The Effects of Hepatotoxic Agents and of Liver Growth on the Urinary Excretion of the \(N\)-Hydroxy Metabolite of 2-Acetylaminofluorene by Rats*

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SUMMARY

Adult rats subjected to a number of treatments which cause liver damage and/or growth (administration of hepatotoxic agents, partial hepatectomy, or protein depletion-repletion) and young growing rats excreted in the urine a greater percentage of a test dose of 2-acetylaminofluorene (AAF) as the carcinogenic \(N\)-hydroxy metabolite than did normal adult rats. Alterations in the urinary excretion of the noncarcinogenic ring-hydroxy derivatives of AAF under these conditions were much smaller and, in most cases, negligible.

Rats fed certain thermally oxidized derivatives of corn oil excreted 1\(^\frac{1}{2}\)–2 times as much \(N\)-hydroxy-AAF in the urine after a test dose of AAF as rats fed fresh corn oil. These effects are correlated with the increased carcinogenicity of AAF when fed with the thermally oxidized products (Sugai et al.).

In view of the importance of \(N\)-hydroxy-AAF as a proximate carcinogenic metabolite of AAF (11–13), knowledge of the factors which affect its formation or degradation is desirable. One approach to this problem has been through a study of the urinary excretion of \(N\)-hydroxy-AAF which reflects in a gross manner the amount of this metabolite available to the tissues. In earlier studies (12) the urinary excretion of \(N\)-hydroxy-AAF was found to increase greatly as the dietary administration of AAF to rats was continued. Further, when 3-methylcholanthrene was administered simultaneously with AAF the excretion of \(N\)-hydroxy-AAF, the degree of hepatic damage, and the tumor incidences at various sites were all markedly reduced (10, 11, 17). These observations and the \textit{in vitro} demonstrations of the major role of the liver in the metabolism of AAF and its derivatives (2, 6–8, 18, 20, 22) suggested the possible importance of the progressive liver damage and regenerative changes to the altered metabolic pattern. The present studies were undertaken primarily to investigate the effects of other hepatotoxic agents and of normal forms of liver growth on the excretion of \(N\)-hydroxy-AAF after administration of a test dose of AAF. The effect of certain fractions of thermally oxidized corn oil, shown by Sugai et al. (21) to increase tumor induction by a very low level of AAF, was also investigated. In most cases the urinary excretion of the noncarcinogenic phenolic metabolites of AAF was also determined.

MATERIALS AND METHODS

Treatment of rats.—Rats from the Holtzman Rat Co., Madison, Wis., were housed in screen-bottomed cages; they were fed a semi-purified diet containing 18 per cent of casein (1) and water ad libitum. For the chronic administration of AAF (14), DL-ethionine (California Corp. for Biochemical Research), or thioacetamide (Distillation Products Co.) the compounds were added to the basal diet as 10 gm. of a glucose triturate per kg. of diet to give final concentrations of 0.03, 0.3, and 0.05 per cent, respectively. 2-Methyl-4-dimethylaminoazobenzene (15), 4-aminoazobenzene (Distillation Products Co., recrystallized from an ethanol-water solution), and 3'-methyl-4-dimethylaminoazobenzene (3) were dissolved in the corn oil of the diet with mild heat and incorporated in the diets at a final concentration of 0.03, 0.3, and 0.05 per cent, respectively. 2-Methyl-4-dimethylaminoazobenzene (15), 4-aminooazobenzene (Distillation Products Co., recrystallized from an ethanol-water solution), and 3'-methyl-4-dimethylaminoazobenzene (3) were dissolved in the corn oil of the diet with mild heat and incorporated in the diets at a final concentration of 0.06 per cent. In one experiment the diet contained 0.054 per cent of 3'-methyl-4-dimethylaminoazobenzene. Carbon tetrachloride was injected subcutaneously (0.1 ml., undiluted, every 3d day). Partial

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\(\text{AAF} = 2\text{-acetylaminofluorene or } N\text{-2-fluorenylacetamide.}\)
hepatectomies were performed by the method of Higgins and Anderson (5), with the removal of two-thirds of the liver; all operations were performed between 3 and 5:30 P.M.

In one experiment AAF was administered orally; 1.5 mg AAF/100 gm body weight was added in acetone to 0.5 gm. of the basal diet, the acetone was evaporated at room temperature, and the diets were offered to rats which had been starved for 18 hours. After 3 hours the diets were removed, and any residue (no more than 15 per cent of the initial amount) was weighed so that the dose could be calculated. In all other experiments the test dose of AAF was suspended at a final concentration of 2–3 mg/ml in a solution containing 1.75 or 7 per cent of gum acacia and was injected intraperitoneally. One to 3 mg. of AAF were administered per 100 gm. of body weight.

