Influence of Riboflavin on Disturbances in Tryptophan Metabolism and Hepatoma Production After a Single Dose of Aflatoxin B₁

F. J. Lemonnier,² J. M. Scotto,³ and C. Thuong-Trieu ², ³

SUMMARY—Female Wistar rats were given a single oral dose of aflatoxin B₁, either alone or with a large amount of riboflavin. Biochemical and histologic studies were performed for 30 months. Nine animals of 19 in the aflatoxin-treated group and only 5 of 18 in the riboflavin-aflatoxin-treated group developed hepatomas. The number of rats was insufficient for tests of statistical significance to be fruitful. Urinary excretion of tryptophan metabolites was studied in aflatoxin- and riboflavin-treated rats after an oral administration of 10 mg tryptophan/100-g rat. Riboflavin did not affect the percentage of aflatoxin-treated animals with abnormal urinary excretion patterns, but did increase the magnitude of the disturbances in elimination of kynurenic and xanthurenic acids. The hepatic tryptophan-oxygenase activity was increased only in the two groups given riboflavin, and the levels of nucleic acids were the same in all groups.—J Natl Cancer Inst 55: 1085–1087, 1975.

Recently (1), we studied, in female rats, the modifications of tryptophan metabolism induced by long-term intoxication with a single dose of aflatoxin B₁ (7 mg/kg rat), given either alone or with CCl₄. In groups given CCl₄ 50% developed hepatomas. The association of the two toxic substances increased all biochemical effects and induced earlier histologic changes.

We now report the influence of a riboflavin-rich diet on the same histologic and biochemical abnormalities as those induced by aflatoxin B₁. The study was initiated because 1) riboflavin acts as a coenzyme in tryptophan metabolism (2), and 2) this vitamin has an important role in carcinogenesis, and earlier studies seem contradictory (3, 4).

Our purpose was to determine if riboflavin modified the action of aflatoxin on hepatic tissue and on tryptophan metabolism.

MATERIALS AND METHODS

Animals.—Female Wistar rats were used. Surviving animals were killed at 30 months. The standard base diet was No. 103 from the "Union d’Alimentation Rationnelle." The diet contained 9 mg riboflavin/kg food corresponding to 90 µg riboflavin/rat/day.

Control group: Twenty-one 3-week-old rats received by gastric gavage, 7 ml dimethyl sulfoxide (DMSO)/kg body weight. The three other groups received the same amount of DMSO with or without aflatoxin.

Aflatoxin group: Twenty-one 3-week-old rats were given, by gastric gavage, a single dose of aflatoxin B₁ (7 mg/kg) dissolved in DMSO, which was approximately equivalent to half lethal dose 50.

Riboflavin: Twenty 3-week-old rats received about 500 µg riboflavin/rat/day in their water, which was available ad libitum.

Riboflavin-aflatoxin group: Twenty 3-week-old rats were given the same amount of riboflavin as was the preceding group. One week later, they were given a single dose of aflatoxin B₁ (7 mg/kg) in DMSO by gastric gavage.

1. Tryptophan load tests.—About every 15 days, a dose of L-tryptophan (10 mg/100 g/rat) was administered by gavage to 5 animals from each group. Urine specimens were collected 24 hours before and after gavage.

Measurement techniques for kynurenine, kynurenic acid, and xanthurenic acid excreted in the urine were described in (1).

Tryptophan-oxygenase activity and nucleic acid concentrations were measured in whole-liver homogenates immediately after the animals were killed (1).

Histologic controls.—Three or four biopsies were performed on each rat during the experiment. At the end of the study, the animals were killed and a final histologic assay was performed.

RESULTS

Control Group

No histologic abnormality was observed. The average urinary elimination of tryptophan metabolites is shown in table 1. The kynurenine excretion (22.3 µg ± 3.6) was the same in all 4 groups. A test was considered abnormal when the data went beyond quoted averages ± 3 sd. Results of tests for tryptophan-oxygenase and nucleic acids are given in table 2.

Aflatoxin Group

The first megalocytosis was observed at month 9; all rats were affected. The first hepatoma was seen at the 17th month; 9 lesions were found (table 1). The percentage of abnormal tests was significantly different from that of control rats (table 1).

<table>
<thead>
<tr>
<th>Group (No. of rats)</th>
<th>Percent of rats with hepatomas</th>
<th>Percent of abnormal tests</th>
<th>Kynurenine acid: mean ± 2sd (µg/24 hr)</th>
<th>Xanthurenic acid: mean ± 2sd (µg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0 2.5 97±6 77±3</td>
<td>(21) (76) (277)</td>
<td>97±6 77±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin 0 2.5 97±6 77±3</td>
<td>(20) (47) (243)</td>
<td>110±12 87±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin 47.5% 13.6 268±34 154±24</td>
<td>(19) (72) (257)</td>
<td>268±34 154±24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin–</td>
<td>27% 17.4 420±74 204±32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aflatoxin (18) (54) (265)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* These percentages are based on number of rats still alive after first hepatoma in each group. Numbers in parentheses correspond to total number of biopsies and tryptophan load tests performed.

