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# Interconversion of Aflatoxin $B_1$ and Aflatoxicol by Several Fungi

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**Four fungal strains, namely, *Aspergillus niger*, *Eurotium herbariorum*, a *Rhizopus* sp., and non-aflatoxin (AF)-producing *Aspergillus flavus*, which could convert AF-$B_1$ to aflatoxicol (AFL), could also reconvert AFL to AF-$B_1$. The interconversion of AF-$B_1$ to AFL and of AFL to AF-$B_1$ was ascertained to occur during proliferation of the fungi. These reactions were distinctly observed in cell-free systems obtained from disrupted mycelia of *A. flavus* and the *Rhizopus* sp., but they were not observed in culture filtrates from intact (nondisrupted) mycelia of the same strains. The interconversion activities of AF-$B_1$ and AFL were not observed when the cell-free systems were preheated at 100°C. These findings strongly suggest that the interconversion of AF-$B_1$ and AFL is mediated by intracellular enzymes of *A. flavus* and the *Rhizopus* sp. In addition, the isomerization of AFL-A to AFL-B observed in culture medium was also found to occur by the lowering of the culture pH.**

Aflatoxicol (AFL) is formed by reduction of the cyclopentenone carbonyl of aflatoxin (AF)-$B_1$ and has two types of stereoisomers, AFL-A and AFL-B, determined by the stereoconfiguration of the OH group in the cyclopentenol ring (2) (Fig. 1).

AFL is well known (1) as a main in vitro metabolite produced from AF-$B_1$ by reductase in the supernatant of liver homogenates of several avian and mammalian species. It has also been reported that AFL is produced from the biological reduction of AF-$B_1$ by microorganisms such as *Rhizopus* spp. (3), *Dactylium dendroides* (4–6), *Absidia repens* (5), *Mucor griseo-cyanus* (5), *Aspergillus niger* (8, 9), *Mucor ambiguus* (8, 9), *Trichoderma viride* (8, 9), *Streptococcus lactis* (10), and *Tetrahymena pyriformis* (14).

These facts suggest the possibility of detecting AFL in foods and feeds contaminated with AF-$B_1$. Saito et al., in this laboratory (16), reported the simultaneous contamination of commercial pistachio nuts and corn with AFL and AF-$B_1$ in 1984, in the first report on natural contamination of food with AFL.

To elucidate the cause of natural AFL contamination, we reported the abilities of various species of non-AF-$B_1$-producing fungi which were isolated from AFL-contaminated corn to convert AF-$B_1$ to AFL. As a result, it was found (11) that *A. niger*, *Eurotium herbariorum*, the *Rhizopus* sp., etc., had fairly potent conversion abilities. It was also found (12) that several strains of AF-$B_1$-producing *Aspergillus flavus* isolated from AFL-contaminated corn showed a fairly high AFL-producing ability.

As mentioned above, the conversion of AF-$B_1$ to AFL by microorganisms has already been demonstrated, but little is known about the reverse conversion, namely, that from AFL to AF-$B_1$.

This paper deals with the metabolic interconversion of AFL and AF-$B_1$ by non-AF-producing fungi isolated from AFL-contaminated corn.

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## MATERIALS AND METHODS

**Organisms.** The fungal strains used in this study were the following: *A. niger* C-41-i, *E. herbariorum* C-41-f, *Rhizopus* sp. C-40-m, and non-AF-producing *A. flavus* C-40-d, which were isolated from AFL-contaminated corn (11).

**Culture medium and reagents.** Low-salt (SL) medium (13) was used to study the ability of the fungi to convert from AFL to AF-$B_1$. The medium consisted of the following components: sucrose, 85 g; asparagine, 10 g; $(NH_4)_2SO_4$, 3.5 g; $KH_2PO_4$, 750 mg; $MgSO_4 \cdot 7H_2O$, 350 mg; $CaCl_2 \cdot 2H_2O$, 75 mg; $ZnSO_4 \cdot 7H_2O$, 10 mg; $MnCl_2 \cdot 4H_2O$, 5 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 2 mg; $Na_2B_4O_7$, 2 mg; $FeSO_4 \cdot 7H_2O$, 2 mg; and distilled water, 1,000 ml. AFL-A, AFL-B, and AF groups, i.e., $B_1$, $B_2$, $B_2a$, $G_1$, $G_2$, $M_1$, $M_2$, $P_1$, and $Q_1$, were obtained from Makor Chemicals Ltd. (Jerusalem, Israel). It was confirmed by high-performance liquid chromatography (HPLC) that AFL-A did not contain AFL-B and that AFL-B did not contain AFL-A.

