On the Correlation between the Hepatocarcinogenicity of the Carcinogen, N-2-Fluorenylacetamide, and Its Metabolic Activation by the Rat

H. R. Gutmann, D. Malejka-Giganti, E. J. Barry, and R. E. Rydell

INTRODUCTION

Hepatocarcinogenesis by 2-FAA appears to require a 2-step mechanism of activation. In the 1st step, 2-FAA is N-hydroxylated to the hydroxamic acid, N-hydroxy-2-FAA. In the 2nd step, N-hydroxy-2-FAA is sulfonated by hepatic arylsulfotransferase to N-sulfoxy-2-FAA, which is considered to be an "ultimate" carcinogen (25, 26). Resistance to hepatocarcinogenesis by 2-FAA would be expected if an animal fails to perform 1 or both of these activation steps.

SUMMARY

The carcinogenicity of N-2-fluorenylacetamide (2-FAA) for the female Sprague-Dawley and female Fischer rats was compared. The arylamide administered i.p. induced a large number of mammary tumors in the female Sprague-Dawley rat but was not carcinogenic for the female Fischer rat. The lack of carcinogenicity of the arylamide coincided with a low capacity of the female Fischer rat for N-hydroxylation of 2-FAA to N-hydroxy-2-fluorenylacetamide. N-Hydroxy-2-fluorenylacetamide administered i.p. to the female Fischer rat yielded primarily malignant hepatic lesions. This observation provided further evidence that N-hydroxylation of 2-FAA is a requirement for the carcinogenicity of this compound. The susceptibility to hepatocarcinogenesis by N-hydroxy-2-fluorenylacetamide correlated with the extent of sulfonation of the hydroxamic acid by hepatic arylsulfotransferase in vitro and in vivo. The activity of this enzyme in the female Sprague-Dawley rat, which was refractory to hepatocarcinogenesis, was very low, whereas the arylsulfotransferase of the female Fischer rat appeared to be highly active. The evidence supports the view that hepatocarcinogenesis by 2-FAA depends on a sequential two-step mechanism of activation involving N-hydroxylation of the arylamide to an arylhydroxamic acid and sulfonation of the hydroxamic acid. Resistance to hepatocarcinogenesis in different strains of the same species appears to be due to the limited capacity to perform one of the activation steps.

It has recently been shown that the activity of the arylsulfotransferase in the liver of the female CD rat, a descendant of the Sprague-Dawley rat, is very low (4). Since the female Sprague-Dawley rat does N-hydroxylate 2-FAA, as shown below, the resistance of the female Sprague-Dawley rat to hepatocarcinogenesis by 2-FAA is presumably referable to the low level of formation of N-sulfoxy-2-FAA.

There are reports in the literature that female rats of other strains are prone to hepatocarcinogenesis. Thus, Dunning et al. (5) observed that female Fischer rats developed benign as well as malignant liver tumors after the p.o. intake of 2-FAA. This observation suggested that the female Fischer rat metabolizes the carcinogen in a different fashion than the female Sprague-Dawley rat. The female Fischer rat would appear to be capable of performing the complete activation sequence. Accordingly, we compared the metabolism of 2-FAA by the female rats of the 2 strains with the intent of testing the concept of the 2-step activation mechanism. Since the susceptibility of the female Fischer rat toward hepatocarcinogenesis by 2-FAA rests on the single report cited, the carcinogenicity of 2-FAA for the 2 strains was reinvestigated. These carcinogenicity tests and the associated metabolic studies form the basis of this report.

MATERIALS AND METHODS

Preparation of Unlabeled Compounds. 2-FAA [m.p. 196–198° (30)], 2-FA [m.p. 127–129° (18)], and N-hydroxy-2-FAA [m.p. 150–151° (24)] were prepared by the published procedures. 3-Methylthio-2-FAA was obtained from the reaction of N-acetoxy-2-FAA with DL-methionine (21). The crude thioether was recrystallized from ethanol:water until the melting point was the same as that of authentic 3-methylthio-2-FAA (168–169°) (7). The infrared absorption spectrum of the compound was identical with that of authentic 3-methylthio-2-FAA (7).

