β, 17α, 21 trihydroxypregn-4-ene-3-11-20 trione (cortisone) have all been identified and a quantitative estimate made in a number of samples of liquor from full-term normal pregnancies.

The extraction of these extremely polar corticosteroids has been previously described[1–3]. The preliminary purification and identification procedures and the quantitative determination of 6β hydroxycortisol by gas-liquid chromatography (GLC) and the reproducibility of the assay has also been described previously[3]. The identification of other cortisol metabolites more polar than 6β hydroxycortisol has been concluded, following initial purification of the ethyl acetate extract by thin-layer chromatography in a distilled water system. The 20α and 20β derivatives of 6β hydroxycortisol were isolated as a mixture following subsequent thin-layer chromatography in a system comprising chloroform–methanol 84:16 by volume (Rf 0.30). They were then separated by chromatography on boric acid impregnated plates (3% boric acid in water) in a solvent comprising chloroform–methanol 84:16 by volume. In this system 17α, 20β-diols moved considerably faster than 17α, 20α-diols. The UV-absorbing zones (254 nm) were eluted and separately purified by thin-layer chromatography in a system comprising diethyl ether–ethanol 9:1 by volume, when a wide separation of the compounds occurred.

Alternatively it was found possible to separate the 2 epimers by preliminary paper chromatography in a benzene–ethyl acetate–methanol–water system (7:3:10:10 by volume) and following elution of the single spot subsequent paper chromatography in a system comprising benzene–tertiary butanol–water (70:43:86 by volume) with a 4 h overrun. 2 UV-absorbing zones were observed which corresponded to the markers of 20α and 20β dihydro 6β hydroxycortisol; the 20α epimer being more polar than the 20β.

Identification procedures on the eluted compounds were carried out as previously described[4, 5] and by comparative programmes of thin-layer, paper and gas liquid chromato-

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