**Inoculation and incubation.** Fungal strains were inoculated previously on potato dextrose agar slants (Eiken Chemical Co., Ltd., Tokyo, Japan) and cultivated for 7 days at 25°C until they were well sporulated. The spores were harvested in a 0.001% Tween 80 solution to make a spore suspension containing $10^4$ spores per ml. Samples (100 μl) of the spore suspension were inoculated onto 10 ml of SL culture medium containing AFL-A and AFL-B (2,000 ng/ml each), and the medium was cultured for 6 and 10 days at 25°C under stationary conditions. After the incubation, AFL metabolites were investigated.

To study the time course of conversion from AFL to AF-$B_1$, each strain of fungus was cultured for 2 to 20 days, under the same conditions.

**Weighing of mycelial mat and measurement of pH.** After incubation, the culture was filtered through a glass filter. The fungal mat was dried at 50°C for 48 h and then stored over silica gel for 1 week. The weight of the dried fungal mat was then determined. The pH of the culture filtrate was measured with a pH meter.

**Extraction of AFLs and AFs and preparation of test solution.** Chloroform (100 ml) was added to the whole culture.

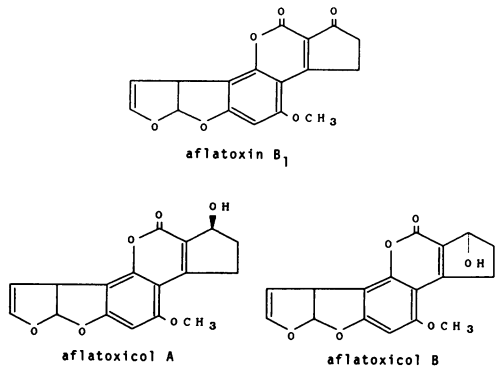


FIG. 1. Structures of AF-$B_1$, AFL-A, and AFL-B.

The mixture was homogenized for 10 min and centrifuged to separate the chloroform layer. The chloroform layer was dehydrated with anhydrous sodium sulfate and filtered through filter paper. The solvent was evaporated under reduced pressure. The residue was dissolved with an appropriate amount of chloroform and used as the test solution for thin-layer chromatography (TLC). After the detection of AFLs and AFs by TLC, a constant quantity of the TLC test solution was separated into an AFL fraction and an AF-$B_1$ fraction by column chromatography (15) and each fraction was used in the assay. The test solution was applied onto the column containing 10 g of silica gel. The column was washed with 150 ml of *n*-hexane, and then AFL was eluted with 180 ml of ethyl ether. Then, AF-$B_1$ was eluted with a mixed solution of methanol-chloroform (3:97). The solvent was evaporated from the respective eluates. The residue was dissolved in an appropriate amount of chloroform to make the test solution for HPLC. AF-$B_2$a was directly analyzed by using the filtrate from another culture by HPLC.

**TLC.** Qualitative analysis of the AF groups, i.e., $B_1$, $B_2$, $G_1$, $G_2$, $M_1$, $M_2$, $P_1$, $Q_1$, and AFLs was carried out by TLC using preparative Kieselgel 60 plates (gel thickness, 0.25 mm; E. Merck AG, Darmstadt, Federal Republic of Germany) under the development conditions described by Saito et al. (15).

**HPLC.** The HPLC system (Shimadzu, Kyoto, Japan) was composed of an LC-5A pump, an RF-530 fluorescence detector, and a C-R3A data processing unit. HPLC conditions for the determination of AF-$B_1$ were as follows: column, Finepack SIL (particle size, 5 μm; 4.6-mm inside diameter by 25-cm length; Jasco, Tokyo, Japan); mobile phase, toluene-ethyl acetate-formic acid-methanol (712:60:16:28); flow rate, 1.2 ml/min; and detection, fluorescence (excitation, 365 nm; emission, 425 nm). The determination of AFL-A and AFL-B was as described in a previous report (11). HPLC conditions for the determination of AF-$B_2$a were as follows: column, Finepack SIL $C_{18}$ (particle size, 10 μm; 4.6-mm inside diameter by 25-cm length); mobile phase, methanol-acetonitrile-water (20:20:60); flow rate, 1.0 ml/min; detection, fluorescence (excitation, 370 nm; emission, 435 nm).

**Confirmation of AF-$B_1$, AF-$B_2$a, and AFL.** Confirmation of AF-$B_1$ was carried out by the method of Haghighi et al. (7). Namely, AF-$B_1$ was converted to AF-$B_2$a by treatment with trifluoroacetic acid and AF-$B_2$a was analyzed by HPLC. Confirmation of AFL and AF-$B_2$a was carried out by reading excitation and emission spectra of the peaks separated by HPLC. The spectra were compared with those of standard substances.

