hydes derived from alcohols of higher molecular weight which are present in bourbon. After such inhibition the resultant delay in the oxidation of acetaldehyde would be reflected by its accumulation in blood. The average daily consumption of bourbon per drinker was 649 ml which contained 34 mg of acetaldehyde. If we assume that bourbon was consumed only during a 12-hour period per day, the dose of administered acetaldehyde would be 0.041 mg per kilogram per hour (0.49 mg per kilogram per 12 hours). However, in previous studies on humans (*11*) 124 to 164 mg of acetaldehyde was infused during 6 to 8 minutes with no significant effects.

Figure 2 presents representative data for two subjects who consumed grain ethanol and bourbon for 10 and 13 consecutive days, respectively. Although blood ethanol concentrations peaked above 400 mg per 100 ml and were sustained above 300 mg per 100 ml for long periods of time, blood acetaldehyde concentrations remained relatively stable throughout the course of long-term drinking.

Figure 3 presents consecutive hourly blood ethanol and blood acetaldehyde concentrations for a subject who consumed grain ethanol. On initiation of grain ethanol intake, blood ethanol concentrations rose to 360 mg per 100 ml after 8 consecutive hours of drinking. However, blood acetaldehyde concentrations remained relatively low with no dose or dose-time relationship to ascending blood ethanol concentrations. On the first day of withdrawal from alcohol, blood ethanol concentrations fell from 335 mg per 100 ml to almost 0 within 14½ hours. No descending dose or dose-time relationship was found between blood ethanol and blood acetaldehyde concentrations immediately after cessation of drinking.

The findings of this study indicate that some effects of the presence of acetaldehyde observed in animal and in in vitro experiments may also occur in alcoholics during long-term drinking. For example, in vitro kinetic studies (*4*) with rat brain mitochondrial aldehyde dehydrogenase revealed an inhibition constant K_I for acetaldehyde of $2.62 \times 10^{-6} M$, whereas the Michaelis constant K_m for 5-hydroxyindoleacetaldehyde was $5.44 \times 10^{-6} M$. These data suggest that a similar type of inhibition may occur in the human brain during long-term alcohol intake with the blood acetaldehyde concentrations ($5 \times 10^{-5} M$) reported here. However, the concentrations of acetaldehyde reported here are relatively low in comparison to dose levels used by a number of other investigators in studies of intermediary metabolism and biotransformation of catecholamines (*2, 3*).

Both tolerance and physical dependence have dose and dose-time relationships to blood ethanol concentrations (*12*). Such dose-response relationships were not found for blood acetaldehyde concentrations reported here. However, it remains to be determined if sustained elevations in acetaldehyde concentrations at neural tissue sites may be related to the addictive process in alcoholism.

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Ochratoxin A: Inhibition of Mitochondrial Respiration

Abstract. *Ochratoxin A is a fungal metabolite which induces pathological changes in animals. The toxin was isolated from cultures of* Aspergillus ochraceus *and purified by thin-layer chromatography. Ochratoxin A and one of its hydrolysis products, dihydroisocoumarin, severely inhibited coupled respiration when applied at low concentration to rat liver mitochondria.*

Ochratoxin A is a fungal toxin produced primarily by certain isolates of *Aspergillus ochraceus* Wilh. (*1*), although its production by a species of *Penicillium* has also been reported (*2*). On acid hydrolysis, it yields L-phenylalanine and dihydroisocoumarin (*3*) (Fig. 1).

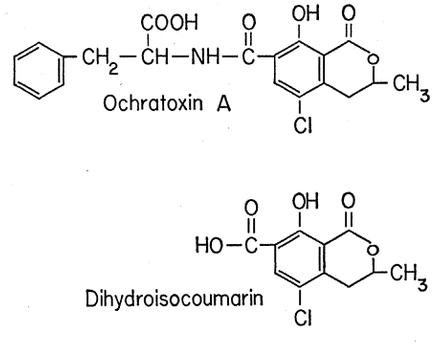

Fig. 1. Structure of ochratoxin A and the dihydroisocoumarin derivative (5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin-7-carboxylic acid) (*3*).

Aspergillus ochraceus is widely distributed in nature and has been isolated from soils, decaying vegetation, and animal feeds, and from foodstuffs intended for human consumption (*1, 4*). In culture, the fungus will synthesize the toxin in quantity (*5*). Ochratoxin A was reported to be a natural contaminant (110 to 150 ppb) of poor-grade corn in storage (*4*).

Both ochratoxin A and feeds infested with toxin-producing strains of the fungus are highly toxic to experimental animals (*6, 7*). The toxicity of a single dose of ochratoxin A toward ducklings is similar to the toxicity of aflatoxin B_1 (a liver poison produced by *A. flavus*) (*1*). Pathological changes were found in the liver cells of day-old ducklings (*6*) 4 hours after the ducklings were given 100 μg of crystalline ochratoxin A. Mild fatty infiltration of

the hepatocytes was observed, and electron microscopy revealed gross changes in the mitochondria and endoplasmic reticulum. At later stages, organelles were found free in the sinusoids apparently as a result of their being discharged through gaps in the sinusoidal endothelium. Purchase and Nel (*8*) studied the effects of the toxin on rats; the major macroscopic changes at autopsy were tubular necrosis of the kidney, mild liver degeneration, and enteritis. When rats were given an intraperitoneal injection of ochratoxin A (10 mg/kg), both dihydroisocoumarin and unchanged ochratoxin A were recovered in feces and urine collected over a 72-hour period. Ochratoxin A and minute quantities of the dihydroisocoumarin derivative were also found in the blood and liver (*8*).

