Effect of Aflatoxin B_1 on the in vitro Incorporation of ^{14}C-Acetate into Human Skin Lipids

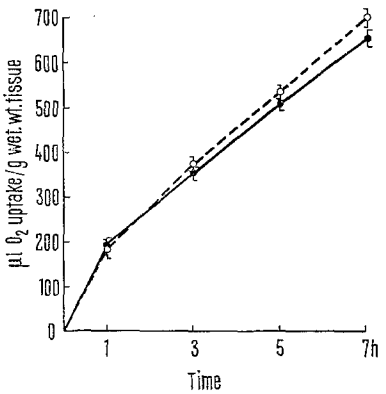
Aflatoxin B_1 is the most potent of the carcinogenic metabolites produced by strains of *Aspergillus flavus*[1]. This fungal product causes liver tumors in numerous species of experimental animals and appears to be a rather specific inhibitor of protein synthesis[2-5]. Protein synthesis is presumably inhibited as the result of the interaction of the toxin and DNA and the resulting inhibition of messenger RNA formation[4].

The manifestations of aflatoxin poisoning appear almost exclusively in the liver, the primary target organ[6]. It has been shown that although aflatoxin is readily absorbed through the skin of the rat, no gross toxic effects occur in this tissue[7]. Contrary to results obtained with rat skin, JOFFE and UNGAR[8] have demonstrated that exposure of rabbit skin to very low levels of the toxin results in severe epidermal lesions. These authors have emphasized the possibility of dermatoses occurring in humans who come in contact with aflatoxins. The possible biochemical lesions in this tissue resulting from aflatoxin poisoning have received little attention. This paper reports the effects of aflatoxin B_1 on the in vitro incorporation of ^{14}C-acetate into human skin lipids.

Materials and methods. Fresh human skin specimens were obtained from the lower abdomen of male Caucasians. The area was anesthetized with 1% lidocaine and the skin removed with a 6 mm dermal punch. Subcutaneous tissue was trimmed and discarded. The skin was rinsed with buffer, blotted dry and weighed, and placed in 15 ml manometric reaction flasks containing 2.0 ml Kreb's-Ringer phosphate buffer, pH 7.4, and 50 µg aflatoxin B_1. The toxin was either dissolved in ethanol or ethanol-propylene glycol (1:1 v/v). Ethanol levels did not exceed 1% of the total volume and the control contained an equal volume of alcohol or alcohol-propylene glycol. The flasks were incubated for 1 h at 37 °C after which 0.5 ml buffer containing 1 µC 1-^{14}C-acetate was added to the incubation medium from the side-arm of the reaction flask. Incubation continued for an additional 6 h at which time the reaction was halted. Respiratory rates were determined manometrically for the total incubation period. The skin specimens were sectioned (50 µ diameter) with a freezing stage microtome and the total lipid extracted by the method of FOLCH et al.[9]. Phospholipids were isolated by the method of SINCLAIR and DOLAN[10] and free sterols, as the digitonides, by the method of SPERRY and WEBB[11]. Neutral fats were isolated after the precipitation of phospholipids and the values in the Table have been corrected for sterols. Radioactivity of each fraction was counted in a liquid scintillation spectrometer. Total content of digitonide-precipitable sterols per unit weight of tissue was determined gravimetrically. Inorganic phosphorous was determined by the method of FISKE and SUBBAROW[12].

Results and discussion. As seen in the Table, aflatoxin effectively inhibits the incorporation of ^{14}C-acetate into the total lipid, phospholipid, free sterol, and neutral fat fractions of human skin. Because the rates of isotope incorporation into all the fractions examined were significantly lower than controls, it appeared that the effect of the toxin might be due to general cellular injury. However, the respiratory rates of toxin-treated tissues were not greatly affected and were only 6% less than controls at the end of the experiment (Figure).

SHANK and WOGAN[13] demonstrated that marked reductions in precursor incorporation into liver lipids occurred in ducklings treated with aflatoxin. The lowered rate of precursor incorporation was accompanied with a rise in total lipid content. Similarly, KATO et al.[14] have shown



Effect of aflatoxin B_1 on the respiratory rates of human skin. Values represent the mean of 6 experiments ± S.E.M. Respiratory rates are expressed on an accumulative basis.

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Effect of aflatoxin B_1 on the incorporation of 1-^{14}C-acetate into human skin lipids

Treatment	Total lipid (dpm × 10^{-3}/100 mg wet wt.)	Phospholipid (dpm × 10^{-3}/µg Pi)	Free sterols (dpm × 10^{-3}/mg digitonide)	Neutral fats (dpm × 10^{-3}/100 mg wet wt.)
Control	40.02	3.69	15.29	19.46
Aflatoxin	23.55 (—41%)	2.49 (—32%)	8.89 (—42%)	14.01 (—28%)

Values expressed for the total lipid and neutral fat fractions represent the mean of 6 experiments and are significant at the 1% and 5% levels respectively. Specific activities for the phospholipid and free sterol fractions represent values obtained from the pooled extracts of 6 experiments. Values for these fractions, based on wet weights of the 6 experiments, were significant at the 1% level.

that aflatoxin B_1 affects the rate of ^{14}C-acetate incorporation into cholesterol in rat liver and in their studies aflatoxin treatment also resulted in elevated levels of cholesterol. These investigators suggested that the inhibitory action of aflatoxin on acetate incorporation into cholesterol was specific and not the result of general hepatic injury.

