Activation of the Carcinogen, N-Hydroxy-2-fluorenylbenzenesulfonamide, by Desulfonylation to N-2-Fluorenylhydroxylamine in Vivo

D. Malejka-Giganti, H. R. Gutmann, R. E. Rydell, and Y. Yost

Laboratory for Cancer Research, Veterans Administration Hospital, Minneapolis, Minnesota 55417 [D. M-G., H. R. G., R. E. R., Y. Y.], and the Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455 [D. M-G., H. R. G.]

SUMMARY

The carcinogenicity of N-hydroxy-2-fluorenylbenzenesulfonamide (N-HO-2-FBS), a potent carcinogen for the young male and female rat by i.p. administration, was confirmed by p.o. administration of the compound to the adult male rat. The compound produced hepatic lesions that were macroscopically and microscopically very similar to those induced by N-hydroxy-2-fluorenylacetamide. Examination of the urine after the administration of N-HO-2-FBS to male and female rats revealed several metabolites indicative of desulfonylation of N-HO-2-FBS to the intermediate, N-2-fluorenylhydroxylamine. Determination of the 35S:14C ratio in the tissues after i.p. injection of N-HO-2-FBS 35S and N-hydroxy-2-fluorenyl-14C-benzenesulfonamide to the female rat disclosed rapid and extensive desulfonylation of the carcinogen by the mammary gland, liver, and kidney. The mammary gland, which is the primary target of N-HO-2-FBS in the female rat, appeared to be more active in hydrolyzing the compound than the other tissues. The carcinogenicity of i.p. administered N-phenyl-2-fluorenylhydroxylamine, N-phenyl-4-biphenylylhydroxylamine, and the corresponding amines was tested in the male and female rat. N-Phenyl-2-fluorenylhydroxylamine displayed only weak activity in the female rat and was inactive in the male rat. N-Phenyl-4-biphenylylhydroxylamine was not carcinogenic either for the male or female rat. These as well as previously published data support the view that N-2-fluorenylhydroxylamine is an obligatory intermediate for the carcinogenicity of N-acyl-2-fluorenylhydroxylamines. Replacement of the hydrolyzable acyl or benzenesulfonyl group by the phenyl group results in a molecule with greatly diminished carcinogenic properties.

INTRODUCTION

In a previous study (11) of the structural requirements for the carcinogenicity of N-acylarylhdroxylamines, we found that the inactive arylamide, 2-FBS, was converted to the potent carcinogen, N-HO-2-FBS, by synthetic N-hydroxylation. The compound administered i.p. was highly active toward the mammary gland of the young female rat and, in the young male rat it produced a variety of neoplastic lesions in the intestinal tract, the pancreas, the abdominal cavity, and the skin at very low doses. However, the compound was poorly tolerated and caused a large number of premature deaths. In the present study, the carcinogenicity of N-HO-2-FBS for the male adult rat, which tolerates relatively large amounts of the compound, has been examined by gastric intubation. N-HO-2-FBS decomposes appreciably to 2-nitrosofluorene in aqueous and nonaqueous media, and the possibility was considered that the nitroso compound might account for the activity of N-HO-2-FBS. Accordingly, the carcinogenicity of 2-nitrosofluorene for the male rat p.o. was also evaluated in the current experiments.

There is considerable evidence that N-2-fluorenylhydroxylamines, such as N-HO-2-FAA and N-hydroxy-2-fluorenylbenzamide, are deacylated by rat tissues in vitro (7, 13, 14, 16, 17) and in vivo (10, 39). The carcinogenicity of N-2-fluorenylhydroxylamines might have been attributable, therefore, to the action of the product of the hydrolysis, N-2-fluorenylhydroxylamine, or of a derivative thereof. N-2-Fluorenylhydroxylamine, which is a carcinogen (30), reacts with amino acids (25), proteins (3, 6, 7, 17), and nucleic acids (6, 16, 17, 19, 20), and the interaction results in stable (3, 6, 16, 17, 21, 25) as well as unstable adducts (18, 21). In order to substantiate a mechanism of action of N-2-fluorenylhydroxylamines involving the formation of N-2-fluorenylhydroxylamine, we pursued two approaches. First, we investigated the urinary excretion of compounds that would arise from the desulfonylation of N-HO-2-FBS by the rat. In addition, we examined the splitting of N-HO-2-FBS by rat tissues in vivo by means of N-HO-2-FBS labeled with 14C in the fluorene portion of the molecule or with 35S in the benzenesulfonyl group. As a second approach, we prepared N-phenyl-2-fluorenylhydroxylamine and N-phenyl-4-biphenylylhydroxylamine and tested these compounds along with the aromatic amines derived therefrom for their carcinogenicity in the rat. These compounds differ from N-acyl-2-fluorenyl- or N-acyl-4-biphenylylhydroxylamines in that the potentially hydrolyzable acyl group has been replaced by the phenyl group, which theoretically would be more refractory to removal from the molecule than the acetyl, benzoyl, or benzenesulfonyl group. Consequently, the amounts of N-2-fluorenylhydroxylamine liberated from these...
diarylhydroxylamines by the tissues would be expected to be less than the amounts released by the hydrolysis of N-acyl-2-fluorenylhydroxylamines; hence, the carcinogenicity of N-phenyl-2-fluorenyl- or N-phenyl-4-biphenylhydroxylamine compared to that of N-acyl-2-fluorenylhydroxylamines or of N-acyl-4-biphenylhydroxylamines should be demonstrably diminished, if not abolished. The results of these metabolic studies and the associated carcinogenicity tests form the basis of this report.