Each of the rats from the experiments on the hepatotoxic agents was killed by exsanguination at the end of the 24-hour urine collection, and pieces of liver were fixed in 10 per cent neutral formalin or Bouin's fixative and cut into 6-μ sections.*

Estimation of urinary metabolites of AAF.—Twenty-four-hour urine samples were collected under toluene in ice-cooled containers from one or two adult rats or groups of three or four weanling rats housed together in metabolism cages equipped with glass or stainless-steel funnels and wire screens for separation of the feces. Water was available ad libitum. In general, no food was available in the metabolism cages, but the rats were sometimes fed in other cages for 30 minutes 12 hours after the administration of the test dose of AAF.

The urine samples were analyzed as described earlier (12) by procedures adapted from those of Weisburger et al. (24). In brief, the urine was incubated with β-glucuronidase and Taka-diastase, and the ether-extractable metabolites were chromatographed on Whatman No. 1 filter paper. After elution with ethanol the ultraviolet spectra of the metabolites were determined with a Beckman recording spectrophotometer; before spectral analysis the N- and 1-hydroxy derivatives were separated from AAF by extraction with 0.5 N NaOH. The data for the urinary metabolites were corrected on the basis of the recoveries obtained by the addition of known amounts of the synthetic compounds† to normal urine (12). The amounts of 1-hydroxy-AAF were generally too low to quantitate adequately, and the data on this metabolite have therefore been omitted.

RESULTS

Effect of previous treatment with hepatotoxic agents.—As previously observed for rats fed AAF for several weeks (12) the administration of various other hepatotoxic and hepatocarcinogenic agents also increased the excretion of N-hydroxy-AAF after a test dose of AAF (Tables 1 and 2).

* We are indebted to Dr. Henrik Hartmann, Department of Pathology, who supervised the preparation of these sections and assisted in the interpretation of the pathology.
† The N-hydroxy-AAF was prepared in this laboratory (11).
‡ The 1-hydroxy-AAF and 1-hydroxy-AAF were not quantitated.
† Tr (trace) indicates that a faint yellow color was detected at the expected Rf after the chromatogram was sprayed with the acidic p-dimethylaminobenzaldehyde reagent. However, there was insufficient N-hydroxy-AAF to quantitate by spectral analysis.

Since the colored metabolites of the aminoazo dyes interfered with the determination of the metabolites of AAF, the rats were transferred, in most cases, from the diets containing the hepatotoxic agents to the basal diet 5 or 6 days before administration of the test dose of AAF. This procedure resulted in a much lower excretion of N-hydroxy-AAF from a test dose of AAF as compared with that obtained with rats fed AAF continuously (12), but the excretions of N-hydroxy-AAF were still 2–8 times the levels observed in untreated rats.

As shown in Table 1, rats fed the hepatotoxic and hepatocarcinogenic agents ethionine and thioacetamide for 3 weeks or 3'-methyl-4-dimethylaminoazobenzene for 3 or 9 weeks, followed by the basal diet for 6 days, excreted 3–6 times as much N-hydroxy-AAF after intraperitoneal injection of a test dose of AAF as did rats fed only the control diet. On the other hand the administration of two noncancergenic and essentially nonhepatotoxic aminoazo dyes (2-methyl-4-dimethylaminoazobenzene and 4-amino-

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**TABLE 1**

The Levels of the Urinary Metabolites of AAF Excreted by Rats Given a Test Dose of AAF Intraperitoneally After Chronic Treatment with Hepatotoxic Agents

Male rats with an initial weight of 180–200 gm. were fed the diets for the times indicated. The rats were then fed the basal diet alone for 6 days prior to an intraperitoneal injection of 1 mg AAF/100 gm body weight. Urine was collected for 24 hours. There were two rats per group; their urines were pooled for analysis.