Preparation of Labeled Compounds. 2-FAA-9,14C (Lot 31-299, 2.97 mCi/mmmole) and N-hydroxy-2-FAA-9,14C (Lot 485-120, 10 mCi/mmmole) were obtained from New England Nuclear, Boston, Mass. For use in the experiments described in Table 4, the N-hydroxy-2-FAA-9,14C was diluted with the appropriate amount of unlabeled N-hydroxy-2-FAA. N-Hydroxy-2-FAA-1,14C (specific radioactivity, 0.38 mCi/mmmole) was prepared as described previously (2). The
radiopurity of the labeled compounds was demonstrated by paper chromatography and thin-layer chromatography (2).

Animals. Female Sprague-Dawley rats were obtained from the Holtzman Company, Madison, Wis. Female Fischer 344 rats were purchased from A. R. Schmidt and Company, Madison, Wis., and from the Charles River Breeding Laboratories, Inc., North Wilmington, Mass. The animals were caged individually in an air-conditioned room (26°) and were maintained on a 20% casein diet (15). Food and water were allowed ad libitum.

Carcinogenicity Tests. Rats of the 2 strains weighing initially 75 to 90 g received the compounds under test by i.p. injection. The compounds dispersed in 0.9% NaCl solution:acacia (11) (1 mg compound/0.1 ml vehicle) were injected 3 times weekly for 1 month. The rats were weighed on the day of injection and once weekly after the administration of the compounds had been completed. All animals were autopsied immediately after death or at the termination of the carcinogenicity tests (12 months). The tumors as well as the lungs, liver, spleen, kidneys, stomach, and intestine were sectioned, and the sections (5 to 6 μm thick) were stained routinely with hematoxylin-eosin.

Determination of the Biliary and Urinary Excretion of N-Hydroxy-2-FAA-9-14C by the Female Fischer Rat after i.p. Administration of 2-FAA-9-14C or of N-Hydroxy-2-FAA-9-14C. The bile ducts of rats weighing on the average 150 g were cannulated, and the rats were kept postoperatively on the regimen previously described (10). Each rat received 2-FAA-9-14C (1.3 to 1.6 mg; specific radioactivity, 2.97 mCi/mmmole) or N-hydroxy-2-FAA-9-14C (0.9 to 1.1 mg; specific radioactivity, 7.8 mCi/mmmole) in propylene glycol (0.3 to 0.5 ml) by single i.p. injections, and the bile was collected as previously described (10). The O-glucuronides in the bile were then hydrolyzed with bacterial β-glucuronidase (10). The specific activity of the β-glucuronidase assayed with phenolphthalein glucuronide as the substrate was 58,000 Fishman units/g of protein. For the estimation of the urinary excretion of N-hydroxy-2-FAA-9-14C, the urine was collected for 3 days after the i.p. injection of the labeled compounds. During the collection, the rats were kept up in stainless steel metabolism cages and maintained on Purina chow and water ad libitum. The urinary O-glucuronides and O-sulfates were hydrolyzed with O-glucuronidase and Taka-Diastase, respectively (10). The specific radioactivity of the chromatographed 2-FAA-9-14C was used for the calculation of the amounts of N-hydroxy-2-FAA-9-14C in the bile or urine.

Determination of the Hepatic Arylsulfotransferase Activity of the Sprague-Dawley and Fischer Rat. The enzymatic sulfonation of N-hydroxy-2-FAA-1-14C by the cytoplasmic arylsulfotransferase from the livers of adult Sprague-Dawley and Fischer rats was measured by the arylamidation of tRNA (16, 17). The soluble enzyme was separated from compounds of low molecular weight by gel filtration, and the tRNA adduct was purified through the cetyltrimethylammonium salt as previously described (17, 35).

