N-Hydroxy Metabolites of 2-Acetylaminophenanthrene and 7-Fluoro-2-acetylaminofluorene as Proximate Carcinogens in the Rat

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Summary

N-Hydroxy-7-fluoro-2-acetylaminofluorene (N-hydroxy-7-fluoro-AAF) and N-hydroxy-2-acetylaminophenanthrene (N-hydroxy-AAP) were identified as urinary metabolites of 7-fluoro-2-acetylaminofluorene and 2-acetylaminophenanthrene, respectively, in the rat.

When administered as 0.010-0.012% of the diet for 10-15 weeks N-hydroxy-7-fluoro-AAF proved to be a much more potent carcinogen for the rat than 7-fluoro-AAF. The N-hydroxy derivative produced high incidences of squamous cell carcinomas of the forestomach, adenocarcinomas of the small intestine, carcinomas of the liver, and carcinomas of the mammary gland (females). In addition, this compound induced significant incidences of carcinomas of the ear duct gland and urinary bladder. On an average each rat had more than 2 primary tumors of different tissue origins. Under these conditions 7-fluoro-AAP induced primarily liver and mammary carcinomas; even at these sites the amide was less active than the N-hydroxy derivative.

When administered by s.c. injection in young female rats N-hydroxy-AAF induced sarcomas at the site of injection and a high incidence of mammary carcinomas. Under these conditions AAP induced only a few mammary carcinomas and no sarcomas.

Repeated i.p. injections of the N-hydroxy derivatives of aniline and N-ethylaniline (total doses of 58 and 72 mg) did not result in mammary tumor induction in female rats.

These data, in addition to those reported earlier, indicate that N-hydroxylation is of general importance in the neoplastic processes induced by carcinogenic aromatic amines and amides.

Syntheses are reported for the following new compounds: N-hydroxy-7-fluoro-2-acetylaminofluorene and N-hydroxy-2-acetylaminophenanthrene.

Introduction

Studies on the metabolism and carcinogenicity of 2-acetylaminofluorene (AAF) and of certain other aromatic amines and amides have led to the identification of their N-hydroxy derivatives as proximate carcinogenic metabolites of these compounds. Thus, AAF is metabolized to N-hydroxy-AAF by all of the species known to be susceptible to its carcinogenic action (see references in (14)). The N-hydroxy derivative is more carcinogenic than AAF, especially at sites of local application, in rats, mice, and hamsters (14, 15, 17, 25). Furthermore, the guinea pig, which apparently cannot convert AAF to N-hydroxy-AAF and which does not develop tumors on administration of AAF, is susceptible to the carcinogenic activity of the N-hydroxy derivative (14). Similar observations have also implicated the N-hydroxy derivatives of 4-acetaminobiphenyl (21), 4-acetaminostilbene (1, 2), and 2-aminonaphthalene (4) as proximate carcinogenic metabolites of these compounds.

The data to be presented in this paper extend these observations to 2 other potent carcinogenic amines, 7-fluoro-AAF and 2-acetylaminophenanthrene (AAP) (9, 20). Both of these amines are metabolized in vivo to their N-hydroxy derivatives, and both N-hydroxy-7-fluoro-AAF and N-hydroxy-AAP are more carcinogenic for the rat than the parent amines. These data, in addition to those reported earlier, indicate that N-hydroxylation is of general importance in the carcinogenic processes induced by aromatic amines and amides (13, 19).

Materials and Methods

Preparation of Compounds

7-Fluoro-AAF (m.p. 197°-198°C) (20) and 2-AAP (m.p. 226°-227°C) (20) were prepared as described previously. The syntheses of the corresponding N-hydroxy derivatives, which are new compounds, are described below.

N-HYDROXY-7-FLUORO-2-ACETYLMINOFUORENE (N-HYDROXY-7-FLUORO-AAF). A solution of 2.29 gm (0.01 mole) of

1 Received January 28, 1966; accepted May 17, 1966.

2 Chemical Abstracts nomenclature for the carcinogens is as follows: 7-fluoro-2-acetylaminofluorene, N-(7-fluoro-2-fluorenyl) acetamide; N-hydroxy-7-fluoro-2-acetylaminofluorene, N-(7-fluoro-2-fluorenyl) acetohydroxamic acid; 2-acetylaminophenanthrene, N-2-phenanthrylacetamide; N-hydroxy-2-acetylaminophenanthrene, N-2-phenanthrylacetoxyhydroxamic acid.