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Riboflavin apparently affected tumor growth in terms of the causal agent. It accelerated spontaneous mammary cancer and lymphosarcomas in mice (7, 8). In the chemical carcinogenesis process, riboflavin inhibited the development of hepatomas (9) and epitheliums (10). The growth rate of azo dye-induced tumors was increased in cases of riboflavin deficiency (11, 12). This may be due partly to a decreased activity of flavin-dependent microsomal enzymes (13, 14) that inactivated the azo dyes.

The number of hepatomas observed in both treated groups is not significant because of the small number of rats involved; with twice the number, we believe percentages would be significant. Other observations include 1) the intensity of megalocytosis; 2) the time of discovery of the first hepatoma in each group (at 18 mo 8 hepatomas were counted in the aflatoxin group and none in the riboflavin–aflatoxin group).

We think riboflavin might either inhibit the conversion by enzymes (15) of aflatoxin B1 to an active compound, X, or promote its detoxification into less active metabolites, M1 and P1 (16–18). Whatever the involved mechanism, it would be important to examine the effect of riboflavin in a larger experiment with both deficiency and excess of the vitamin.

Confirmation of our results would be particularly important in that some authors found a relationship between the presence of aflatoxin in oil cakes of various seeds and the incidence of human liver cancer (19, 20).

### REFERENCES

(7) MORRIS HP, ROBERTSON WV: Growth rate and number of spontaneous mammary carcinomas and riboflavin concentration of liver, muscle, and tumor of C3H mice as influenced by dietary riboflavin. J Natl Cancer Inst 3:479–489, 1943

**Table 2.—Levels of nucleic acids and tryptophan–oxygenase in rat liver tissues**

<table>
<thead>
<tr>
<th>Group (No. of rats)</th>
<th>RNA (mean ± 2 sd; mg/g protein)</th>
<th>DNA (mean ± 2 sd; mg/g protein)</th>
<th>Tryptophan–oxygenase (mean ± 2 sd; amoles kynurenine/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
<td>46.9 ± 11.6</td>
<td>13.1 ± 4.3</td>
<td>11.5 ± 3.5</td>
</tr>
<tr>
<td>Aflatoxin (10)</td>
<td>51.8 ± 8.4</td>
<td>12.5 ± 2.7</td>
<td>15.8 ± 2.3</td>
</tr>
<tr>
<td>Riboflavin (10)</td>
<td>57.4 ± 4.4</td>
<td>16.2 ± 3.2</td>
<td>23.6 ± 4.1</td>
</tr>
<tr>
<td>Riboflavin–aflatoxin (10)</td>
<td>55.7 ± 17</td>
<td>17.6 ± 7.0</td>
<td>21.0 ± 6.2</td>
</tr>
</tbody>
</table>

observed in the control group (P ≤ 0.001). The results were not significant for tryptophan–oxygenase and nucleic acids (table 2).

### Riboflavin Group

Three animals showed only a slight megalocytosis. The average elimination of the tryptophan metabolites in urine was not different from that of the control group (table 1). There was a significant increase of enzyme activity as compared with that of the control (P ≤ 0.01) and aflatoxin groups (P ≤ 0.02) (table 2).

### Riboflavin–Aflatoxin Group

Megalocytosis affected 75% of the animals to a lesser degree. The first hepatoma appeared at the 22d month, 5 months later than in the aflatoxin-treated group. Five hepatomas were found (table 1). The intensity of the abnormal tests was more important in comparison with the group given aflatoxin alone (P ≤ 0.01) (table 1). During the first 15 months, there were few abnormal tryptophan tests: 6.3%. In the second period, this rose to 27.3%. These two percentages were significantly different (P < 0.001). The enzyme activity (table 2) was significantly increased in comparison with that of the control group (P ≤ 0.02).

### DISCUSSION

Previously we had observed abnormalities in tryptophan dose tests in human liver cancers and other neoplasms of digestive organs (5). Riboflavin improved the results of these tests, even in some patients not affected by pyridoxine treatment (6).

In this work, the riboflavin apparently did not change the abnormalities of tryptophan metabolism induced by aflatoxin B1. However, the intensity of the positive tests and their distribution during the experiment were different in the two treated groups. The average of abnormal responses was generally higher in the riboflavin–aflatoxin group than in the aflatoxin group. Furthermore, in the second part of the experiment, the number of positive tests increased.

The results in the riboflavin-treated group were the same as in the control group. Thus the association of high doses of riboflavin and aflatoxin led to an increase of disturbances in tryptophan metabolism. It was as if the riboflavin enhanced the aflatoxin effect on this metabolism, but only after a period of delay. Indeed, the changes were marked only after a time lapse.

The level of nucleic acids was unchanged, in contrast to that observed in the association CCl4–aflatoxin (7).