MATERIALS AND METHODS

Preparation of Unlabeled Compounds

N-HO-2-FBS, m.p. 138—140°; 2-FBS, m.p. 202—204° (11); N-HO-2-FAA, m.p. 150—151° (27); 2-FAA, m.p. 196—198° (34); 3-HO-2-FAA, m.p. 247—249° (40); 5-HO-2-FAA, m.p. 217—218° (41); 7-HO-2-FAA, m.p. 230—232° (43); 2-nitrofluorenone, m.p. 79—81° (22); and p-hydroxybenzenesulfonamide, m.p. 172—174° (literature m.p. 176°) (37), λ_max (e, 14,750) μm, were prepared by the published procedures. Benzzenesulfonamide was purchased from the Aldrich Chemical Co., Inc., Milwaukee, Wis., and was recrystallized from 70% ethanol (with charcoal), m.p. 153—154°, λ_max (e, 9,520) μm. The UV and infrared absorption spectra of the compounds were checked routinely against those of authentic samples. N-Phenyl-2-fluorenylhydroxylamine, m.p. 107—109° (with decomposition), and N-phenyl-4-biphenylhydroxylamine, m.p. 90° (with decomposition), were prepared by treatment of 2-nitrofluorene or 4-nitrobiphenyl with phenylmagnesium bromide (46). N-Phenyl-2-fluorenamine, m.p. 135—136°, and N-phenyl-4-aminobiphenyl, m.p. 113—114°, were obtained by zinc dust reduction of the diarylhydroxylamines. All compounds were characterized by elemental analysis, UV and infrared absorption spectra, and in several instances by the mass spectra (46).

Preparation of Labeled Compounds

N-HO-2-FAA-9-14C (Lot 31-301, 7.0 mCi/mmole) and 2-FAA-9-14C (Lot 31-299, 2.9 mCi/mmole) were obtained from New England Nuclear, Boston, Mass. The radioactivity of the labeled compounds was determined by paper and thin-layer chromatography as described previously (3).

N-HO-2-FBS-9-14C. N-2-Fluorenylhydroxylamine-9-14C (22) was prepared by the partial reduction of 2-nitrofluorene-9-14C, m.p. 157—158° (Mallinkrodt Nuclear, St. Louis, Mo.; Lot 2761, 13.38 mCi/mmole). The nitro compound was radiochemically pure as indicated by a single peak (R_f 0.60) on radiochromatograms of the compound on silica gel. The radioactive nitrofluorene (0.30 g) was diluted with chromatographically pure 2-nitrofluorene (0.12 g). The labeled compound was dissolved in 25 ml of dimethylformamide and 25 ml of 95% ethanol and was cooled through the solution for 30 and 15 min, respectively. The reaction mixture stood overnight at 4°, and the N-2-fluorenylhydroxylamine-9-14C was precipitated with 140 ml of cold water saturated with nitrogen. The precipitate was washed with water and dried in a vacuum at 4°. The N-2-fluorenylhydroxylamine-9-14C (0.35 g; 87.3% yield; specific activity, 8.04 mCi/mmmole), m.p. 175° (with decomposition), had UV absorption spectrum (λ_max 284 μm) identical with that of an authentic sample. The labeled compound (0.34 g; 1.74 mmoles) was suspended in 20 ml of ethanol saturated with N2. Pyridine (0.055 ml, 0.68 mmoles) and benzenesulfonyl chloride (0.1 ml, 0.77 mmoles) were added dropwise to the stirred suspension, which was kept under N2 atmosphere and at 35° throughout the reaction. After 2.5 hr, a yellow product was removed by filtration, and cold water (25 ml) saturated with N2 was added to the filtrate. The suspension was left overnight at 4° (0.25 g; 37.4% yield based on 2-nitrofluorenone). The compound was recrystallized from 50% ethanol (20 ml) (0.14 g; 20% yield; m.p. 130—131°; specific activity, 8.63 mCi/mmmole). The UV absorption spectrum of the radioactive compound in ether was superimposable on that of an authentic sample (11).

N-HO-2-FB35S. This compound was prepared from N-2-fluorenylhydroxylamine (0.42 g, 2.4 mmoles) and benzenesulfonyl-35S chloride (0.17 g, 0.94 mmole, 13.6 mCi/mmmole; Lot 557163, Radiochemical Centre, Amersham, England) in the same way as the 14C-labeled compound. The labeled compound (m.p. 134—135°; 0.1 g; 11% yield; specific activity, 6.97 mCi/mmmole) had UV absorption spectrum identical with that of an authentic compound (11).

Administration of Compounds

For injection or intubation, the compounds (1.0 to 1.5 mg) were dispersed in 0.1 ml of 0.9% NaCl solution containing 1.75% acacia by homogenization in a Potter-Elvehjem homogenizer. In the experiments in which the content of 35S and 14C in the tissues was determined, 0.6 mg of labeled compound was dispersed in 0.1 ml of 0.9% NaCl solution containing 7% acacia.

Animal Experiments

The rats used in the experiments were purchased from the Holtzman Co., Madison, Wis. All rats were maintained on a semisynthetic 20% casein diet (15). Food and water were allowed ad libitum.

Carcinogenicity Tests

The male rats used in the p.o. carcinogenicity tests (10 to 12/group) weighed 200 to 220 g each at the start of the experiment. The compounds were administered by stomach tube for 6 to 7 months. During the initial 4 months, the rats received N-HO-2-FBS (4 mg/100 g of body weight), 2-FABS (3.8 mg/100 g of body weight), and 2-nitrosofluorene (2.3 mg/100 g of body weight). From 4 to 6 months, the dosages were doubled, and during the last month the animals were dosed at the initial level. When the toxicity of compounds resulted in a loss of body weight, the administration was suspended until the animals resumed normal weight gain. The
tests were terminated at the end of 12 months. The male and female rats that received the compounds i.p. weighed 75 to 80 g each at the start of the experiments. The compounds were injected 3 times weekly for 4 weeks at a dosage of 4.5 mg of compound/100 g of body weight. The rats were caged individually and weighed daily. After the administration of the compounds had been completed, the animals were weighed once weekly. All animals were autopsied immediately after death or at the termination of the experiments. The tumors, lungs, liver, spleen, kidney, stomach, and intestines were fixed in buffered formalin and sectioned 5 to 6 μ thick. The sections were stained with hematoxylin and eosin.