<table>
<thead>
<tr>
<th>CHRONIC TREATMENT</th>
<th>WEEKS</th>
<th>BODY WT. AT INJ. OF AAF (gm.)</th>
<th>N-HO-AAF</th>
<th>1-HO-AAF</th>
<th>3-HO-AAF</th>
<th>5-HO-AAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Basal diet)</td>
<td>3</td>
<td>280</td>
<td>0.4</td>
<td>1.3</td>
<td>3.6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>370</td>
<td>0.6</td>
<td>1.3</td>
<td>3.7</td>
<td>10</td>
</tr>
<tr>
<td>0.03% AAF</td>
<td>3</td>
<td>235</td>
<td>0.9</td>
<td>1.8</td>
<td>5.9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>260</td>
<td>1.8</td>
<td>1.8</td>
<td>3.4</td>
<td>17</td>
</tr>
<tr>
<td>0.06% 3'-Me-DAB</td>
<td>3</td>
<td>180</td>
<td>2.2</td>
<td>3.2</td>
<td>4.4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>200</td>
<td>3.9</td>
<td>6.8</td>
<td>6.7</td>
<td>30</td>
</tr>
<tr>
<td>0.06% 2-Me-DAB</td>
<td>3</td>
<td>240</td>
<td>0.6</td>
<td>†</td>
<td>3.1</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>220</td>
<td>tr†</td>
<td>†</td>
<td>4.2</td>
<td>†</td>
</tr>
<tr>
<td>0.06% AB</td>
<td>3</td>
<td>260</td>
<td>0.4</td>
<td>0.9</td>
<td>2.9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>330</td>
<td>tr</td>
<td>1.6</td>
<td>4.9</td>
<td>14</td>
</tr>
<tr>
<td>0.3% Ethionine</td>
<td>3</td>
<td>250</td>
<td>2.2</td>
<td>3.0</td>
<td>5.5</td>
<td>25</td>
</tr>
<tr>
<td>0.06% Thioacetamide</td>
<td>3</td>
<td>215</td>
<td>1.3</td>
<td>2.1</td>
<td>5.1</td>
<td>18</td>
</tr>
</tbody>
</table>

* The urinary AAF and 1-hydroxy-AAF were not quantitated.
† The value was low, but the spectrum was not good enough to permit quantitation.
‡ Tr (trace) indicates that a faint yellow color was detected at the expected Rf after the chromatogram was sprayed with the acidic p-dimethylaminobenzaldehyde reagent.
The levels of urinary metabolites of AAF excreted by rats given a test dose of AAF orally after chronic treatment with hepatotoxic agents

Male rats with an initial weight of approximately 200 gm. were fed the diets for 4 weeks. The rats were then fed a test dose of AAF either immediately or after being fed the basal diet for 5 days. The test dose of AAF consisted of 1.5 mg/100 gm body weight administered in 0.5 gm. of diet. The consumption of the test dose required about 3 hours, after which the urine was collected for 24 hours. There were two rats per group; their urines were pooled for analysis.

**Table 2**

<table>
<thead>
<tr>
<th>Chronic Treatment (4 weeks)</th>
<th>Days on Basal Diet*</th>
<th>Body Wt. at Admin. AAF (gm.)</th>
<th>% of Dose Excreted as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Basal diet)</td>
<td>0</td>
<td>302</td>
<td>0.5 2.0 6.7 17</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>300</td>
<td>0.7 3.2 7.6 13</td>
</tr>
<tr>
<td>0.05% AAF</td>
<td>5</td>
<td>234</td>
<td>2.3 3.7 6.8 16</td>
</tr>
<tr>
<td>CCl₄ (subcu.)†</td>
<td>0</td>
<td>253</td>
<td>tr 2.1 2.3 10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>272</td>
<td>0.6 1.9 4.6 17</td>
</tr>
<tr>
<td>0.05% 3'-Me-DAB</td>
<td>5</td>
<td>201</td>
<td>4.7 5.0 7.2 18</td>
</tr>
<tr>
<td>0.3% Ethionine</td>
<td>0</td>
<td>246</td>
<td>tr 0.9 4.2 20</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>256</td>
<td>1.5 2.7 5.8 18</td>
</tr>
<tr>
<td>0.05% Thioacetamide</td>
<td>0</td>
<td>171</td>
<td>tr 2.5 4.4 15</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>255</td>
<td>1.3 4.2 9.1 15</td>
</tr>
</tbody>
</table>

* Days on basal diet between chronic treatment and administration of test dose of AAF.
† The amount of the AAF offered which was consumed ranged from 85 to 100 per cent. The excretion data are calculated on the basis of the amount actually consumed.
‡ 0.1 ml. of CCl₄ was administered subcutaneously every 3d day. The last dose of CCl₄ was given 24 hours before the administration of the first test dose of AAF and 6 days before the administration of the later test dose.

Diethylaminoazobenzene (ethionine) had no effect on the metabolism of subsequently administered AAF.