**Preparation of cell-free system.** Each strain of *Rhizopus* sp. and non-AF-producing *A. flavus* was inoculated onto 500 ml of SL culture medium and cultured for 4 and 6 days, respectively. After the incubation, the cultures were separated into fungal mycelial mat and culture fluid. The culture fluid was filtered through a membrane filter (pore size, 0.2 μm; Gelman Sciences, Inc., Ann Arbor, Mich.). The filtrate was saved for measurement of the abilities to convert AF-$B_1$ to AFL and AFL to AF-$B_1$. The mycelial mat was rinsed three times with distilled water and lyophilized. Freeze-dried mycelium (1 g) was ground with 20 g of glass beads (diameter, 150 to 210 μm) and mixed with 20 ml of cold 0.05 M phosphate buffer (pH 7.2). The mixture was vibrated for 10 min in an ultrasonic bath filled with ice water (model UT204; Sharp, Tokyo, Japan) and then filtered through Toyo no. 5A filter paper. The filtrate was centrifuged at 15,000 × *g* for 20 min with a high-speed refrigerated centrifuge (model CX-250; Tomy Seiko, Tokyo, Japan). The supernatant was used as the cell-free system and was employed in the conversion assay. The protein content in the cell-free system was determined by bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, Ill.).

**Assay of interconversion activity of cell-free system and culture filtrate.** The cell-free system and culture filtrate (10 ml each) were pipetted into a 20-ml test tube. The reaction was started by adding 200 μl of AF-$B_1$ or AFL in *N*,*N*-dimethylformamide (200 ppm) to the test tube. The reaction mixture was incubated at 30°C in a water bath. After 10 to 180 min of incubation, 1 ml of the reaction mixture prepared from the cell-free system was extracted with 5-ml portions of chloroform. AF-$B_1$ and AFL in the chloroform solution were chromatographed by HPLC. In the case of the reaction mixture prepared from culture filtrates, after 1 to 72 h of incubation, 1 ml of the reaction mixture was placed in a test tube and then treated as mentioned above.

## RESULTS

**Conversion of AFL-A and AFL-B to AF-$B_1$.** AF-$B_1$ was detected in the cultures of all four species, *A. niger*, *E. herbariorum*, the *Rhizopus* sp., and *A. flavus*. AF-$B_2$a was detected only in the *A. niger* culture. Other AF groups, i.e., $B_2$, $G_1$, $G_2$, $M_1$, $M_2$, $P_1$, and $Q_1$ were not detected in any cultures (Table 1).

AF-$B_1$ was detected in all cultures to which AFL-A and AFL-B were added. In both cultures inoculated with the same strain of fungus, the residual AFL was almost the same and the produced AF-$B_1$ was also nearly the same. When *A. flavus* and *E. herbariorum* were used as inocula, the amounts of AF-$B_1$ in cultures incubated for 10 days were greater than those in cultures incubated for 6 days. In *A. niger* and the *Rhizopus* sp., larger amounts of AF-$B_1$ were found in the 6-day cultures than in the 10-day cultures. In all cultures, AF-$B_1$ increased with a decrease in AFL. This finding suggested that AF-$B_1$ was produced by the conversion of added AFL.

In all of the cultures, both AFL-A and AFL-B were observed. AFL-B was detected in the cultures supplemented with AFL-A, and AFL-A was detected in the cultures supplemented with AFL-B. The ratio of AFL-A to AFL-B was nearly the same in all of the cultures. On the other hand, similar results were also observed in the control medium which was not inoculated with fungus. This finding suggested that the interconversion between AFL-A and AFL-B occurred independently of fungal metabolic activity.

**Time course of conversion of AFL to AF-$B_1$.** In the exper-

TABLE 1. Residual amounts of AFL-A and AFL-B and detected amounts of AF-$B_1$ and AF-$B_2$a in culture medium inoculated with four species of fungi