Identification of the Urinary Metabolites of N-HO-2-FBS

In these experiments, 6 male rats weighing 110 g each received 4.0 mg of N-HO-2-FBS/100 g of body weight 3 times weekly for 8 weeks by stomach tube. In another series, 6 female rats weighing 80 g each received 4.5 mg of N-HO-2-FBS/100 g of body weight 3 times weekly for 4 weeks by i.p. injections. The urine from each rat was collected for a period of 24 hr in containers packed in Dry Ice. The urines excreted by the animals of each group were pooled, and the pooled samples were stored at −20° until they were processed. O-Glucuronides and O-sulfates were hydrolyzed with β-glucuronidase (Sigma Chemical Co., St. Louis, Mo.) and Taka-diastase (Parke, Davis and Co., Detroit, Mich.). Two preparations of β-glucuronidase with specific activities of 93,400 and 75,000 Fishman units/g, respectively, were used. The preparations of β-glucuronidase with specific activities of 93,400 and 75,000 Fishman units/g, respectively, were used. The compounds were estimated by direct isotope dilution.

The 24-hr excretion of the urinary metabolites after a single i.p. injection of 2.3 mg of N-HO-2-βS or N-HO-2-FBS-9-14C/100 g of body weight was determined by inverse isotope dilution. Each radioactive compound was administered to 2 female rats weighing 180 to 200 g each. The urines of the rats receiving the compound with the same label were pooled, and the metabolites were fractionated as above. In addition, the excretion of inorganic sulfate-35S derived from N-HO-2-βS was determined by precipitation of the sulfate with barium chloride. The radioactivity of the dry precipitate was measured in a gas flow counter (Nuclear-Chicago, Des Plaines, Ill.) with a counting efficiency of 8%.

Distribution of 35S and 14C from N-HO-2-βS and N-HO-2-FBS-9-14C in Rat Tissues and Urine

Sixteen female rats weighing 180 to 200 g each received a single i.p. injection of 2.3 mg of N-HO-2-βS/100 g of body weight, and an equal number were given the same dose of N-HO-2-FBS/100 g. At 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hr after the injection, 2 rats from each of the 2 groups were killed by heart puncture. The blood was drawn into syringes rinsed with heparin and was centrifuged at 2000 rpm. Duplicate aliquots (0.4 ml) of the plasma were diluted with 1% NaOH solution and the radioactivity of the samples was measured by liquid scintillation spectrometry in the scintillation liquid described below. The mammary glands from each rat were excised, and the glandular tissue was separated from the fat pad with scissors. The liver was perfused with cold 0.9% NaCl solution prior to excision. Following excision, all tissues were washed in cold 0.9% NaCl solution and then minced with scissors. Each mince was partially solubilized with cold (4°) 1% NaOH. One ml of the NaOH solution was used per 10 mg of mammary gland, and 10 ml were used per gram of the other tissues. The suspensions were then homogenized for 5 min in a VirTis “23” homogenizer at the highest speed, and the radioactivity of duplicate aliquots (1 ml) was determined in the scintillation

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Silica gel&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cellulose&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>System A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>System B</td>
</tr>
<tr>
<td>N-HO-2-FBS</td>
<td>0.57</td>
<td>0.14</td>
</tr>
<tr>
<td>2-FBS</td>
<td>0.28</td>
<td>0.34</td>
</tr>
<tr>
<td>N-HO-2-FAA</td>
<td>0.20</td>
<td>0.37</td>
</tr>
<tr>
<td>3-HO-2-FAA</td>
<td>0.22</td>
<td>0.31</td>
</tr>
<tr>
<td>5-HO-2-FAA</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>7-HO-2-FAA</td>
<td>0.83</td>
<td>0.79</td>
</tr>
<tr>
<td>2-Nitrosofluorene</td>
<td>0.60</td>
<td>0.71</td>
</tr>
<tr>
<td>Benzenesulfonamide</td>
<td>0.41</td>
<td>0.53</td>
</tr>
<tr>
<td>p-Hydroxybenzenesulfonamide</td>
<td>0.60</td>
<td>0.80</td>
</tr>
</tbody>
</table>

<sup>a</sup> Silica Gel GF<sub>54</sub> was heated to 110° for 1 hr prior to application of the compounds.
<sup>b</sup> Precoated sheets manufactured by Macherey Nagel and Co., Düren, West Germany, and distributed by Brinkmann Instruments, Inc., Westbury, N. Y.
<sup>c</sup> Solvent systems: A, petroleum ether:chloroform:ethanol (50:45:5, v/v) (3); B, petroleum ether:chloroform:ethanol (80:18:2, v/v) (3); C, ethyl acetate:benzene (70:30, v/v) (3); D, chloroform:ethanol (95:5, v/v) (3); E, benzene; F, cyclohexane:tert-butyl alcohol:glacial acetic acid:water (160:5:15:20, v/v) (upper phase).
Table 2

Carcinogenicity of 2-FBS, N-HO-2-FBS, and 2-nitrosofluorene administered p.o. to the male Holtzman rat

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Total dose\textsuperscript{a} (mmoles/rat)</th>
<th>Duration of administration (mo.)</th>
<th>Weight gain at 2 mo.\textsuperscript{b} (g)</th>
<th>No. of tumor-bearing rats/no. of rats used</th>
<th>Of mammary gland</th>
<th>Of liver</th>
<th>At other sites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>7.0</td>
<td>235 ± 24</td>
<td>0/12</td>
<td>0/12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-FBS</td>
<td>2.46 ± 0.40</td>
<td>6.1</td>
<td>205 ± 26</td>
<td>0/12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-HO-2-FBS</td>
<td>2.41 ± 0.45</td>
<td>6.7</td>
<td>110 ± 37</td>
<td>6/10</td>
<td>0</td>
<td>3(3)</td>
<td>4(7)\textsuperscript{d} 60</td>
</tr>
<tr>
<td>N-HO-2-FBS</td>
<td>0.486 ± 0.019</td>
<td>2.0</td>
<td>141 ± 14</td>
<td>2/5</td>
<td>1(1)</td>
<td>1(1)</td>
<td>2(2)\textsuperscript{e} 40</td>
</tr>
<tr>
<td>2-Nitrosofluorene</td>
<td>1.95 ± 0.51</td>
<td>7.1</td>
<td>134 ± 15</td>
<td>0/10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Mean ± S.D.
\textsuperscript{b} Mean ± S.D. of the difference between the initial weight of the rat and the weight at 2 months.
\textsuperscript{c} No. in parentheses, no. of tumors.
\textsuperscript{d} Four ear duct tumors, 1 squamous cell carcinoma of the forestomach, and 2 adenocarcinomas of small intestine.
\textsuperscript{e} One squamous cell carcinoma of the forestomach and 1 squamous cell carcinoma of the duodenum.