We report here the effects of ochratoxin A and its metabolites on the respiration of isolated rat-liver mitochondria.

Aspergillus ochraceus (NRRL 3174) was grown on a semisynthetic medium, and ochratoxin A was isolated and identified (*5*). Ochratoxin A was eluted from thin-layer chromatography (TLC) plates, and its concentration was measured spectrophotometrically (*3*). Dilutions of this eluate were prepared to give the required concentrations for testing.

Dihydroisocoumarin was obtained from ochratoxin A by acid hydrolysis (*3*). Completeness of hydrolysis was checked by TLC, and the concentration of the extracted dihydroisocoumarin was measured spectrophotometrically. This extract was used to prepare solutions of dihydroisocoumarin for testing.

Liver mitochondria were isolated from female weanling rats (Charles River CDA) (*9*). Respiration rates and respiratory control ratios were measured polarographically (*10*, *11*). Mitochondria were added to a medium containing no phosphate acceptor, and, when a steady state of respiration was recorded, state 3 respiration (*12*) was initiated by the addition of adenosine diphosphate (ADP) as phosphate acceptor. Initial state 3 respiration rates recorded with the different preparations had an average respiratory control ratio of 2.8 (range 2.2 to 3.6). After transition to state 4 respiration, the appropriate concentration of ochratoxin A, dihydroisocoumarin, or L-phenylalanine solution was added, and the effects on state 4 respiration were observed before a further addition of ADP was made. Respiration rate during the second state 3 was recorded. The effect of ochratoxin A or its metabolites on the second state 3 rate was calculated as the percentage of inhibition of the initially measured state 3 rate. When the respiration rate was constant, 2,4-dinitrophenol, at a concentration which uncouples electron transport from a requirement for the phosphate acceptor ADP, was added and the effect recorded (Table 1).

Table 1. Effect of ochratoxin A, dihydroisocoumarin, and L-phenylalanine on rat liver mitochondrial respiration. Experiments were conducted at 30°C in 3 ml of medium (*p*H 7.2) containing: 0.25*M* sucrose; 3.3 m*M* succinate; 20 m*M* KCl; 5 m*M* $MgSO_4$; 10 m*M* KH_2PO_4; 3 mg of bovine serum albumin, and mitochondria (approximately 0.4 mg nitrogen). 2,4-Dinitrophenol (DNP) and ADP were added to give final concentrations of 37.5 and 40 μmole/liter, respectively. Ochratoxin A and dihydroisocoumarin were added in ethanol, and an equal volume of ethanol was added to the controls. Data are mean values from at least three, in most cases five or six, replicates.

Concentration (*M*)	Inhibition of respiration (%)		Release of state 3 inhibition by DNP
	State 3	State 4	
Ochratoxin A			
4.2×10^{-4}	100 ± 0	28 ± 4.6	−
4.2×10^{-5}	89 ± 4.6	0	+
2.1×10^{-5}	55 ± 2.4	0	+
2.1×10^{-6}	43 ± 7.7	0	+
2.1×10^{-7}	34 ± 11.7	0	+
Dihydroisocoumarin			
1.0×10^{-4}	100 ± 0	20 ± 8.5	−
1.0×10^{-5}	100 ± 0	0	+
1.0×10^{-6}	64 ± 11.8	0	+
1.0×10^{-7}	21 ± 11.5	0	+
L-Phenylalanine			
3.0×10^{-3}	0	0	

Ochratoxin A, at low concentration, inhibited ADP-stimulated (state 3) respiration. The dihydroisocoumarin fraction was more inhibitory than the parent compound, and L-phenylalanine was not inhibitory. The rate of state 4 respiration was reduced only by the highest concentrations of ochratoxin A and dihydroisocoumarin. When 2,4-dinitrophenol was added, inhibition of state 3 respiration by either ochratoxin A or dihydroisocoumarin was overcome, except when the highest toxin concentrations were used. This indicates that the block due to ochratoxin A or dihydroisocoumarin is at some point—in the sequence of reactions leading to adenosine triphosphate formation—beyond the action site of dinitrophenol.

Because a metabolite of ochratoxin A has been detected, in feces and urine from rats injected intraperitoneally (*8*), we investigated the possibility that mitochondria might metabolize ochratoxin A to produce the inhibitory dihydroisocoumarin derivative. Mitochondria were incubated at 30°C in the medium described in Table 1 along with ochratoxin A at concentrations of 4.2×10^{-4} and $2.1 \times 10^{-5}M$ (100 and 55 percent inhibition of respiration, respectively). At regular intervals for 30 minutes, samples were removed and examined for dihydroisocoumarin by TLC (*3*). Ochratoxin A was apparently not degraded; consequently, data for ochratoxin A in Table 1 probably represent inhibition due to the parent molecule.

No information is available on the absorption of ochratoxin A by animal tissues, and we believe this is the first report of any direct effect of ochratoxin A or its metabolites on the metabolism of a cell organelle. The obvious histological changes produced by the toxin in animals indicate that many metabolic and physiological systems are affected. How serious a problem ochratoxin A may be is not known (*7*); however, the free toxin has now been isolated from stored corn (*4*), and foodstuffs that contained the organism have been purchased (*7*). Further studies on the distribution and growth of the fungus and the effects of ochratoxin A on animal metabolism seem warranted.

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