| Fungus | Incubation time (days) | Initial concn of AFLs (ng/ml) | | Concn detected (ng/ml) | | | |
|---|---|---|---|---|---|---|---|
| | | | | AFL | | AF | |
| | | A | B | A | B | $B_1$ | $B_2$a |
| *Aspergillus niger* C-41-i | 6 | 2,000 | | 180 | 190 | 620 | 37 |
| | 10 | 2,000 | | 28 | 32 | 84 | 33 |
| | 6 | | 2,000 | 140 | 160 | 490 | 44 |
| | 10 | | 2,000 | 34 | 35 | 55 | 23 |
| *Aspergillus flavus* C-40-d | 6 | 2,000 | | 570 | 550 | 190 | ND[a] |
| | 10 | 2,000 | | 180 | 180 | 450 | ND |
| | 6 | | 2,000 | 570 | 650 | 190 | ND |
| | 10 | | 2,000 | 220 | 230 | 400 | ND |
| *Eurotium herbariorum* C-41-f | 6 | 2,000 | | 650 | 600 | 290 | ND |
| | 10 | 2,000 | | 280 | 270 | 520 | ND |
| | 6 | | 2,000 | 470 | 570 | 310 | ND |
| | 10 | | 2,000 | 230 | 260 | 470 | ND |
| *Rhizopus* sp. C-40-m | 6 | 2,000 | | 200 | 190 | 240 | ND |
| | 10 | 2,000 | | 45 | 56 | 58 | ND |
| | 6 | | 2,000 | 170 | 180 | 260 | ND |
| | 10 | | 2,000 | 37 | 48 | 43 | ND |
| None (control) | 6 | 2,000 | | 910 | 830 | ND | ND |
| | 10 | 2,000 | | 830 | 810 | ND | ND |
| | 6 | | 2,000 | 870 | 890 | ND | ND |
| | 10 | | 2,000 | 850 | 860 | ND | ND |

[a] ND, Not detected.

iments mentioned above, AF-$B_1$ increased more in the 10-day cultures than in the 6-day cultures with *A. flavus* and *E. herbariorum*. On the contrary, AF-$B_1$ decreased in 10-day cultures with *A. niger* and *Rhizopus* sp. In cultures with *A. niger*, AF-$B_2$a was detected. Considering these facts, we investigated the time courses of growth of the fungal mycelial mat, the change in the pH of culture medium, and the production of AF-$B_1$ and AF-$B_2$a. Since almost the same levels of AF-$B_1$ were converted from either AFL-A or AFL-B, only AFL-A was used in the following experiment. Each of five tubes from the respective strains was cultured, and two of them were used for the measurement of pH and the weighing of mycelia and three of them were used for the assaying of AF and AFL. The results are summarized in Fig. 2. AFL is shown as the sum of AFL-A and AFL-B.

In the *A. niger* culture, AFL decreased rapidly and was scarcely detected on day 20. AF-$B_1$ was detected on and after day 2, and it reached a maximum level of 870 ng/ml on day 4. This finding suggests that 40% or more of the added AFL was converted to AF-$B_1$ on day 4. After day 4, the amount of AF-$B_1$ decreased rapidly. After the appearance of AF-$B_1$, AF-$B_2$a was detected, showing a maximum level on day 7. In this case, the drop in pH of the culture filtrate was marked, compared with the culture filtrates inoculated with other fungi, reaching a pH of 2 on day 7 of culturing; even on day 20, the pH was still 2.9.

In the *Rhizopus* sp. culture, about half the added AFL was converted to AF-$B_1$ despite only slight growth of the mycelia (11 mg) on day 2. AF-$B_1$ showed a maximum on day 2. Afterwards, it decreased rapidly and disappeared completely from the culture on day 20. AF-$B_2$a was not detected in the *Rhizopus* sp. culture.

In the non-AF-producing *A. flavus* culture, AFL decreased slowly, compared with *A. niger* and the *Rhizopus* sp. AF-$B_1$ accumulated in cultures without showing rapid decreases in the cases of *A. niger* and the *Rhizopus* sp. These tendencies were observed in the case of *E. herbariorum*.

In any case, the sum of the residual amount of AFL and the detected amount of AF-$B_1$ was about equal to the amount of AFL added at the initial stage of incubation: however, afterwards, the sum of AFL and AF-$B_1$ decreased with time. These findings suggest that both AF-$B_1$ and AFL were further metabolized to unknown substances by the fungi.

This experiment was repeated once more, and the reproducibility was satisfactory.

**Interconversion of AF-$B_1$ and AFL in cell-free system and culture filtrate.** The ability to interconvert AF-$B_1$ and AFL was not observed in culture filtrates prepared from *A. flavus* and the *Rhizopus* sp. cultures; however, in the cell-free systems prepared from these fungi, it was clearly observed (Fig. 3).

The cell-free system prepared from *A. flavus* converted about 13% of AFL-A to AF-$B_1$ and about 5% of AF-$B_1$ to AFL-A during a 180-min incubation.