Isolation of o-CH3-S-2-FAA-9-14C and of 2-FA-9-14C from the Hepatic Cytoplasmic Proteins of Sprague-Dawley and Fischer Rats after i.p. Injection of N-Hydroxy-2-FAA-9-14C. In these experiments, N-hydroxy-2-FAA-9-14C (1.0 mg/100 g body weight) in propylene glycol (0.2 ml) was injected i.p. into 5 groups of male and female Fischer and Sprague-Dawley rats and into 1 group of ovariec-tomized Sprague-Dawley rats. Each group consisted of 3 animals. The average weight of the animals in each group is listed in the legend to Chart 2. The pooled livers from each group were homogenized in 0.25 M sucrose and 0.003 M CaCl2 (3). The cytoplasmic proteins were isolated by differential centrifugation and purified by gel filtration and ion exchange chromatography on DEAE-cellulose (1). The purified proteins were then partially hydrolyzed with 3 N NaOH (21), and the amounts of o-CH3-S-2-FAA-9-14C and of 2-FA-9-14C released from the proteins were estimated by inverse isotope dilution (2). o-CH3-S-2-FAA designates the mixture of isomers, 3-methylthio- and 1-methylthio-2-FAA, which are liberated by alkaline hydrolysis from protein adducts formed by the interaction of N-sulfoxyl- or N-acetoxy-2-FAA with methionine in proteins (27). Although the hydrolysate contains a mixture of the 2 isomers, on a quantitative basis 3-methylthio-2-FAA appears to be the predominant compound (4). Since the isomers are not separable chromatographically (4, 27), inverse isotope dilution with 3-methylthio-2-FAA measures the sum of these isomers in the protein hydrolysate. Labeled o-CH3-S-2-FAA and 2-FA were

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separated and purified to constant specific radioactivity by repeated thin-layer chromatography as previously described (2).

Analytical Procedures. The radioactivity of all samples was determined by liquid scintillation spectrometry with the use of a toluene-based scintillator (1) or of Scintisol-Complete (5 to 10 ml), purchased from Isolab, Inc., Elkhart, Ind. All samples were counted in duplicate with an error not exceeding 5%, and the counts were corrected for quenching. The counting efficiencies were 70 to 80%. Radioactive compounds on thin-layer chromatograms were located by scanning the chromatograms with a radioscanner (Model LB-271, Berthold Laboratories, Wildbad, West Germany). The protein content of samples in the experiments described in Table 4 and Chart 1 were determined by the modified Folin method with crystalline bovine serum albumin as a standard (22).

RESULTS AND DISCUSSION

The carcinogenilities of 2-FAA for the female Sprague-Dawley rat and the female Fischer rat are compared in Table 1. 2-FAA administered i.p. to the female Sprague-Dawley rat at the dose level used routinely in this laboratory (2.3 mg/100 g body weight, 3 times weekly for 1 month) (11) gave a high incidence (92%) of mammary adenocarcinomas. These data are in agreement with earlier observations regarding the tumor incidence and site of action of 2-FAA (24). In contrast, a similar dose of 2-FAA produced no tumors in the female Fischer rat. It has been suggested that arylamines that are not N-hydroxylated by a given species are also not carcinogenic for that species (11, 23). One might therefore infer from the present data that the female Fischer rat does not N-hydroxylate 2-FAA. In order to substantiate

| Table 1 |
The susceptibility of the Sprague-Dawley and Fischer rats to hepatocarcinogenesis by 2-FAA and N-hydroxy-2-FAA