Similar results were obtained in a second experiment (Table 2) in which the hepatotoxic compounds were administered for 4 weeks. Rats fed AAF, 3'-methyl-4-dimethylaminoazobenzene, ethionine, or thioacetamide for 4 weeks and then maintained on the basal diet for 5 days prior to the oral administration of AAF excreted 2-7 times as much N-hydroxy-AAF as the controls fed the basal diet for 5 weeks. On the other hand, rats given injections of 0.1 ml. of carbon tetrachloride every 3d day for 4 weeks metabolized an oral test dose of AAF in the same manner as the controls.

The data in Table 2 also show that the time of analysis influenced the results obtained. Thus, although rats fed ethionine or thioacetamide for 4 weeks and then maintained on the basal diet for 5 days prior to the feeding of the test dose of AAF excreted more N-hydroxy-AAF than did normal rats, the rats did not excrete more N-hydroxy-AAF than the controls when the test dose of AAF was administered immediately after the cessation of feeding of ethionine or thioacetamide.

There seemed to be no consistent variation in the amounts of 3- and 5-hydroxy-AAF excreted by the rats treated with the hepatotoxic agents (Tables 1 and 2). However, there was a tendency for the amount of 7-hydroxy-AAF to be greater when a hepatotoxic agent had been fed for several weeks. A threefold increase in the excretion of 7-hydroxy-AAF was previously observed for rats fed AAF for 18 weeks (12).

Histological studies were made of the livers from the rats in each group after the urine collections had been completed. According to the experimental design (Tables 1 and 2) most of the rats had been removed from the experimental diets for 6 days. The livers from the rats fed 3'-methyl-4-dimethylaminoazobenzene showed the most damage; at 3 weeks there were perilobular proliferation of ductular cells, increased basophilia of the cytoplasm of the parenchymal cells, and small regenerative nodules of parenchymal cells. By 9 weeks the livers showed cholangiofibrosis and more extensive regenerative liver nodules; a benign hepatoma was found in one liver. The livers from the rats fed AAF for 3 weeks showed marked basophilia, primarily in the centrilobular areas; by 9 weeks some ductular cell proliferation, as well as regenerative nodules and distortion of the architecture, was seen. The livers from the rats fed either ethionine or thioacetamide for 3 weeks showed little change except that the lobules were somewhat irregular in size and the livers from the rats fed thioacetamide had cells with a "starry" appearance. The livers of rats fed 2-methyl-4-dimethylaminoazobenzene had a benign hepatoma was found in one liver.
Male rats with an initial weight of 150-160 gm. were fed diets varying from 0% to 50% casein. Similar changes were noted in the second experiment (Table 2), except that the proliferation of ductular cells was more prominent in the rats killed immediately after continuous administration of AAF or 3'-methyl-4-di-methylaminoazobenzene than after 6 days on the basal diet. The livers of the rats treated with carbon tetra-chloride showed hydropic degeneration, especially in the middle zone of the lobules and focal areas of round-cell infiltration in the periportal areas 48 hours after the last injection. Seven days later the livers of the carbon tetra-chloride-treated rats were essentially normal.

Effect of partial hepatectomy.—Rats which were given injections intraperitoneally of AAF 24 or 48 hours after removal of two-thirds of the liver excreted approximately 7 times as much N-hydroxy-AAF in the subsequent 24 hours as did control rats (Chart 1). When the AAF was injected 3-6 days after the operation, the rats excreted 2-3 times as much N-hydroxy-AAF as rats not operated upon; by the 27th day the excretion was normal. Rats subjected to partial hepatectomy 24-48 hours prior to the injection of AAF also excreted more of the phenolic metabolites of AAF than did the control rats. However, in contrast to the seven-fold rise observed for N-hydroxy-AAF excretion, the increase in the urinary phenolic metabolites was no more than 1.5- to threefold.