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2-nitro-7-fluorofluorene (20) in a mixture of 150 ml of ethyl acetate, 10 ml of acetic anhydride, 2.2 gm of piperidine (freshly distilled), and 1 gm of 5% palladium on charcoal (Matheson, Coleman, and Bell) in a 500-ml bottle was hydrogenated with shaking at room temperature in a Parr low pressure hydrogenation apparatus until 0.015 mole of hydrogen was taken up (approximately 25 min). This is an arbitrary point. Several runs indicated that a longer reaction time resulted in an increase in the formation of amide and a decrease in the final yield of hydroxamic acid. The catalyst was filtered off, and the filtrate was boiled under reflux with 300 ml of 9 n ammonium hydroxide with stirring for 1 hr and then boiled without a condenser for 10 min.

The aqueous layer was removed and the ethyl acetate solution was mixed with 250 ml of n-hexane. This was allowed to stand overnight, and the solution was carefully decanted from a white precipitate. The solution was extracted 3 times with 100-ml volumes of 1 n NaOH. The precipitate was taken up in ethyl ether and extracted once with 25 ml of 1 n NaOH. The combined alkaline solutions were extracted 2 times with ethyl ether. Acidification of the aqueous phase to pH 6 with 6 n HCl caused the precipitation of the N-hydroxy derivative (1.1 gm, 48% of theory based on the starting compound). The melting point of 157°-159°C, with slight softening at 155°C, rose to 168°-169°C after treatment with Darco charcoal and 2 recrystallizations from benzene (overall yield of pure product was 0.7 gm, 30% of theory).

C₂₁H₁₂FNO₂
Calculated: C, 70.63; H, 4.70; F, 7.39; N, 5.45
Found: C, 69.65; H, 4.57; F, 7.09; N, 5.68

N-hydroxy-2-acetylaminoacenaphthene (N-hydroxy-AAP). This compound was prepared by the reductive acetylation of 2-nitroacenaphthene. An unequivocal synthesis of small amounts of the latter compound was reported by Bavin and Dewar (3), and the following modifications of their synthesis were employed.

9-Formylfluorene and 9-fluorenylmethyl acetate: Dry fluorene, 100 gm, and 54 ml of dry neutral ethyl formate were dissolved in 950 ml of anhydrous ethyl ether and placed in a dry flask containing a magnetic stirring bar and 41 gm of fresh dry sodium ethylate (Skellysolve C) for 2 hr. The flask was attached to a drying tube which contained benzene (overall yield of pure product was 0.7 gm, 30% of theory).

9-Formylfluorene and 9-fluorenylmethyl acetate: Dry fluorene, 100 gm, and 54 ml of dry neutral ethyl formate were dissolved in 950 ml of anhydrous ethyl ether and placed in a dry flask containing a magnetic stirring bar and 41 gm of fresh dry sodium ethylate (Skellysolve C) for 2 hr. The flask was attached to a drying tube which contained benzene (overall yield of pure product was 0.7 gm, 30% of theory).

Metabolic Studies

Weanling male rats were given injections i.p. of 8.5 mg of AAP of 3.3 mg of 7-fluoro-AAP/100 gm body weight. The compounds were suspended by homogenization in a solution containing 0.9% of sodium chloride and 1.75% of gum acacia; 1.0 ml was injected/100 gm body weight. Twenty-four-hr urine samples from groups of 5 rats were collected in tubes immersed in Dry Ice.

After the urine was hydrolyzed with bacterial \( \beta \)-glucuronidase and Taka-diastase, the metabolites were extracted and chromatographed on paper according to procedures described previously.
Unsprayed strips were cut with visualization of the metabolites under ultraviolet light and as guided by other strips sprayed with the Folin-Ciocalteu phenol reagent or acidic p-dimethylaminobenzaldehyde, and the metabolites were eluted with ethanol. The N-hydroxy metabolites were separated from the parent amides (which have approximately the same Rf's) by extraction from ethyl ether into 0.5 N NaOH and were then re-extracted into ethyl ether after acidification of the basic solutions. The spectra of the N-hydroxy metabolites were determined 1st in ethanol and a 2nd time after addition of 0.1 ml of 0.1 N KOH/5 ml of ethanolic solution. The spectra of the other metabolites were determined in ethanol solution only.