On the other hand, the cell-free system prepared from the *Rhizopus* sp. was better able to convert AFL-A to AF-$B_1$ and AF-$B_1$ to AFL-A than was *A. flavus*. It was capable of converting 85% of AFL-A to AF-$B_1$ during a 180-min incubation, and it converted 43% of AF-$B_1$ to AFL-A in a 60-min incubation. After 60 min, AFL-A decreased rapidly and was reconverted to AF-$B_1$. In any case, the sum of AFL and AF-$B_1$ was approximately equal to the amount of added substance.

No AFL-B was detected in any of the cell-free systems. This result suggests that the AFL isomer produced from AF-$B_1$ by the microorganism was only AFL-A. No interconversion activity of AF-$B_1$ or AFL-A was observed when the cell-free system was preheated at 100°C for 5 min.

**Isomerization between AFL-A and AFL-B.** From the results of the experiment mentioned above, it was found that isomerization between AFL-A and AFL-B occurred in culture medium containing no fungal inocula and that the AFL level in the medium decreased with time. This tendency of isomerization was similar to the isomerization of AFL in the cultures inoculated with fungi. However, only the AFL-A isomer was found when AFL was converted from AF-$B_1$ by the cell-free systems from the two strains of the *Rhizopus* sp. and *A. flavus*. Accordingly, it was thought that the isomerization of AFL was caused by environmental factors, such as the ingredients of the culture medium or changes in the pH of the culture medium. Consequently, the causes of isomerization of AFL-A to AFL-B and of ALF-B to AFL-A were investigated.

Solutions of individual ingredients of SL medium were prepared with distilled water so as to have concentrations corresponding to those of component ingredients of the SL medium, and AFL-A and AFL-B were added to these solutions. The solutions were incubated at 25°C for 5 days in a dark room, and AFL-A and AFL-B were assayed. Corresponding isomers of AFL were detected in all solutions in which the pH was less than 5.5, such as in solutions of asparagine, ammonium sulfate, and potassium dihydrogen phosphate. The lower the pH was, the more marked was the isomerization observed. From this experiment, it was supposed that the hydrogen ion concentration in the culture medium affected the isomerizations of AFL-A to AFL-B and of AFL-B to AFL-A.

Therefore, buffer solutions ranging from pH 2.2 to 8.0 were prepared by using solutions of 0.1 M citric acid and 0.2 M sodium phosphate dibasic. AFL-A was added to these