TABLE 3
THE LEVELS OF THE URINARY METABOLITES OF AAF EXCRETED BY RATS GIVEN A TEST DOSE OF AAF INTRAPERITONEALLY AFTER BEING FED LOW OR HIGH PROTEIN DIETS

Male rats with an initial weight of 150-160 gm. were fed diets containing 0, 18, or 50 per cent casein for 5 days. After this time groups of three rats from each diet were given injections intraperitoneally of 2 mg AAF 100 gm body weight. Other rats fed the no-protein diet for 5 days were then fed the 50 per cent casein diet for 24 or 72 hr., after which they also received an intraperitoneal injection of AAF. Urine collections were made for 24 hr. The urines from each group of rats were pooled for analysis.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body wt. (gm.)</th>
<th>0.0-HO-AAF</th>
<th>3.0-HO-AAF</th>
<th>5.0-HO-AAF</th>
<th>7.0-HO-AAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Casein</td>
<td>180</td>
<td>0.3</td>
<td>3.4</td>
<td>6.9</td>
<td>15</td>
</tr>
<tr>
<td>18% Casein</td>
<td>189</td>
<td>0.4</td>
<td>3.9</td>
<td>7.9</td>
<td>24</td>
</tr>
<tr>
<td>0% Casein</td>
<td>136</td>
<td>0.4</td>
<td>5.4</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>0% Casein, then 50% casein 24 hr.</td>
<td>146</td>
<td>0.8</td>
<td>7.8</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>0% casein, then 50% casein 72 hr.</td>
<td>154</td>
<td>1.3</td>
<td>5.0</td>
<td>7.3</td>
<td>19</td>
</tr>
</tbody>
</table>

In a second experiment in which the urines were collected for only 12 hours after the intraperitoneal injection of AAF, the excretions of N-hydroxy-AAF were 3, 6, 9, 6, and 4 times, respectively, the control level of 0.3 per cent of the dose when the AAF was injected 12, 24, 36, 48, or 60 hours after partial hepatectomy. In this experiment the control rats were subjected to laparotomy and manipulation of the liver (sham hepatectomy) 24 hours prior to injection of AAF.

Effects of protein starvation and refeeding.—Administration of diets containing 0, 18, or 50 per cent casein to adult male rats for 5 days prior to the intraperitoneal injection of a test dose of 2 mg AAF/100 gm body weight had little influence on the levels of metabolites excreted in the urine (Table 3). However, the excretion of N-hydroxy-AAF was increased to 2-3 times the normal level in rats deprived of dietary protein for 5 days and then fed the 50 per cent casein diet for 2 or 3 days prior to the administration of AAF.

Comparison of young and adult rats.—Although the amounts of 3-, 5-, and 7-hydroxy-AAF excreted in the urine after a test dose of AAF were not significantly different between young and adult male rats, the amount of the N-hydroxy metabolite was about fivefold greater with the young rats (Table 4).

TABLE 4
THE LEVELS OF URINARY METABOLITES OF AAF EXCRETED BY YOUNG OR ADULT RATS GIVEN A TEST DOSE OF AAF INTRAPERITONEALLY*

<table>
<thead>
<tr>
<th>Body wt. (gm.)</th>
<th>No. ANALYSES</th>
<th>N-HO-AAF</th>
<th>3-HO-AAF</th>
<th>5-HO-AAF</th>
<th>7-HO-AAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-60</td>
<td>12</td>
<td>4.0 ± 0.94.9</td>
<td>1.38.0</td>
<td>1.515</td>
<td>± 6.2</td>
</tr>
<tr>
<td>180-190</td>
<td>5</td>
<td>0.8 ± 0.22.4</td>
<td>0.07.0</td>
<td>2.214</td>
<td>± 1.9</td>
</tr>
</tbody>
</table>

* All rats were fed the basal diet; they were given injections intraperitoneally of 3.0 mg AAF/100 gm body wt. Two young rats or one adult rat were used for each analysis. Urine samples were collected for 24 hr. Data expressed as averages with standard deviations.

TABLE 5
THE URINARY EXCRETION OF N-HYDROXY-AAF BY RATS FED THERMALLY OXIDIZED OILS AND GIVEN A TEST DOSE OF AAF INTRAPERITONEALLY

<table>
<thead>
<tr>
<th>Diet</th>
<th>Per cent of dose excreted as N-hydroxy-AAF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Corn oil</td>
<td>0.6 ± 0.15</td>
</tr>
<tr>
<td>5% Thermally oxidized corn oil plus 5% corn oil</td>
<td>0.9 ± 0.24</td>
</tr>
<tr>
<td>2.5% Nonurea adduct fraction of thermally oxidized corn oil plus 7.5% corn oil</td>
<td>1.1 ± 0.32</td>
</tr>
</tbody>
</table>