Carcinogenicity Studies

7-Fluoro-AAF and its N-hydroxy derivative were fed to young adult male and female rats from the Holtzman Rat Co., Madison, Wisconsin. The male and female rats of Experiment 1 had average initial weights of 200 gm; in Experiment 2 the average initial weights were 210 and 195 gm for the males and females, respectively. In Experiment 1 the compounds were fed for a total of 15 weeks with a 3-week period on the carcinogen-free diet interposed after 9 weeks of carcinogen administration. This rest period was instituted because a few of the rats fed the N-hydroxy derivative were in poor health. In Experiment 2 the compounds were fed for 10.5 weeks. After these times the rats were maintained on the same diet without the carcinogens until the experiments were terminated at 11 and 13 months, respectively. The compounds were incorporated in the diets as glucose triturates (5-10 gm/kg). The rats in these 2 experiments received terramycin in their drinking water (80 mg/liter) for 6 days at 5-8-week intervals throughout the experiments. This treatment was instituted to minimize the respiratory infection which sometimes occurs in these rats.

Female rats from the Charles River Breeding Laboratories, Wilmington, Mass, with initial weights of 85-95 gm were used for the study on AAP and its N-hydroxy derivative. The compounds (2.35 mg of AAP or 2.5 mg of N-hydroxy-AAP) were suspended without heat in 0.2 ml of tricaprylin (trioctanoin, Eastman Organic Chemicals) with the aid of a magnetic stirrer; the suspensions were injected s.c. in the right hind leg with a 22-gauge needle. In all of the experiments the rats were housed in groups of 2-4 in screen-bottomed cages and received a grain diet (25) and water ad libitum. All of the animals were weighed at 1-2-week intervals during administration of the compounds and monthly thereafter. They were examined for tumors at 2- to 4-week intervals. All animals were subjected to careful gross autopsy of the mammary tissue, s.c. injection site, and abdominal and thoracic organs. All gross tumors or other abnormal tissues were fixed in neutral formalin, sectioned at 6 µ, and stained with hematoxylin and eosin.

Results

Characterization and estimation of N-hydroxy-7-fluoro-AAF and N-hydroxy-AAP as metabolites of the amides. In a previous study (16) N-hydroxy-7-fluoro-AAF was identified in the urine of rats fed 7-fluoro-AAF by comparison of the properties of the metabolite with those of the synthetic compound.

The properties compared included the Rf on chromatography on paper, reactions with acidic p-dimethylaminobenzaldehyde, and the Folin-Ciocalteu reagents, and the ultraviolet absorption spectrum in ethanol. The N-hydroxy-7-fluoro-AAF excreted by rats after i.p. injection of the amide was similarly identified in this study, except that the spectra of the synthetic compound and the metabolite were compared both in neutral and in alkaline ethanol (Chart 1, Table 1). The properties compared included the Rf on chromatography on paper, reactions with acidic p-dimethylaminobenzaldehyde, and the Folin-Ciocalteu reagents, and the ultraviolet absorption spectrum in ethanol. The N-hydroxy-7-fluoro-AAF excreted by rats after i.p. injection of the amide was similarly identified in this study, except that the spectra of the synthetic compound and the metabolite were compared both in neutral and in alkaline ethanol (Chart 1, Table 1).
TABLE 1

EXCRETION OF N-HYDROXY METABOLITES OF 7-FLUORO-2-ACETYLAMINOFLUORENE (7-FLUORO-AAF) AND OF 2-ACETYLAMINOPHENANTHRENE (AAP) *

<table>
<thead>
<tr>
<th>COMPOUND INJECTED</th>
<th>URINARY N-HYDROXY METABOLITE, (% of dose excreted in 24 hr)</th>
<th>RF's OF N-HYDROXY DERIVATIVE * *</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Fluoro-AAF</td>
<td>2.5, 4.0 **</td>
<td>0.88-0.92</td>
</tr>
<tr>
<td>AAP</td>
<td>0.3, 0.04, 0.5</td>
<td>0.78-0.86</td>
</tr>
</tbody>
</table>

* Weanling male rats were given i.p. injections of 3.3 mg of 7-fluoro-AAF or 8.5 mg of AAP/100 gm body weight.