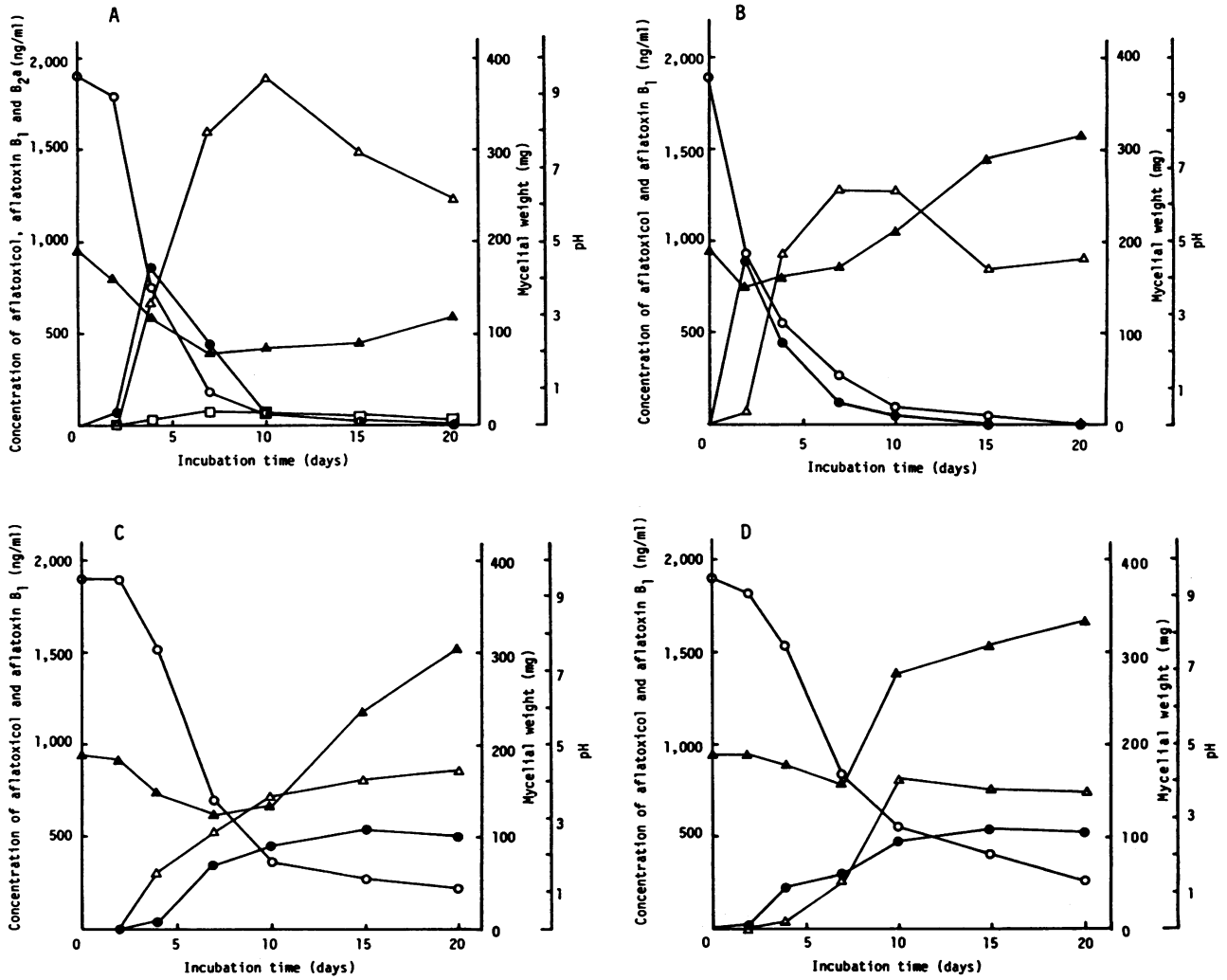


FIG. 2. Time course of fungal growth, pH of culture filtrates, and detected amount of AFL, AF-$B_1$, and AF-$B_2$a in the cultures inoculated with four fungal strains. (A) *A. niger*; (B) *Rhizopus* sp.; (C) *A. flavus*; (D) *E. herbariorum*. Symbols: ○, AFL; ●, AF-$B_1$; □, AF-$B_2$a; △, mycelial weight; ▲, pH. The values of mycelial weight and pH are means of two determinations, and the values of AFs and AFL are means of three determinations.

buffer solutions to make a solution of 2,000 ng/ml, and after 3 and 24 h and 3, 6, 10, and 15 days, the quantities of AFL-A and AFL-B were examined at each pH level. The results are shown in Fig. 4. It was confirmed that the lower the pH was, the more marked was the isomerization of AFL. At the same time, it was proved that the lower the pH was, the more markedly AFL itself decreased. No AF-$B_1$ was detected at all under these conditions. The chloroform extracts of the buffer solutions were analyzed by normal-phase HPLC, and two new peaks (retention times, 17 and 19 min, respectively) appeared after the AFL-A and AFL-B peaks (retention times, 8.5 and 9.8 min, respectively) on chromatograms. Consequently, it was conjectured that AFL was converted to a substance with greater polarity than AFL. At pH 7.0 or 8.0, no isomerization occurred and no decrease in AFL was observed at all. The new substance was detected only in the *A. niger* culture.

## DISCUSSION

In this study, we found that four organisms, *A. niger*, *A. flavus*, a *Rhizopus* sp., and *E. herbariorum*, which had the ability to convert AF-$B_1$ to AFL, also possessed the ability to reconvert AFL to AF-$B_1$. In the conversion of AF-$B_1$ to AFL, AFL accumulated in cultures and reached a maximum level in the middle stage of incubation (11). In the reverse reaction from AFL to AF-$B_1$, AF-$B_1$ accumulated in the cultures attained a maximum level between the initial to middle stages when the fungi were proliferating. These observations indicate that the reciprocal reactions, i.e., the reduction from AF-$B_1$ to AFL and the oxidation from AFL to AF-$B_1$, occur simultaneously. These reactions were distinctly observed in the cell-free systems obtained from disrupted mycelia of *A. flavus* and the *Rhizopus* sp.; however, they were not observed in the culture filtrates from nondisrupted mycelia of the same strains. Accordingly, these reactions are considered to be controlled by fungal enzymatic systems.