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Strain</th>
<th>Sex</th>
<th>Total dose (mmole/rat)</th>
<th>No. of tumor-bearing rats/no. of rats used</th>
<th>Of liver</th>
<th>Of mammary gland</th>
<th>At other sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Male</td>
<td>0/11</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Female</td>
<td>0/11</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>F</td>
<td>Female</td>
<td>0/12</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-FAA</td>
<td>SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Female</td>
<td>0.17</td>
<td>11/12</td>
<td>0</td>
<td>11 (29)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 (2)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-FAA</td>
<td>F</td>
<td>Female</td>
<td>0.12</td>
<td>0/11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-hydroxy-2-FAA</td>
<td>SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Female</td>
<td>0.10</td>
<td>12/12</td>
<td>0</td>
<td>12 (41)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 (1)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-hydroxy-2-FAA</td>
<td>SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Female</td>
<td>0.17</td>
<td>9/9</td>
<td>0</td>
<td>9 (43)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>N-hydroxy-2-FAA</td>
<td>F</td>
<td>Female</td>
<td>0.10</td>
<td>7/11</td>
<td>6 (12)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1 (1)</td>
<td>3 (4)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-hydroxy-2-FAA</td>
<td>SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Male</td>
<td>0.33</td>
<td>9/9</td>
<td>4 (4)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3 (3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7 (11)&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> SD, Sprague-Dawley rat; F, Fischer rat.

<sup>b</sup> Numbers in parentheses are the number of tumors.

<sup>c</sup> Two lung metastases.

<sup>d</sup> Ten hepatocellular carcinomas, 2 cholangiomas.

<sup>e</sup> One probable adenocarcinoma of intestine, 1 ear duct carcinoma, 1 oviduct adenoma, and 1 bladder papilloma.

Table 2

Biliary and urinary excretion of N-hydroxy-2-FAA-9-<sup>14</sup>C after single i.p. injections of 2-FAA-9-<sup>14</sup>C to the female Fischer rat

<table>
<thead>
<tr>
<th>2-FAA-9-&lt;sup&gt;14&lt;/sup&gt;C administered&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Period of collection (hr)</th>
<th>% administered radioactivity excreted&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% 2-FAA-9-&lt;sup&gt;14&lt;/sup&gt;C excreted as N-hydroxy-2-FAA-9-&lt;sup&gt;14&lt;/sup&gt;C&lt;sup&gt;b,c&lt;/sup&gt;</th>
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<tr>
<td>mg dpm</td>
<td>Of bile</td>
<td>Of urine</td>
<td>In bile</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>1.3 3.7 x 10&lt;sup&gt;7&lt;/sup&gt; dpm</td>
<td>40</td>
<td>61.3</td>
<td>1.94&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.3 3.7 x 10&lt;sup&gt;7&lt;/sup&gt; dpm</td>
<td>72</td>
<td>74.5</td>
<td>1.46&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.6 4.4 x 10&lt;sup&gt;7&lt;/sup&gt; dpm</td>
<td>42</td>
<td>51.6</td>
<td>1.02</td>
</tr>
<tr>
<td>1.4 4.0 x 10&lt;sup&gt;7&lt;/sup&gt; dpm</td>
<td>42</td>
<td>49.3</td>
<td>0.72</td>
</tr>
</tbody>
</table>

<sup>a</sup> The 2-FAA-9-<sup>14</sup>C was administered in propylene glycol (0.5 ml).

<sup>b</sup> The values represent the excretion of the radioactivity and of N-hydroxy-2-FAA-9-<sup>14</sup>C by single animals.

<sup>c</sup> Determined by inverse isotope dilution as described in the text.