* The metabolites were chromatographed in a solvent system composed of cyclohexane, t-butanol, acetic acid, and water (18:2:2:1).

* Each figure is the result obtained on analysis of the pooled urine from 5 rats.

the acidic p-dimethylanobenzaldehyde reagent, and ultraviolet spectra in neutral and alkaline ethanol (Chart 2, Table 1).

Other metabolites which reacted with the Folin-Ciocalteu reagent were also detected on chromatography of the urine extracts from rats treated with either 7-fluoro-AAF or AAP. The metabolites observed in the urine from rats given injections of 7-fluoro-AAF were similar to those previously reported for rats fed this amide (16). The most obvious metabolite of AAP, in addition to N-hydroxy-AAP, had an RF of 0.53-0.67 and gave a purple color when the chromatogram was sprayed with diazotized 7-nitro-2-aminofluorene. The latter reaction suggested that this metabolite was an ortho-hydroxy derivative.

CARCINOGENICITY OF 7-FLUORO-AAF AND N-HYDROXY-7-FLUORO-AAF. When fed in the diet N-hydroxy-7-fluoro-AAF proved to be a much stronger carcinogen for the rat than its parent amide (Table 2). Under the conditions of these experiments, 7-fluoro-AAF induced tumors almost exclusively in the livers of rats of either sex and in the mammary glands of female rats. N-Hydroxy-7-fluoro-AAF was more active in each of these tissues and, in addition, induced moderate to high incidences of malignant tumors in 3 other tissues.

A striking feature was the induction of squamous cell carcinomas in the forestomachs of most of the rats fed the N-hydroxy derivative, while no tumors were found in the forestomachs of rats fed 7-fluoro-AAF. Many of these squamous cell carcinomas showed evidence of mucus production as seen by vacuolated cells containing periodic acid-Schiff-positive and mucicarmine-positive material. Sections of these tumors are seen in Figs. 1 and 2. Adenocarcinomas of the small intestine were found in 36 of the 60 rats of Experiment 1 fed N-hydroxy-7-fluoro-AAF, while only 1 of 40 rats fed 7-fluoro-AAF developed this type of tumor. In the 2nd experiment in which the compounds were fed for a shorter time 8 of 28 rats fed the N-hydroxy derivative developed adenocarcinomas of the small intestine, while none were found in the rats fed 7-fluoro-AAF. Generally, the small intestinal adenocarcinomas were of 2 varieties. One type (Fig. 3) exhibited an extensive amount of mucus-like material, and the cells were considerably more anaplastic than the other more differentiated type. In some of the mucin-producing tumors osseous metaplasia was also found. The metaplasia was mostly restricted to areas of mucus production. The other type of small intestinal carcinoma seen was very highly differentiated and contained cells which were histologically compatible with Paneth cells. Examples of such lesions are seen in Figs. 4 and 5. Dunn and Kessel (5) reported the occurrence of Paneth cells in 2 isolated small intestine carcinomas, 1 from a mouse and 1 from a rat. In the series reported here at least 8 animals receiving N-hydroxy-7-fluoro-AAF had well-differentiated adenocarcinomas in which, in some areas, more than 50% of the cells were Paneth cells. Grossly, these lesions were usually firm and round and did not erode through the serosal surface. On cut section no mucoid structures were seen.
### TABLE 2
Cumulative Incidences of Tumors in Rats Fed 7-Fluoro-2-acetylaminofluorene (7-fluoro-AAF) or Its N-Hydroxy Metabolite

<table>
<thead>
<tr>
<th>Compound and % in diet</th>
<th>Time compound fed (wk.)</th>
<th>No. of rats, sex</th>
<th>No. of rats with tumors of a</th>
<th>Negative survivors b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>Mammary gland</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 mo.</td>
<td>11 mo.</td>
</tr>
<tr>
<td><strong>Experiment 1</strong> 0.01% N-hydroxy-7-fluoro-AAF</td>
<td>15</td>
<td>30 male</td>
<td>9 M</td>
<td>12 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 female</td>
<td>3 M</td>
<td>6 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 male</td>
<td>2 M</td>
<td>14 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 female</td>
<td>0</td>
<td>5 M</td>
</tr>
<tr>
<td><strong>Experiment 2</strong> 0.0004% 7-fluoro-AAF</td>
<td>15</td>
<td>14 male</td>
<td>1 M</td>
<td>2 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 female</td>
<td>1 M</td>
<td>3 M</td>
</tr>
<tr>
<td>0.01% 7-fluoro-AAF</td>
<td>10.5</td>
<td>14 male</td>
<td>0 M</td>
<td>1 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 female</td>
<td>0 M</td>
<td>1 M</td>
</tr>
</tbody>
</table>

a For each type of tumor those rats that had 1 or more malignant tumors are designated under M; those rats which had only 1 or more benign tumors of the given type are designated under B. Thus, the total number of rats with tumors in a given organ would be the sum of the numbers listed under M and B.