Table 3

Histological changes in the liver after p.o. administration of 2-FBS, N-HO-2-FBS, and 2-nitrosofluorene to male Holtzman rats

<table>
<thead>
<tr>
<th>Compound administered\textsuperscript{a}</th>
<th>No. of rats with:</th>
<th>Diffuse, focal hyperplasia</th>
<th>Nodular hyperplasia</th>
<th>Small hepatocellular carcinoma</th>
<th>Mild fibrosis</th>
<th>Cirrhosis</th>
<th>Cystic dilation of bile ducts\textsuperscript{b}</th>
<th>Cysto-adenoma, &gt;5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-FBS</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-HO-2-FBS</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>1\textsuperscript{c}</td>
<td>6</td>
<td>1</td>
<td>10\textsuperscript{d}</td>
<td>2\textsuperscript{e}</td>
</tr>
<tr>
<td>N-HO-2-FBS</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>5\textsuperscript{f}</td>
<td>4\textsuperscript{g}</td>
</tr>
<tr>
<td>2-Nitrosofluorene</td>
<td>10</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2\textsuperscript{h}</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} For total dose administered and duration of administration, see Table 2.
\textsuperscript{b} Cystic dilation of bile ducts was classified in 5 categories: trace, 1+ (random), 2+ (numerous with enlargement of the foci), 3+ (multiple focal changes and distortion of normal configuration of liver), and 4+ (liver essentially replaced by cystic dilation of bile ducts).
\textsuperscript{c} Multiple foci of histological carcinoma, all of which were <5 mm in diameter.
\textsuperscript{d} One rat with trace, 2 rats with 1+, 3 rats with 2+, 2 rats with 3+, and 2 rats with 4+ cystic dilation of bile ducts.
\textsuperscript{e} Discrete nodular tumors >5 mm in diameter (1 in rat with 2+ and 1 in rat with 3+ cystic dilation of bile ducts).
\textsuperscript{f} Two rats with trace, 2 rats with 1+, and 1 rat with 2+ cystic dilation of bile ducts.
\textsuperscript{g} Discrete nodular tumor >5 mm in diameter in rat with 2+ cystic dilation of bile ducts.
\textsuperscript{h} Two rats with trace cystic dilation of bile ducts.

RESULTS

Toxicity and Carcinogenicity of N-HO-2-FBS, 2-FBS, and 2-Nitrosofluorene. 2-FBS administered p.o. appeared to be relatively nontoxic for the rat. Thus, 2 months after the start of the tests the weight gain of the rats receiving 2-FBS was only 12% less than the weight gain of the controls (Table 2). A similarly low toxicity of 2-FBS had been observed after i.p. administration of the compound (11). 2-FBS was noncarcinogenic p.o., and this confirms the lack of activity of the compound that was noted after i.p. injection. These data are in agreement with earlier results indicating that the analogous sulfonamide, N-2-fluorenyl-p-toluenesulfonamide, was not carcinogenic for the rat (1, 33). In contrast to 2-FBS, N-HO-2-FBS administered p.o. in amounts of 0.49 and 2.41 mmoles depressed the growth of the rat by 40 and 53%, respectively. Unlike 2-FBS, N-HO-2-FBS displayed marked carcinogenicity p.o. Thus, 0.057 m mole of N-HO-2-FBS injected i.p. into the male rat caused tumors in 63% of the animals (11), it would appear that N-HO-2-FBS is a more potent carcinogen i.p. than p.o. The hepatic lesions produced by N-HO-2-FBS, as well as by the other compounds, were classified by their histological appearance according to Reuber (35). N-HO-2-FBS produced a diffuse, focal hyperplasia of the liver in all rats. This lesion progressed to the formation of clearly defined hyperplastic nodules in about 70% of the animals. One of 10 rats developed...
Malejka-Giganti, Gutmann, Rydell, and Yost

a hepatocellular carcinoma with a diameter of <5 mm, and 1 of 10 rats showed cirrhosis of the liver. All of these changes are regarded as preneoplastic (35). The cystic dilation of the bile ducts observed in all animals receiving N-HO-2-FBS was graded in 5 categories, as defined in Table 3. In about 70% of the animals receiving N-HO-2-FBS, the dilation was rated 2+ or greater. Three rats developed discrete nodular tumors of the bile ducts. The diameter of these tumors was >5 mm. The data also imply that the rat does not N-hydroxylate 2-FBS acids are more carcinogenic than the corresponding amides.

Because N-HO-2-FBS tends to decompose to 2-nitrosofluorene at 37° in phosphate buffer, pH 7.4, as well as in methyl or ethyl alcohol, the nitroso compound was considered accountable for the carcinogenicity of N-HO-2-FBS and was therefore tested concurrently with N-HO-2-FBS p.o. The nitroso compound inhibited the growth of the rat almost to a significant extent.

Urinary Metabolites of N-HO-2-FBS. In an attempt to determine whether N-HO-2-FBS is converted to a proximate carcinogen by the rat, the metabolism of the compound was investigated. Previous work had indicated that certain N-acylarylhydroxylamines are deacylated by the rat to the carcinogen, N-2-fluorenylhydroxylamine (10, 39). This compound reacts with proteins and nucleic acids (3, 6, 7, 16, 17, 19, 20) and has been under consideration as a proximate carcinogen of 2-FAA (8, 30). Accordingly, the cleavage of the benzenesulfonyl group from N-HO-2-FBS by tissues was examined in vivo. Male and female rats received N-HO-2-FBS in the amounts and by the routes stated in Table 4. The following metabolites, which are indicative of the cleavage of the benzenesulfonyl group from the molecule, were isolated from the urine of male as well as female rats: N-hydroxy-2-FAA, N-(3-hydroxy)-2-FAA, N-(5-hydroxy)-2-FAA, N-(7-hydroxy)-2-FAA, and 2-FAA. When N-HO-2-FBS was administered for 1 to 2 months to the rat, N-HO-2-FAA was a major excretion product accounting for as much as 70% of the isolated urinary metabolites. The hydroxamic acid arose very likely from the cleavage of N-HO-2-FBS to N-2-fluorenylhydroxylamine and from the subsequent acetylation of the hydroxylamine (42). The metabolites with the hydroxyl group on the carbon atom contributed up to 35% of the isolated metabolites. The amidofluorenols were very likely referable to the microsomal