* Average data with standard deviations from four experiments in each of which there were two or four male rats with average weights of 225-280 gm. per group. The composition of the diets was identical to that of the usual basal diet, except that the fat content was raised to 10% at the expense of glucose. The diets were fed 1 or 2 weeks prior to the injection of 1.87 mg AAF/100 gm body weight; urine samples were collected for 24 hours.
**Administration of thermally oxidized fat in the diet.**—
Sugai *et al.* (21) observed that the incorporation of 2.5 per cent of the nonurea adduct fraction from thermally oxidized corn oil greatly increased the number of tumors which developed in rats fed 0.005 per cent of AAF. As shown in Table 5 rats fed this fraction of thermally oxidized corn oil excreted nearly twice as much N-hydroxy-AAF in the urine as the controls after a test dose of AAF. Rats fed the unfraccionated thermally oxidized corn oil also excreted more N-hydroxy-AAF than did the controls in each of the four experiments, although the statistical significance of the difference was less definite. The excretions of the 3-, 5-, and 7-hydroxy derivatives were not significantly altered by the administration of either of the thermally oxidized products.4

**DISCUSSION**

Earlier studies showed that the prolonged administration of AAF to rats resulted in an increased excretion of the N-hydroxy metabolite (12), whereas the prolonged administration of 4-acetylaminobiphenyl, which causes no gross hepatic changes, resulted in a decrease in the level of its N-hydroxy metabolite in the urine (16). The present studies have shown that a number of other hepatotoxic agents cause a similar increased excretion of N-hydroxy-AAF after a test dose of AAF and suggest that the effect may be related to the regenerative responses of the liver to toxic compounds.

Although the histological examinations of the livers were not particularly helpful in delineating among the morphological changes those responsible for the altered metabolism, it is clear that two compounds, 4-aminooazobenzene and 2-methyl-4-dimethylaminoazobenzene, which do not cause degeneration and regenerative hyperplasia, did not alter the amount of N-hydroxy-AAF excreted after a test dose of AAF. In these experiments administration of carbon tetrachloride also had no effect on the excretion of N-hydroxy-AAF, but the histological study suggested that most of the regenerative response had occurred by the time the AAF was administered. From the studies of Weisburger *et al.* (23) it appears that rats administered larger doses of carbon tetrachloride did excrete increased amounts of N-hydroxy-AAF after a test dose of AAF. The effect of liver growth on the excretion of N-hydroxy-AAF was particularly evident from the seven-fold increase in excretion of the metabolite when AAF was administered 1 or 2 days after partial removal of the liver and in the several-fold greater excretion of N-hydroxy-AAF by weaning as compared with adult rats given single doses of AAF. Likewise, rats depleted of protein and then fed a high protein diet for a short period prior to the injection of AAF responded with an increased excretion of the N-hydroxy metabolite. Since regrowth of the liver after protein depletion apparently involves little or no cell division (9), it is unlikely that the increased excretion of N-hydroxy-AAF after these various treatments has any relationship to the mitotic rate of the liver. Rather, the altered urinary pattern seems to be associated with the presence of liver cells actively engaged in protein synthesis or other metabolic functions associated with cytoplasmic growth.

Alterations in the amounts of the urinary metabolites of AAF must reflect differences in the activities of one or more of the enzyme systems which metabolize AAF or its metabolites. Because of the several reactions to which AAF is susceptible (ring-hydroxylation, N-hydroxylation, and deacetylation), because N-hydroxy-AAF and 2-amino-fluorene can be reconverted to AAF, and because the majority of the metabolites in the urine are excreted as conjugates, detailed in vitro studies will be necessary to discern the metabolic variables responsible for these alterations in urinary metabolic patterns. Since the liver is the major site of metabolism of AAF (2, 6–8, 18, 20, 22), it is probable that the urinary pattern of AAF metabolites reflects changes in the enzymatic pattern of the liver cells.

Sugai *et al.* (21) observed that certain fractions of thermally oxidized corn oil, which had no carcinogenic activity by themselves, greatly facilitated the induction of tumors when a very low level of AAF was administered over a long period. It is therefore of interest that the administration of one of these materials, the nonurea adduct fraction, prior to the injection of a test dose of AAF increased the urinary excretion of N-hydroxy-AAF. In view of the greater carcinogenic activity of the N-hydroxy metabolite than of the parent amide, the increased carcinogenic activity of AAF fed simultaneously with this fraction most probably reflects an increase in the tissue levels of N-hydroxy derivatives or of further carcinogenic metabolites.

**REFERENCES**


The Effects of Hepatotoxic Agents and of Liver Growth on the Urinary Excretion of the N-Hydroxy Metabolite of 2-Acetylaminofluorene by Rats

Alfredo Margreth, Prabhakar D. Lotlikar, Elizabeth C. Miller, et al.

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