b Experiments 1 and 2 were terminated at 11 and 13 months, respectively. The negative survivors are the rats which were killed tumor-free at these times. The urinary bladder tumors and "other tumors" are those observed during the entire experimental period.
TABLE 3
CUMULATIVE INCIDENCE OF TUMORS IN FEMALE RATS THAT RECEIVED S.C. INJECTIONS
OF 2-ACETYLAMINOPHENANTHRENE OR ITS N-HYDROXY METABOLITE

<table>
<thead>
<tr>
<th>Groups of 20 female rats with initial weights of 85-95 gm received 4 s.c. injections at weekly intervals of 0.2 ml of triacrylaminophenone which contained 2.35 mg of 2-acetylaminophenanthrene or 2.5 mg of N-hydroxy-2-acetylaminophenanthrene.</th>
<th>AV. Wt. GAIN (gm)</th>
<th>NO. OF RATS WITH MAMMARY CARCINOMAS AT</th>
<th>NO. OF RATS WITH SARCOMAS AT INJECTION SITE</th>
<th>NEGATIVE SURVIVORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Hydroxy-2-acetylaminophenanthrene</td>
<td>1 mo.</td>
<td>3 mo.</td>
<td>4 mo.</td>
<td>5 mo.</td>
</tr>
<tr>
<td>2-Acetylaminophenanthrene</td>
<td>100</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>None (solvent only)</td>
<td>120</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* In addition, 2 rats injected with 2-acetylaminophenanthrene and 2 injected with N-hydroxy-2-acetylaminophenanthrene each had a single benign mammary tumor; none of these 4 rats bore malignant mammary tumors.

One control rat died with an anaplastic carcinoma of the ovary at 7 months.

Carcinomas of the ear duct gland occurred in 16 rats fed N-hydroxy-7-fluoro-AAP and in only 2 rats fed 7-fluoro-AAP. Most of these tumors were of sebaceous gland origin. Of the rats fed the N-hydroxy derivative 5 developed carcinomas of the urinary bladder, 1 developed a papilloma of the bladder, and 1 had a kidney carcinoma (Fig. 6). The final incidences of mammary carcinomas were about twice as great in the female rats fed N-hydroxy-7-fluoro-AAP as for the rats fed 7-fluoro-AAP.

In Experiment 2 these averages were 1.9 for the male rats and 2.3 for the female rats. The average numbers of malignant tumors of different tissue origins/rat were 0.7 and 0.6 for the male and female rats fed 7-fluoro-AAP in Experiment 1 and 0.4 and 0.3 in Experiment 2.

Carcinogenicity of AAP and its N-hydroxy derivative.

When the carcinogenic activities of these compounds were compared by s.c. injection into young female rats, the greater activity of N-hydroxy-AAP was apparent from the incidences of mammary carcinomas and of sarcomas at the injection site (Table 3). Of the 20 rats that received s.c. injections of N-hydroxy-AAP (total dose of 10 mg) 14 rats developed a total of 52 mammary carcinomas, and 2 other rats each had a single benign mammary tumor. More than 50% of these 16 rats received an N-hydroxy compound had 1 or more mammary tumors within 3 months after the 1st injection. Of the 20 rats that received equimolar amounts of AAP, 4 rats developed single mammary carcinomas and 2 others each had a single benign mammary tumor. Furthermore, while 6 of the rats that received injections of N-hydroxy-AAP developed sarcomas at the site of injection with an average latent period of 7.5 months, no sarcomas developed in the rats injected with the amide.