<table>
<thead>
<tr>
<th>Compound administered (mg)</th>
<th>Route of administration</th>
<th>Duration of experiment (days)</th>
<th>No. and sex of rats used</th>
<th>Urinary metabolites isolated after the administration of N-HO-2-FBS to the rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-HO-2-FBS (957.3)</td>
<td>p.o.</td>
<td>56</td>
<td>6 d</td>
<td>N-HO-2-FAA 23.73 e 5.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-FAA 0.42 a 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-, 5-, 7-HO-2-FAA 4.07 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-FBS 2.10 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Benzenesulfonamide 2.32 0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-Hydroxy-benzenesulfonamide 0.83 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N-HO-2-FAA 12.43 a 7.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-FAA 0.13 d 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-, 5-, 7-HO-2-FAA 8.31 4.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-FBS 0.47 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Benzenesulfonamide 1.25 1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-Hydroxy-benzenesulfonamide 0.84 0.66</td>
</tr>
<tr>
<td>N-HO-2-FBS (248.3)</td>
<td>i.p.</td>
<td>28</td>
<td>6 g</td>
<td>N-HO-2-FAA 0.12 c 1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-FAA 0.01 e 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-HO-2-FAA 0.07 e 1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-HO-2-FAA 1.09 e 16.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7-HO-2-FAA 0.57 e 8.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-FBS &lt;0.01 e 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Benzenesulfonamide &lt;0.01 c 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-Hydroxy-benzenesulfonamide &lt;0.01 e 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inorganic sulfate e 0.57</td>
</tr>
</tbody>
</table>

These metabolites were assayed by direct isotope dilution. The other metabolites were estimated spectrophotometrically.

b N-HO-2-FBS-9-14C (1.7 x 106 dpm/mg) was administered to each of 2 rats (2.3 mg/100 g of body weight).

c These metabolites were estimated by inverse isotope dilution in the pooled urines from 2 rats.

d N-HO-2-FB3 S (7.5 x 104 dpm/mg) was injected into each of 2 rats (2.3 mg/100 g of body weight).

e The inorganic sulfate was counted as the Ba2+ salt.
Activation of Carcinogenic N-Acylarylhydroxylamines

Compounds may likewise be due to the cleavage of the benzenesulfonyl group from N-HO-2-FBS and to the subsequent reaction with ammonium ion or with an amino group donor, such as glutamine and asparagine (23, 24). However, the possibility cannot be ruled out that these metabolites resulted from the scission of the carbon-2—nitrogen bond. This type of cleavage would theoretically yield fluorene or a phenolic derivative thereof as well as N-benzenesulfonylhydroxylamine or benzenesulfonylamide. Such a mechanism seems highly unlikely, since we were unable to find any evidence for the excretion of N-benzenesulfonylhydroxylamine, which is a stable compound (32, 44). Moreover, to our knowledge there is no precedent that the rat metabolizes arylamides or arylhydroxamic acids in this fashion.

Only trace amounts of 2-FBS were detected in the urine of the rat after the administration of N-HO-2-FBS. Similarly, very little N-2-fluorenylbenzamide has been isolated from rat urine after the administration of N-hydroxy-2-fluorenylbenzamide (10). In contrast, 2-FAA was a major urinary metabolite of N-HO-2-FAA (28). This suggests that the reduction of N-acylarylhydroxylamines in the rat proceeds only with difficulty unless the acetyl group constitutes the acyl moiety.

Distribution of $^3$S and $^{14}$C from $^3$S and $^{14}$C in Rat Tissues and Urine. The urinary excretion of metabolites indicative of the desulfonylation of N-HO-2-FBS prompted us to obtain evidence for the occurrence of this reaction in the tissues of the rat and particularly in the mammary gland, which is the principal target for the action of N-HO-2-FBS in the female rat. In these experiments, N-HO-2-FBS labeled either with $^3$S in the sulfonamido group or with $^{14}$C in position 9 was administered by a single i.p. injection to female rats, and the content of $^3$S or $^{14}$C in the mammary gland, liver, kidney, plasma, and urine was determined at various times thereafter. The fraction of the administered $^3$S or $^{14}$C in the fat-free mammary gland was at all times very much smaller than in any other tissue.

hydroxylation of 2-FAA (5, 38), which was also isolated in small amounts. 2-FAA has been identified as the major metabolite of N-HO-2-FAA in the rat (22, 28). The same metabolites accounted for virtually all of the $^{14}$C excreted in the urine within 24 hr after a single i.p. dose of N-HO-2-FBS-9-$^{14}$C. However, under these conditions, the amidofluorenols comprised the major fraction of the urinary metabolites, while N-HO-2-FAA was excreted only in relatively minor amounts. It would appear that N-HO-2-FAA is the predominant urinary excretion product only after prolonged intake of N-HO-2-FBS. The reasons for the different excretion patterns are not understood at this time. 2-Nitrosofluorene, the major product of the chemical decomposition of N-HO-2-FBS, was not detected in the urine either after a single injection or after prolonged administration of N-HO-2-FBS. This would appear to be additional evidence for the view that the carcinogenicity of N-HO-2-FBS may not be ascribed to 2-nitrosofluorene.