<sup>d</sup> The percentage of a single i.p. dose of 2-FAA-9-<sup>14</sup>C (1.1 mg, 3.2 x 10<sup>7</sup> dpm) excreted in the bile of a female Sprague-Dawley rat was 25.2%.
this inference, we examined by tracer methods the biliary excretion of N-hydroxy-2-FAA-9-14C 40 and 72 hr after a single i.p. injection of 2-FAA-9-14C to female Fischer rats (Table 2). The biliary and urinary excretion of radioactive compounds following the administration of the labeled arylamide appeared to be nearly complete by that time (Chart 1). Irving et al. (14) have shown that the bile is the principal pathway for the elimination of N-hydroxy-2-FAA formed from 2-FAA by the male Sprague-Dawley rat. The finding that 25% of a dose of 2-FAA was excreted in the bile of the female Sprague-Dawley rat as the N-hydroxy compound (Table 2, Footnote d) indicated that the bile is also a major route for the excretion of N-hydroxy-2-FAA by the female Sprague-Dawley rat. In contrast, only 1 to 2% of a comparable dose of 2-FAA-9-14C was eliminated as N-hydroxy-2-FAA-9-14C in the bile of the female Fischer rat, and only 0.7 to 1.0% of the amide was excreted as the hydroxamic acid in the urine (Table 2). The possibility remained that the low levels of N-hydroxy-2-FAA in the bile and urine of the female Fischer rat were attributable to the inability of the animal to conjugate N-hydroxy-2-FAA and to excrete the conjugate(s). This was checked by the i.p. administration of N-hydroxy-2-FAA-9-14C into the female Fischer rat and by the estimation of the hydroxamic acid in the urine and bile. Approximately 8 and 10% of the administered compound were recovered from the hydrolysates of the urine and bile, respectively (Table 3). This value for the urine was comparable to the fraction of the hydroxamic acid (~12%) that was isolated by Weisburger et al. (33) from the hydrolyzed urine of female Fischer rats dosed with N-hydroxy-2-FAA-9-14C. Our data as well as those of Weisburger et al. showed that the female Fischer rat conjugates N-hydroxy-2-FAA and excretes the conjugated hydroxamic acid in the bile and urine. Moreover, the total fraction of the administered hydroxamic acid that was eliminated via the bile and the urine in the present experiments (~18%) was of the same order of magnitude as that reported recently for the Sprague-Dawley rat (~22%) (13). It would appear that the female Fischer rat is able to dispose of an appreciable portion of N-hydroxy-2-FAA by excretion via the bile and urine. The available evidence leads us to conclude that the female Fischer rat, in comparison to the female Sprague-Dawley rat, has only a limited capacity for the N-hydroxylation of 2-FAA. This capacity appears to be insufficient for the formation of tumor-producing amounts of N-hydroxy-2-FAA from the relatively restricted quantities of 2-FAA (0.1 to 0.2 mmole/rat) that were used in the carcinogenicity tests described in Table 1.

Dunning et al. (5) administered a total of 4.0 mmoles 2-FAA per rat over a period of 12 months; i.e., the total amount of carcinogen ingested by the rat was nearly 40 times as large as that administered in our standard test. Under these conditions, it seems possible that the female Fischer rat, although its capacity for N-hydroxylation is limited, formed N-hydroxy-2-FAA in amounts that were adequate for hepatocarcinogenesis. This interpretation of the data presumes that the female Fischer rat is susceptible to the hepatocarcinogenic action of N-hydroxy-2-FAA. This supposition was tested by the i.p. administration of N-hydroxy-2-FAA to female rats of the Fischer strain. These tests showed that N-hydroxy-2-FAA administered to the female Fischer rat by the same schedule that had been ineffective with 2-FAA gave a high incidence of hepatic tumors (Table 1). Seventy % of the tumors produced by the hydroxamic acid in the female Fischer rat were hepatic neoplasms (Table 1). Sixty-five % of the hepatic neoplasms (8/12) presented the typical histological picture of well-differentiated hepatocellular carcinomas (19, 28, 29, 31)

Table 3

<table>
<thead>
<tr>
<th>N-HO-2-FAA-9-14C administered</th>
<th>Period of collection (hr)</th>
<th>% radioactivity excreted</th>
<th>% N-HO-2-FAA-9-14C recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>dpm</td>
<td>Of bile</td>
<td>Of urine</td>
</tr>
<tr>
<td>1.1</td>
<td>8.23 × 10⁷</td>
<td>20</td>
<td>30.3</td>
</tr>
<tr>
<td>1.1</td>
<td>8.23 × 10⁷</td>
<td>72</td>
<td>43.3</td>
</tr>
<tr>
<td>0.9</td>
<td>6.80 × 10⁷</td>
<td>72</td>
<td>72.0</td>
</tr>
</tbody>
</table>

a The N-HO-2-FAA-9-14C was administered in propylene glycol (0.3 to 0.5 ml).

b The values represent the excretion of the radioactivity of N-hydroxy-2-FAA-9-14C by single animals.