Lower doses of N-hydroxy-AAP also induced mammary carcinomas in young female rats. In a small experiment 5 s.c. injections at weekly intervals of 0.75, 1.25, 1.75, or 2.5 mg of N-hydroxy-AAP gave rise to mammary carcinomas in 4 out of 5, 3 out...
of 4, 4 out of 4, and 4 out of 4 rats, respectively, by 7 months. One of the latter group also had a sarcoma at the injection site by 7 months. Of 4 rats given injections 5 times of the solvent alone (tricarprin) and 4 rats given injections of 2.35 mg of AAP none developed tumors by 7 months when the experiment was terminated.

N-Hydroxy-AAP was too toxic when administered i.p. to permit satisfactory carcinogenesis experiments. In trial experiments N-hydroxy-AAP was suspended in 1.75% gum acacia-0.9% sodium chloride solution and injected 3 times weekly into weaning female rats. With 4 rats/group 2 injections of 4 mg/100 gm body weight resulted in death of all 4 rats by 9 days; 4 injections of 3.2 or 2.4 mg/100 gm body weight caused death of 4 and 3 rats, respectively, by 17 days; and 5 injections of 1.3 mg/100 gm body weight resulted in the death of 3 rats by 18 days. In another experiment 24 rats were given injections once of 1.2 mg/100 gm body weight, once of 0.8 mg/100 gm, and 8 times of 0.4 mg/100 gm body weight. Within the 1st month, 7 rats were dead, and 12 died within 3 months from the 1st injection. In nearly all of the rats that received i.p. injections of the N-hydroxy derivative extensive peritoneal adhesions, which in some cases caused intestinal obstruction, were observed on autopsy. Equivalent levels of AAP or of the injection medium alone caused neither adhesions nor death.

**Test for Carcinogenicity of N-Phenylhydroxylamine and Its N-Ethyl Derivative.** N-Phenylhydroxylamine (11) and N-ethyl-N-phenylhydroxylamine (12) were not carcinogenic for female rats when injected i.p. 3 times weekly for 12 weeks from weaning at doses of 1.0 and 1.24 mg/100 gm body weight, respectively (average total doses of 88 and 72 mg). There were 15 rats/group. One rat given injections of N-phenylhydroxylamine developed a mammary carcinoma at 8 months, and 1 control rat injected only with the suspending medium (0.9% sodium chloride) died with a lymphoma at 12 months. Fourteen, 13, and 13 rats treated with N-phenylhydroxylamine, N-ethyl-N-phenylhydroxylamine, or the suspending medium were killed without tumors when the experiment was terminated at 12 months. In another group given injections 3 times weekly for 3 weeks of 4.3 mg of N-hydroxy-AAP/100 gm body weight (total dose about 20 mg/rat) 90% developed mammary carcinomas with an average of 3 such tumors/rat. The doses of N-phenylhydroxylamine and N-ethyl-N-phenylhydroxylamine were the highest that could be injected without acute toxicity (manifested primarily as methemoglobinemia) which resulted in death within 1–2 days. Even at the levels used most of the rats developed methemoglobinemia and extreme lethargy for several hr after each of the 1st few injections.

**Discussion**

The data presented herein provide 2 additional examples of the conversion of aromatic amines to proximate carcinogenic metabolites via N-hydroxylation. Thus, N-hydroxy-7-fluoro-AAF and N-hydroxy-AAP were each identified as urinary metabolites of the corresponding amines, and each hydroxamic acid was much more carcinogenic for rats than the parent amine. Of particular interest is the induction of squamous cell carcinomas of the forestomach in the rats fed N-hydroxy-7-fluoro-AAF and of sarcomas at the site of injection of N-hydroxy-AAP; the corresponding amides were inactive at each of these sites of local application. These data and similar previously reported data on AAF, 4-acetylaminobiphenyl, 4-acetylaminoostilbene, and 2-aminoanaphthalene appear to permit the generalization that carcinogenic aromatic amines and amides produce tumors through mechanisms which involve N-hydroxylation of the parent carcinogens. In addition to the greater carcinogenic activities of the N-hydroxy derivatives their ability to induce tumors at sites of administration, where the amides and amines are not active, indicates that the N-hydroxy derivatives are closer than the parent amides or amines to the ultimate carcinogenic metabolites which interact with tissue macromolecules to initiate neoplasia (13, 19).