In addition to the metabolites derived from the splitting of N-HO-2-FBS at the nitrogen-sulfur bond, minor amounts of benzenesulfonamide and of $p$-hydroxybenzenesulfonamide were isolated from the urine. The presence of these compounds may likewise be due to the cleavage of the benzenesulfonyl group from N-HO-2-FBS and to the subsequent reaction with ammonium ion or with an amino group donor, such as glutamine and asparagine (23, 24). However, the possibility cannot be ruled out that these metabolites resulted from the scission of the carbon-2—nitrogen bond. This type of cleavage would theoretically yield fluorene or a phenolic derivative thereof as well as N-benzenesulfonylhydroxylamine or benzenesulfonylamide. Such a mechanism seems highly unlikely, since we were unable to find any evidence for the excretion of N-benzenesulfonylhydroxylamine, which is a stable compound (32, 44). Moreover, to our knowledge there is no precedent that the rat metabolizes arylamides or arylhydroxamic acids in this fashion.

Only trace amounts of 2-FBS were detected in the urine of the rat after the administration of N-HO-2-FBS. Similarly, very little N-2-fluorenylbenzamide has been isolated from rat urine after the administration of N-hydroxy-2-fluorenylbenzamide (10). In contrast, 2-FAA was a major urinary metabolite of N-HO-2-FAA (28). This suggests that the reduction of N-acylarylhydroxylamines in the rat proceeds only with difficulty unless the acetyl group constitutes the acyl moiety.

Distribution of $^3$S and $^{14}$C from N-HO-2-FBS-3$^3$S and N-HO-2-FBS-9-$^{14}$C in Rat Tissues and Urine. The urinary excretion of metabolites indicative of the desulfonylation of N-HO-2-FBS prompted us to obtain evidence for the occurrence of this reaction in the tissues of the rat and particularly in the mammary gland, which is the principal target for the action of N-HO-2-FBS in the female rat. In these experiments, N-HO-2-FBS labeled either with $^3$S in the sulfonamido group or with $^{14}$C in position 9 was administered by a single i.p. injection to female rats, and the content of $^3$S or $^{14}$C in the mammary gland, liver, kidney, plasma, and urine was determined at various times thereafter. The fraction of the administered $^3$S or $^{14}$C in the fat-free mammary gland was at all times very much smaller than in any other tissue.

hydroxylation of 2-FAA (5, 38), which was also isolated in small amounts. 2-FAA has been identified as the major metabolite of N-HO-2-FAA in the rat (22, 28). The same metabolites accounted for virtually all of the $^{14}$C excreted in the urine within 24 hr after a single i.p. dose of N-HO-2-FBS-9-$^{14}$C. However, under these conditions, the amidofluorenols comprised the major fraction of the urinary metabolites, while N-HO-2-FAA was excreted only in relatively minor amounts. It would appear that N-HO-2-FAA is the predominant urinary excretion product only after prolonged intake of N-HO-2-FBS. The reasons for the different excretion patterns are not understood at this time. 2-Nitrosofluorene, the major product of the chemical decomposition of N-HO-2-FBS, was not detected in the urine either after a single injection or after prolonged administration of N-HO-2-FBS. This would appear to be additional evidence for the view that the carcinogenicity of N-HO-2-FBS may not be ascribed to 2-nitrosofluorene.

In addition to the metabolites derived from the splitting of N-HO-2-FBS at the nitrogen-sulfur bond, minor amounts of benzenesulfonamide and of $p$-hydroxybenzenesulfonamide were isolated from the urine. The presence of these compounds may likewise be due to the cleavage of the benzenesulfonyl group from N-HO-2-FBS and to the subsequent reaction with ammonium ion or with an amino group donor, such as glutamine and asparagine (23, 24). However, the possibility cannot be ruled out that these metabolites resulted from the scission of the carbon-2—nitrogen bond. This type of cleavage would theoretically yield fluorene or a phenolic derivative thereof as well as N-benzenesulfonylhydroxylamine or benzenesulfonylamide. Such a mechanism seems highly unlikely, since we were unable to find any evidence for the excretion of N-benzenesulfonylhydroxylamine, which is a stable compound (32, 44). Moreover, to our knowledge there is no precedent that the rat metabolizes arylamides or arylhydroxamic acids in this fashion.

Only trace amounts of 2-FBS were detected in the urine of the rat after the administration of N-HO-2-FBS. Similarly, very little N-2-fluorenylbenzamide has been isolated from rat urine after the administration of N-hydroxy-2-fluorenylbenzamide (10). In contrast, 2-FAA was a major urinary metabolite of N-HO-2-FAA (28). This suggests that the reduction of N-acylarylhydroxylamines in the rat proceeds only with difficulty unless the acetyl group constitutes the acyl moiety.

Distribution of $^3$S and $^{14}$C from N-HO-2-FBS-3$^3$S and N-HO-2-FBS-9-$^{14}$C in Rat Tissues and Urine. The urinary excretion of metabolites indicative of the desulfonylation of N-HO-2-FBS prompted us to obtain evidence for the occurrence of this reaction in the tissues of the rat and particularly in the mammary gland, which is the principal target for the action of N-HO-2-FBS in the female rat. In these experiments, N-HO-2-FBS labeled either with $^3$S in the sulfonamido group or with $^{14}$C in position 9 was administered by a single i.p. injection to female rats, and the content of $^3$S or $^{14}$C in the mammary gland, liver, kidney, plasma, and urine was determined at various times thereafter. The fraction of the administered $^3$S or $^{14}$C in the fat-free mammary gland was at all times very much smaller than in any other tissue.
Table 5
Carcinogenicity of N-phenyl-2-fluorenamine, N-phenyl-4-biphenylamine, and the corresponding N-diarylhydroxylamine after i.p. administration to the Holtzman rat