AF-$B_2$a was also found in the culture inoculated with *A. niger*. Tsubouchi et al. (17) stated that AF-$B_2$a was produced from AF-$B_1$ because of a lower pH of the medium, caused by organic acids produced by *A. niger*. In this experiment, AF-$B_2$a was observed when the pH of culture filtrates

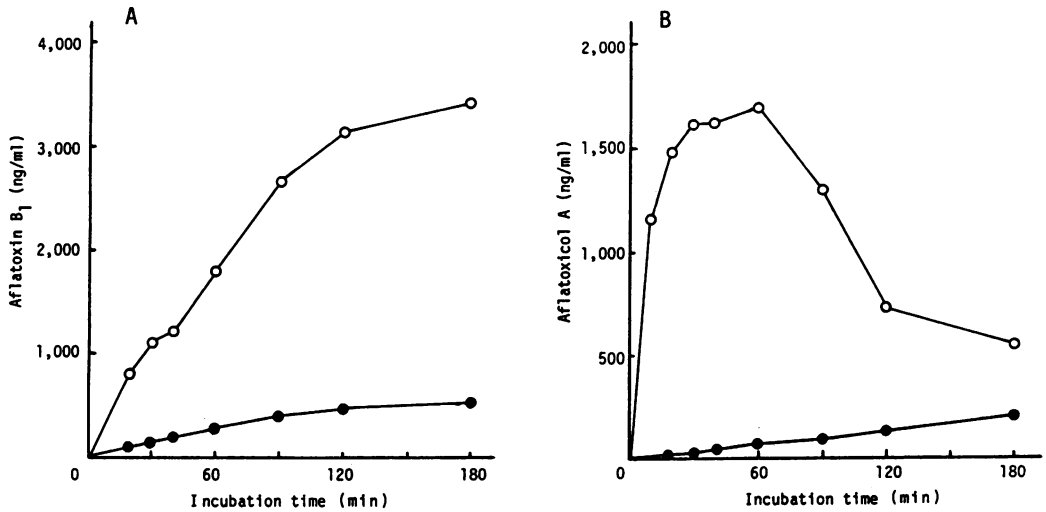


FIG. 3. Interconversion of AF-$B_1$ and AFL-A by cell-free systems of the *Rhizopus* sp. and non-AF-producing *A. flavus*. (A) Concentration of AF-$B_1$ in cell-free system when AFL-A was added; (B) concentration of AFL-A in cell-free system when AF-$B_1$ was added. Symbols: ○, *Rhizopus* sp.; ●, non-AF-producing *A. flavus*. The protein concentrations of the cell-free systems prepared from *A. flavus* and the *Rhizopus* sp. were 6.7 and 5.8 mg/ml, respectively.

decreased. It was supposed that AF-$B_2$a observed in this experiment was also produced from AF-$B_1$, which was converted from AFL, by lower medium pH due to organic acids produced by *A. niger*.

This experiment showed that AFL was converted to a compound with a higher polarity than AFL and decreased not only as a result of fungal metabolic decomposition but also by the actions of organic acids which accumulated in the culture medium. Because the treatment of AF-$B_1$ with a weak acid converts it to AF-$B_2$a, a hemiacetal of AF-$B_1$, it was presumed that the more polar compounds were hemiacetals converted from AFL-A and AFL-B, respectively.

It was also proved that the conversions of AFL-A to AFL-B and of AFL-B to AFL-A were produced by the lowering of the culture pH. It is thought that the interconversion of AFL-A and AFL-B results from the acid-catalyzed isomerization of an allylic alcohol on the cyclopentenol ring. In studies on the conversion of AF-$B_1$ to AFL by fungi, reported by us (11), Cole et al. (3), and Detroy and Hesseltine (5), AFL-A was found at first in the medium and then AFL-B appeared later. On the basis of these results, we conclude that AF-$B_1$ is first converted to AFL-A by fungi and that AFL-A is further converted to AFL-B by the actions of medium components or organic acids produced by the fungi.

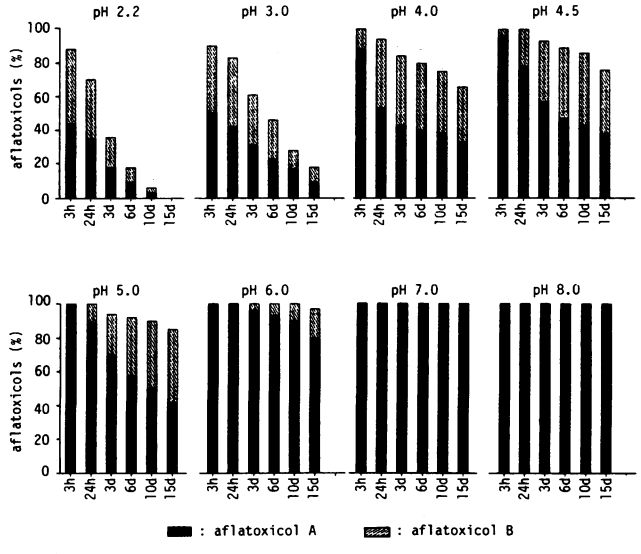


FIG. 4. Conversion of AFL-A to AFL-B and decrease of total AFLs in buffer solutions with the pH adjusted to 2.2 to 8.0. Each buffer solution initially contained 2,000 ng of AFL-A per ml. d, Days of incubation.