In our experiments the level of sterol digitonides increased 22% with a concomitant decrease in specific activity of 42%. The increase of lipid and cholesterol content in the previous studies could, as suggested by SHANK and WOGAN, have resulted from failure of transport of these metabolites from the liver. The present in vitro studies, in which transport is not involved, indicate that the toxin affects both the rate of synthesis and turnover of lipids in the skin. Furthermore, the failure of aflatoxin to affect the respiratory rates of skin also suggests that the effects on lipid metabolism are of a more specific nature than that of general toxicity. CLIFFORD and REES[4] have reported comparable results on the respiratory rates of aflatoxin poisoned liver.

Although it is generally concluded that the toxicity of aflatoxin B_1 is tissue specific, the similarities of results from the present study with human skin to those of previous ones of lipid metabolism in animal liver suggest that a) at least this facet of aflatoxin action may be common to all tissues actively engaged in lipid metabolism via similar biosynthetic pathways and b) that this effect of aflatoxin on lipid metabolism is of a specific nature. The relationship of these findings to the possible occurrence of dermatoses in human skin must await further study[15].

Zusammenfassung. Aflatoxin B_1 hindert den in vitro Einbau des ^{14}C-Azetats in die Gesamtlipide, die freien Sterine, die Phospholipide und die Neutralfette der menschlichen Haut. Die Atmungsgeschwindigkeit der toxinbehandelten Gewebe wurde nicht beeinflusst.

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[15] Supported in part by The Robert A. Welch Foundation Grant No. Q-253, USPHS Grant No. EC00110-03, and Clinical Investigatorship Award of Veterans Administration.

Calcium Sensitivity of Succinate and Pyruvate Dehydrogenases in the Denervated Amphibian Skeletal Muscle

The calcium-sensitive proteins, as well as acidic proteins, of amphibian gastrocnemius muscle showed cathodal migration suggesting a net positive sign of charge on them[1]. Similarly some of the dehydrogenases were also found to possess a net positive sign of charge[2–4]. Since the acidic protein environment is known to elevate the activity of dehydrogenases[2–4] and the denervated muscle has altered calcium levels, it is essential to see whether calcium-sensitive proteins or calcium sensitivity of the dehydrogenases exists in the denervated muscle.

Material and methods. *Rana hexadactyla* of medium size were denervated unilaterally by sciatic nerve section under aseptic conditions. The frogs were maintained in aquarium tanks and were fed with cockroaches or earthworms. After 30 days, the normal and denervated gastrocnemius muscles were isolated and used for enzymatic studies.

5% (wt/vol) homogenates of normal and denervated muscles were prepared in 0.25 *M* sucrose solution and centrifuged at 1500 *g* for 20 min. 2 ml aliquots of each supernatant was transferred into 3 centrifuge tubes. The first tube received 1 ml 0.25 *M* sucrose and this served as the control. The second and third tubes received 1 ml 10 m*M* calcium chloride and 1 ml 1.5 m*M* sodium citrate respectively. The mixtures were centrifuged at 1000 *g* for 10 min and the levels of the succinate dehydrogenase (SDH, EC 1.3.99.1) and pyruvate dehydrogenase (PDH, EC 1.3.4.1) activities were determined in the supernatant, adopting the triphenyl tetrazolium chloride reduction method of SRIKANTAN and KRISHNAMOORTHY[5] as standardised by GOVINDAPPA and SWAMI[3]. The chosen molar concentrations of calcium chloride and sodium citrate were arrived at after several preliminary experiments, where their effects on enzyme activities were found to be optimal.

Results and discussion. Since the binding of calcium to proteins is known to be pH dependent[6], the effects of calcium and citrate on SDH and PDH activities were studied at the pH 5.8, 6.8 and 7.8 (Tables I and II). The pH value of 6.8 represents the muscle homogenate pH and this condition will provide a native environment to the muscle homogenate. The pH values 5.8 and 7.8 were selected so that a relatively acidic and alkaline environment respectively was induced on the enzyme system.

At 6.8 pH both the enzymes are activated by calcium and inactivated by citrate. Thus, it appears that both the enzymes require an increase in the positive charge density in the environmental protein for their activation, since calcium is known for induction of positive charges on protein by neutralizing negative charges[7]. At pH 5.8, the SDH activity of normal and denervated muscles was significantly elevated by calcium and suppressed by citrate. At pH 7.8, the SDH was inhibited both by calcium and citrate, but the inhibition by calcium was more pronounced and statistically significant while the inhibition by citrate was not significant. Increase in the acidity (i.e., pH 5.8) of the medium normally involves the depletion of negative charges contributed by carboxyl and phosphate groups of proteins as some of the groups approach the pKa values in the direction of acidity. Addition of calcium at this pH might have further decreased the negative charge density

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