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>No. and sex of rats used</th>
<th>Total dose ( a ) (mmole/rat)</th>
<th>Weight gain at 2 months ( b ) (g)</th>
<th>No. of tumor-bearing rats</th>
<th>Of mammary gland</th>
<th>At other sites</th>
<th>Tumor incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>11 ♀</td>
<td>255 ± 25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>10 ♀</td>
<td>161 ± 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-Phenyl-2-fluorenamine</td>
<td>12 ♀</td>
<td>0.317 ± 0.020</td>
<td>265 ± 24</td>
<td>1</td>
<td>0</td>
<td>1 ( d ) (1)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>12 ♀</td>
<td>0.235 ± 0.011</td>
<td>158 ± 6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-Phenyl-2-fluorenylhydroxylamine</td>
<td>12 ♀</td>
<td>0.276 ± 0.030</td>
<td>243 ± 23</td>
<td>0</td>
<td>0</td>
<td>3 ( e ) (3)</td>
<td>2 ( f ) (2)</td>
</tr>
<tr>
<td></td>
<td>22 ♀</td>
<td>0.256 ± 0.012</td>
<td>156 ± 18</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-Phenyl-4-biphenylamine</td>
<td>9 ♀</td>
<td>0.296 ± 0.013</td>
<td>266 ± 20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11 ♀</td>
<td>0.245 ± 0.013</td>
<td>137 ± 16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N-Phenyl-4-biphenylhydroxylamine</td>
<td>12 ♀</td>
<td>0.302 ± 0.020</td>
<td>255 ± 11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12 ♀</td>
<td>0.243 ± 0.017</td>
<td>140 ± 15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\( a \) Mean ± S.D.  
\( b \) Mean ± S.D. of the difference between the initial weight of the rat and the weight at 2 months.  
\( c \) No. in parentheses, no. of tumors.  
\( d \) Ear duct tumor.  
\( e \) Three adenocarcinomas of the mammary gland.  
\( f \) Two pleomorphic sarcomas.

Calculations of the amounts of \( ^{14} \text{C} \) per unit of weight of tissue or per unit of volume of plasma showed likewise that the average concentration of label in the mammary gland was one-fifteenth of that in the liver and one-fifth of that in plasma. The amounts or the concentration of compound in the tissues therefore appeared to be unrelated to its carcinogenic effect. The content of \( ^{14} \text{C} \) in the mammary gland and liver was at all times greater than that of \( ^{35} \text{S} \), indicating cleavage of the benzenesulfonyl group from the molecule and preferential retention of the \( ^{14} \text{C} \)-containing fragment by the tissues (Chart 1). Analysis of the kidneys gave data very similar to those for mammary gland and liver. The cleavage of N-HO-2-FBS and the preferential retention of the \( ^{14} \text{C} \)-containing fragment by the tissues was further supported by the observation that the percentage of \( ^{35} \text{S} \) excreted in the urine was at all times significantly greater than that of \( ^{14} \text{C} \). The relative amounts of \( ^{14} \text{C} \) and \( ^{35} \text{S} \) in the plasma in the first 2 hr after the administration of the labeled compound likewise supported the idea that N-HO-2-FBS was cleaved and that the \( ^{35} \text{S} \)-containing cleavage product was transported from the tissues initially at a faster rate than the \( ^{14} \text{C} \)-containing fragment. However, after 8 hr the content of \( ^{14} \text{C} \) in the plasma exceeded that of \( ^{35} \text{S} \) significantly. This change in the labeling pattern may be rationalized by the equilibration of the \( ^{14} \text{C} \)-containing fragment between tissue and plasma and the preferential binding of this fragment by the plasma proteins.

The cleavage of N-HO-2-FBS into \( ^{14} \text{C} \)- and \( ^{35} \text{S} \)-containing fragments was also inferred from a plot of the ratios of \( ^{35} \text{S}:^{14} \text{C} \) per unit weight of tissue or unit volume of urine versus time (Chart 2). The \( ^{35} \text{S}:^{14} \text{C} \) ratio of the administered compound was assigned a value of 1.0. Deviations from 1.0 would denote a disproportion of the relative amounts of \( ^{35} \text{S} \) and \( ^{14} \text{C} \) resulting from the cleavage of the benzenesulfonyl group from the molecule and from the selective concentration of 1 of the fragments. The distance between the lines indicates the range of error at a 68% level of confidence. The marked decrease in the \( ^{35} \text{S}:^{14} \text{C} \) ratios in mammary gland and liver within 2 hr after the injection of the labeled compounds was consistent with the view that during this time the benzenesulfonyl group was split from the molecule and that the \( ^{14} \text{C} \)-containing fragment was retained preferentially by the
N-acyl-2-fluorenylhydroxylamines were deacylated by the rat. N-phenyl-2-fluorenylhydroxylamine and related compounds are potent carcinogens for the rat (9, 10, 11, 27). Since the benzenesulfonyl group was replaced by a group that is likely to result in the hydrolyzable acyl or aromatic amine, the metagenic reaction in the rat. Under these conditions, diarylamines and similar compounds is not known as a major proximate agent of the compounds. This possibility was explored by testing the activity of disubstituted hydroxylamines in which the hydrolyzable acyl or benzenesulfonyl group was replaced by a group that is likely to resist metabolic cleavage. The phenyl group would appear to be a suitable substituent, since the dearylation of diarylamines and similar compounds is not known as a major metabolic reaction in the rat. Under these conditions, N-2-fluorenylhydroxylamine, which is the primary target of N-HO-2-FBS, occurs sooner and to a greater extent in the mammary gland than in the liver and other tissues.

Toxicity and Carcinogenicity of N-Phenyl-2-fluorenylhydroxylamine and Related Compounds.

With the exception of N-acylarylhydroxylamines, which are potent carcinogens for the rat (9, 10, 11, 27), N-phenyl-2-fluorenylhydroxylamines were deacylated by the rat in the present as well as in previous experiments (10, 39), the primary cleavage product, N-2-fluorenylhydroxylamine, which is a carcinogen (30), would seem to be a plausible choice as the proximate agent of the compounds. This possibility was explored by testing the activity of disubstituted hydroxylamines in which the hydrolyzable acyl or benzenesulfonyl group was replaced by a group that is likely to resist metabolic cleavage. The phenyl group would appear to be a suitable substituent, since the dearylation of diarylamines and similar compounds is not known as a major metabolic reaction in the rat. Under these conditions, N-2-fluorenylhydroxylamine, which is the primary target of N-HO-2-FBS, occurs sooner and to a greater extent in the mammary gland than in the liver and other tissues.