## LITERATURE CITED

1. **Campbell, T. C., and J. R. Hayes.** 1976. The role of aflatoxin metabolism in its toxic lesion. Toxicol. Appl. Pharmacol. **35:** 199–222.
2. **Cole, R. J., and R. H. Cox.** 1981. The aflatoxins, p. 1–66. *In* Handbook of toxic fungal metabolites. Academic Press, Inc., New York.
3. **Cole, R. J., J. W. Kirksey, and B. R. Blankenship.** 1972. Conversion of aflatoxin $B_1$ to isomeric hydroxy compounds by *Rhizopus* spp. J. Agric. Food Chem. **20:**1100–1102.
4. **Detroy, R. W., and C. W. Hesseltine.** 1968. Isolation and biological activity of a microbial conversion product of aflatoxin $B_1$. Nature (London) **219:**967.
5. **Detroy, R. W., and C. W. Hesseltine.** 1969. Transformation of aflatoxin $B_1$ by steroid-hydroxylating fungi. Can. J. Microbiol. **15:**495–500.
6. **Detroy, R. W., and C. W. Hesseltine.** 1970. Aflatoxicol: structure of a new transformation product of aflatoxin $B_1$. Can. J. Biochem. **48:**830–832.
7. **Haghighi, B., C. Thorpe, A. E. Pohland, and R. Barnett.** 1981. Development of a sensitive high-performance liquid chromatographic method for detection of aflatoxins in pistachio nuts. J. Chromatogr. **206:**101–108.
8. **Mann, R., and H. J. Rehm.** 1976. Degradation products from aflatoxin $B_1$ by *Corynebacterium rubrum*, *Aspergillus niger*, *Trichoderma viride* and *Mucor ambiguus*. Eur. J. Appl. Microbiol. **2:**297–306.
9. **Mann, R., and H. J. Rehm.** 1977. Degradation of aflatoxin $B_1$ by various microorganisms. Z. Lebensm.-Unters.-Forsch. **163:**39–43.
10. **Megalla, S. E., and M. A. Mohran.** 1984. Fate of aflatoxin $B_1$ in

fermented dairy products. Mycopathologia **88:**27–29.
11. **Nakazato, M., K. Saito, Y. Kikuchi, A. Ibe, K. Fujinuma, M. Nishijima, Y. Naoi, T. Nishima, S. Morozumi, T. Wauke, and H. Hitokoto.** 1985. Conversion of aflatoxin $B_1$ to aflatoxicols by various fungi isolated from aflatoxicols contaminating corn. J. Food Hyg. Soc. Jpn. **26:**33–38.
12. **Nakazato, M., K. Saito, Y. Kikuchi, A. Ibe, K. Fujinuma, M. Nishijima, T. Nishima, S. Morozumi, T. Wauke, and H. Hitokoto.** 1985. Aflatoxicol formation by *Aspergillus flavus* and *A. parasiticus*. J. Food Hyg. Soc. Jpn. **26:**380–384.
13. **Reddy, T. V., L. Viswanathan, and T. A. Venkitasubramanian.** 1971. High aflatoxin production on a chemically defined medium. Appl. Microbiol. **22:**393–396.
14. **Robertson, J. A., D. J. Teunisson, and G. J. Boudreaux.** 1970. Isolation and structure of a biologically reduced aflatoxin $B_1$. J. Agric. Food Chem. **18:**1090–1091.
15. **Saito, K., M. Nishijima, K. Yasuda, H. Kamimura, A. Ibe, T. Nagayama, H. Ushiyama, and Y. Naoi.** 1984. Analytical method for aflatoxins and aflatoxicols in cereals, nuts and their products. J. Food Hyg. Soc. Jpn. **25:**112–117.
16. **Saito, K., M. Nishijima, K. Yasuda, H. Kamimura, A. Ibe, T. Nagayama, H. Ushiyama, and Y. Naoi.** 1984. Investigation of the natural occurrence of aflatoxins and aflatoxicols in commercial pistachio nuts, corns and corn flours. J. Food Hyg. Soc. Jpn. **25:**241–245.
17. **Tsubouchi, H., K. Yamamoto, K. Hisada, Y. Sakabe, and K. Tsuchihira.** 1980. Degradation of aflatoxin $B_1$ by *Aspergillus niger*. Proc. Jpn. Assoc. Mycotoxicol. **12:**33–35.