c Determined by inverse isotope dilution as described in the text.
group of experiments, the activities of the hepatic enzyme in the liver of the female Sprague-Dawley rat. All of these results indicated that the liver of the male rat was more active than the enzyme in the liver of the female rat. In these experiments, the arylsulfotransferase of the male Fischer rat was only twice as active as that of the female rat of this strain. In a separate series of experiments, the arylsulfotransferase activity of the female Sprague-Dawley rat was approximately 10-fold greater than that of the female Sprague-Dawley rat. This compound decomposes rapidly to sulfate and a methionine adduct. It would appear that the susceptibility of the female Fischer rat and the refactoriness of the female Sprague-Dawley rat to the hepatocarcinogenicity of N-hydroxy-2-FAA to the female Fischer rat. In the other approach, we estimated the 2nd step of the activation of 2-FAA by the 2 strains in vivo. In these experiments, the 14C-labeled hydroxamic acid was injected i.p., and the fraction of the bound 14C that was cleaved from hepatic cytoplasmic proteins by alkali as o-CH3-S-2-FAA (21) was determined. Eight % of the bound 14C from the soluble proteins of female Fischer rats and 7% of the bound 14C from the soluble proteins of male Fischer or male Sprague-Dawley rats were associated with o-CH3-S-2-FAA (Chart 2) and were therefore derived, in large part, from N-sulfoxo-2-FAA. On the other hand, only 3% of the 14C bound to the cytoplasmic proteins of the liver of the female Sprague-Dawley rat was degraded by alkali to o-CH3-S-2-FAA. It would appear that the level of o-CH3-S-2-FAA that reflects the formation of N-sulfoxo-2-FAA correlated with the susceptibility of the 2 strains to the hepatocarcinogenic action of N-hydroxy-2-FAA. In agreement with the data of DeBaun et al. (4), the formation of methionine adducts in the livers of the female Sprague-Dawley rats was stimulated by ovariectomy. On the basis of the extent of formation of the methionine adduct by the male Fischer rat, one would anticipate that N-hydroxy-2-FAA is a potent hepatocarcinogen for this animal. However, N-hydroxy-2-FAA at the dosage used in the present study was not tolerated by the male Fischer rat. The extreme toxicity of the hydroxamic acid administered i.p. to the female Fischer rat contains an arylsulfotransferase that, although somewhat less active than that of the male Fischer or Sprague-Dawley rat, exceeds the enzymatic activity of the female Sprague-Dawley rat by several orders of magnitude.

In the other approach, we estimated the 2nd step of the activation of 2-FAA by the 2 strains in vivo. In these experiments, the 14C-labeled hydroxamic acid was injected i.p., and the fraction of the bound 14C that was cleaved from hepatic cytoplasmic proteins by alkali as o-CH3-S-2-FAA (21) was determined. Eight % of the bound 14C from the soluble proteins of female Fischer rats and 7% of the bound 14C from the soluble proteins of male Fischer or male Sprague-Dawley rats were associated with o-CH3-S-2-FAA (Chart 2) and were therefore derived, in large part, from N-sulfoxo-2-FAA. On the other hand, only 3% of the 14C bound to the cytoplasmic proteins of the liver of the female Sprague-Dawley rat was degraded by alkali to o-CH3-S-2-FAA. It would appear that the level of o-CH3-S-2-FAA that reflects the formation of N-sulfoxo-2-FAA correlated with the susceptibility of the 2 strains to the hepatocarcinogenic action of N-hydroxy-2-FAA. In agreement with the data of DeBaun et al. (4), the formation of methionine adducts in the livers of the female Sprague-Dawley rats was stimulated by ovariectomy. On the basis of the extent of formation of the methionine adduct by the male Fischer rat, one would anticipate that N-hydroxy-2-FAA is a potent hepatocarcinogen for this animal. However, N-hydroxy-2-FAA at the dosage used in the present study was not tolerated by the male Fischer rat. The extreme toxicity of the hydroxamic acid administered i.p. to the female Fischer rat contains an arylsulfotransferase that, although somewhat less active than that of the male Fischer or Sprague-Dawley rat, exceeds the enzymatic activity of the female Sprague-Dawley rat by several orders of magnitude.