### Table 6

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>$^{14}$C bound/mg tRNA (nmols)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA + N-HO-2-FBS</td>
<td>0.01</td>
</tr>
<tr>
<td>tRNA + N-phenyl-2-fluorenyl-9$^{14}$C-hydroxylamine</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Soluble proteins + tRNA + N-HO-2-FBS</td>
<td>3.04</td>
</tr>
<tr>
<td>Soluble proteins + tRNA + N-phenyl-2-fluorenyl-9$^{14}$C-hydroxylamine</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**a** The incubations were at 37°C for 1 hr. The incubation medium was Tris-HCl buffer, pH 7.4, and the total volume of the incubation system was 2.3 ml.

**b** Yeast tRNA (Calbiochem, Los Angeles, Calif.) was purified by diethylaminoethyl cellulose chromatography (4) prior to use. After the incubation, the tRNA (5.1 mg incubation system) was precipitated as the potassium salt (6, 16, 17). The salt was dissolved in water (1.0 ml), and the radioactivity of aliquots (0.05 ml) was measured in 5 ml of scintillant (Scintisol Complete, Isolab, Inc., Elkhart, Ind.).

**c** N-HO-2-FBS-9$^{14}$C 0.1 µmole; specific radioactivity, 9.62 x $10^{4}$ dpm/µmole was added in 0.1 ml of methyl Cellosolve.

**d** N-Phenyl-2-fluorenyl-9$^{14}$C-hydroxylamine 0.1 µmole; m.p. 102-104°C; specific radioactivity, 2.08 x $10^{4}$ dpm/µmole was added in 0.1 ml of methyl Cellosolve.

**e** The soluble proteins (3.9 mg incubation system) in 5 ml of the 105,000 x g supernatant fraction of a 20% homogenate from the livers of adult male rats were chromatographed on Sephadex G-10 immediately before use.

**DISCUSSION**

We have suggested previously that the carcinogenicity of N-acetyl-2-fluorenylhydroxylamines for the rat depends on the prior metabolic deacylation of these compounds to the carcinogen, N-2-fluorenylhydroxylamine (10). The present experiments support this view, since the administration of the carcinogen, N-HO-2-FBS, to the rat yielded several urinary metabolites that are very likely derived from N-2-fluorenylhydroxylamine. As judged by the change of the $^{35}$S$^{14}$C ratios in the tissues after the administration of N-HO-2-FB-3$^{35}$S and N-HO-2-FBS-9$^{14}$C to the rat, extensive desulfonylation takes place in a number of tissues. There appears to be a correlation between the extent of desulfonylation of N-HO-2-FBS and its site of action, since the reaction proceeded at a faster rate in the mammary gland, which is the primary target of N-HO-2-FBS, than in the liver or...
N-acylarylhydroxylamine was a more effective carcinogen than N-phenyl-2-fluorenyl- and N-phenyl-4-biphenylylhydroxylamine from N-HO-2-FBS by rat tissues. N-(7-hydroxy-2-fluorenyl)acetamide were isolated after the metabolism of N-2-fluorenylhydroxylamine and the carcinogenicity of N-acyl-2-fluorenylhydroxylamines was also obtained by preliminary studies on the metabolism of nitroso compounds (45). Even though N-2-fluorenylhydroxylamine would be expected as a major urinary metabolite of N-HO-2-FBS, the isolation of N-HO-2-FAA (26, 29, 30) common to compounds that yield the carcinogenicity of N-acyl-2-fluorenylhydroxylamines. The isolation of N-HO-2-FAA from the urine of rats dosed with N-HO-2-FBS would indeed support such a mechanism of action. Irrespective of the ultimate molecular mechanism of action of N-acyl-2-fluorenylhydroxylamines, the present experiments, in conjunction with those already published, indicate that the nitrene derived theoretically from N-2-fluorenylhydroxylamine by dehydration, and this form would undoubtedly be highly reactive toward biological macromolecules (36). Furthermore, N-2-fluorenylhydroxylamine is acetylated to N-HO-2-FAA by the soluble deacylase of rat liver (3, 6, 17). Alternatively, the marginal activity or inactivity of N-2-fluorenylhydroxylamine for the rat may indicate that the Diaryldihydroxylamine is not esterified and is thus unable to furnish the ionic nucleophile species regarded as 1 of the final active forms that results from a 2-step activation of 2-FAA via the N-acylarylhydroxylamine, N-HO-2-FAA (26, 29, 30). Metabolic experiments in vivo and in vitro now in progress should enable us to decide between these alternatives.

The apparent relationship between the carcinogenicity of N-acyl-2-fluorenylhydroxylamines and the removal of the acyl group from the molecule suggests that N-2-fluorenylhydroxylamine is the intermediate required for the initiation of neoplasia (Chart 3). This does not define N-2-fluorenylhydroxylamine as the active form in the mechanism of action of N-acyl-2-fluorenylhydroxylamines. Because of the aforementioned reactivity of the hydroxylamine with proteins and nucleic acids, this remains a distinct possibility. However, the active form could be a nitrene derived theoretically from N-2-fluorenylhydroxylamine by dehydration, and this form would undoubtedly be highly reactive toward biological macromolecules (36). Furthermore, N-2-fluorenylhydroxylamine is acetylated to N-HO-2-FAA by the rat (42), and the hydroxamic acid may be esterified (29, 30). The amidonium ion derived from the ester may be the activated form (26, 29, 30) common to compounds that yield the mechanism of action. Irrespective of the ultimate molecular mechanism of action of N-acyl-2-fluorenylhydroxylamines, the present experiments, in conjunction with those already published, indicate that the size of the ary1 moiety and the position of the acyl group relative to the aromatic system the ease of removal of the acyl or benzenesulfonyl group from the molecule is a determinant of the carcinogenicity of N-acyl-2-fluorenylhydroxylamines.

**ACKNOWLEDGMENTS**

We are indebted to Dr. E. A. Johnson, Department of Biometry, School of Public Health, University of Minnesota, for the statistical evaluation of the data and for advice in their interpretation. We also thank Miss Catherine Sullivan for careful technical assistance.

**REFERENCES**

Activation of Carcinogenic N-Acylarylhydroxylamines


Activation of the Carcinogen, $N$-Hydroxy-2-fluorenylbenzenesulfonamide, by Desulfonylation to $N$-2-Fluorenylhydroxylamine \textit{in Vivo}


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/31/6/778

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.