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Hepatocarcinogenicity and Activation of 2-FAA

The male Fischer rat in amounts similar to those of this study has been noted previously (34). In summary, the correlations between the amounts of 2-FA cleaved from the soluble hepatic cytoplasmic proteins of Fischer (F) and Sprague-Dawley (SD) rats and identified as o-CH$_3$-S-2-FAA and 2-FA. A single dose of N-hydroxy-2-FAA-9-$^1$4C (specific radioactivity, 10 mCi/mmol) in propylene glycol was injected i.p. at a level of 1.0 mg/100 g body weight. The amounts of compound in the hydrolysate were determined by inverse isotope dilution as described in the text. The female and male Fischer rats were adult animals with an average weight of 154 ± 5 and 239 ± 3 g, respectively. The young, female Sprague-Dawley (9 $SD_1$) and adult, female Sprague-Dawley (9 $SD_2$) rats had average weights of 156 ± 10 and 239 ± 5 g, respectively. $SD_1$ denotes adult, ovariectomized Sprague-Dawley rats with an average weight of 227 ± 3 g. The male Sprague-Dawley (9 $SD$) rats weighed 233 ± 4 g. Each bar represents the mean of 3 experiments ± average deviation from the mean.

Chart 2. The fraction of the bound radioactivity of N-hydroxy-2-FAA-9-$^1$4C released by alkaline hydrolysis from the hepatic cytoplasmic proteins of Fischer (F) and Sprague-Dawley (SD) rats and identified as o-CH$_3$-S-2-FAA and 2-FA. A single dose of N-hydroxy-2-FAA-9-$^1$4C (specific radioactivity, 10 mCi/mmol) in propylene glycol was injected i.p. at a level of 1.0 mg/100 g body weight. The amounts of compound in the hydrolysate were determined by inverse isotope dilution as described in the text. The female and male Fischer rats were adult animals with an average weight of 154 ± 5 and 239 ± 3 g, respectively. The young, female Sprague-Dawley (9 $SD_1$) and adult, female Sprague-Dawley (9 $SD_2$) rats had average weights of 156 ± 10 and 239 ± 5 g, respectively. $SD_1$ denotes adult, ovariectomized Sprague-Dawley rats with an average weight of 227 ± 3 g. The male Sprague-Dawley (9 $SD$) rats weighed 233 ± 4 g. Each bar represents the mean of 3 experiments ± average deviation from the mean.

ACKNOWLEDGMENTS

The authors thank Miss Ann McHale for the cannulation of the bile ducts and Mr. Peter Bell for technical assistance.

REFERENCES

15. King, C. M., and Gutmann, H. R. The Action of the Carcinogen...


Fig. 1 and 3. Hepatocellular adenocarcinoma (1.4-cm diameter) with hemorrhagic and necrotic foci in the liver of a female Fischer rat after i.p. administration of N-hydroxy-2-FAA. This carcinoma was poorly differentiated and characterized by large pleomorphic cells with distinct cell boundaries. There were multiple areas of nodular hyperplasia elsewhere in the liver. A discrete sclerosing cholangioma was also present. Fig. 1, X 6.7; Fig. 3, X 180.

Fig. 2 and 4. Cholangioma (7-mm diameter) in the liver of a female Fischer rat after i.p. administration of N-hydroxy-2-FAA. Note the local invasion of the adjacent parenchymal tissue. The irregular ducts are lined by low cuboidal or flattened epithelium separated by proliferating fibrous connective tissue containing chronic inflammatory cells. Occasional areas of neutrophilic infiltration are present within the lumens of the ducts. Many portal areas throughout this liver showed similar foci of fibroblastic hyperplasia and of hyperplasia of the bile ductules. Fig. 2, X 10; Fig. 4, X 180.
On the Correlation between the Hepatocarcinogenicity of the Carcinogen, \( N \)-2-Fluorenylacacetamide, and Its Metabolic Activation by the Rat


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