7-Fluoro-AAF is more potent than AAF, and the latter compound is more carcinogenic than 4-acetylaminobiphenyl when administered p.o. to rats (20). Each of the corresponding N-hydroxy derivatives is more carcinogenic than its parent amide, but the activities of the N-hydroxy derivatives form the same relative series as those of the amides. Enhanced activity on N-hydroxylation is also apparent from the report of Gutmann et al. (8) that the N-hydroxy derivative of the noncarcinogenic 7-hydroxy metabolite of AAF (22, 30) is carcinogenic on oral administration to the rat. Gutmann et al. (7) have also recently observed that N-benzoyl-AF, a weakly active carcinogen, is converted to a highly potent carcinogen by N-hydroxylation. Furthermore, Shirasu (27, 28) has shown that 4-hydroxymaminequinoline-1-oxide is carcinogenic, while 4-aminoquinoline-1-oxide is inactive. However, N-hydroxylation does not necessarily confer carcinogenic activity on noncarcinogenic amines and amides. Thus, aniline appears to be noncarcinogenic (10, 29) and N-ethylaniline, although untested, is probably inactive [cf. N-ethyl-4-aminoazobenzene (18)], and repeated injections of N-phenylhydroxylamine or N-ethyl-N-phenylhydroxylamine into weaning female rats did not induce mammary tumors under conditions suitable for the induction of mammary carcinomas with N-hydroxy-AAF. Similarly, the p.o. administration of N-hydroxy-N-acetyl-4-aminoazobenzene, repeated i.p. injections of N-hydroxy-4-aminoazobenzene or N-hydroxy-N-acetyl-4-aminoazobenzene, and repeated s.c. injections of the cupric chelate of N-hydroxy-N-acetyl-4-aminoazobenzene have not resulted in tumor induction in rats (26). Thus, both the state of oxidation of the nitrogen atom and the ring structures and their substituents appear to be important determinants of the carcinogenic potential of aromatic amines and amides.

The versatile and high carcinogenic activity of N-hydroxy-7-fluoro-AAF should be noted. With total doses of approximately 150 mg administered p.o. over 10–15-week periods all of the rats developed at least 1 malignant tumor and, on average, the rats developed 1.9–2.4 malignant tumors originating from different tissues. High incidences of malignant tumors of the liver, mammary gland, forestomach, and small intestinal epithelium were observed, and appreciable numbers of carcinomas of the urinary bladder and of the ear duct glands were also seen. In these respects N-hydroxy-7-fluoro-AAF appears to be at least as potent as 2,7-diacetylaminofluorene which, in studies by Morris et al. (23), likewise induced a high incidence of primary tumors with a tumor multiplicity similar to that reported here for N-hydroxy-7-fluoro-AAF. While other aromatic amine derivatives, such as N-hydroxy-4-acetylaminoostilbene (1, 2), are
active at some particular sites such as the ear duct gland at lower doses, N-hydroxy-7-fluoro-AAF and 2,7-diacylaminofluorene are remarkable with respect to the number of sites at which doses, A^-hydroxy-7-fluoro-AAF and 2,7-diacylaminofluorene are active at some particular sites such as the ear duct gland at lower doses, A^-hydroxy-7-fluoro-AAF and 2,7-diacylaminofluorene are remarkable with respect to the number of sites at which moderate to high incidences of tumors can be induced in the rat. If, as seems likely, 2,7-diacylaminofluorene is carcinogenic through conversion to a N-hydroxy derivative, this derivative can be expected to be an extremely potent carcinogen.

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References

FIG. 4. Section of highly differentiated adenocarcinoma of small intestine in rat fed \(N\)-hydroxy-7-fluoro-AAF. Numerous Paneth cells containing granules are noted in the acini of columnar and pseudostratified columnar epithelium. PAS-methylene blue, \(\times 200\).

FIG. 5. Oil immersion photomicrograph of tumor seen in Fig. 4. The granules of the Paneth cells in the neoplasm stand out rather sharply. PAS-methylene blue, \(\times 1250\).

FIG. 6. Transitional cell carcinoma of kidney of rat fed 0.012% \(N\)-hydroxy-7-fluoro-AAF. The neoplasm is arising from pelvic epithelium, a segment of which may be seen in 1 corner of the photomicrograph. H & E, \(\times 200\).
N-Hydroxy Metabolites of 2-Acetylaminophenantherene and 7-Fluoro-2-acetylaninofluorene as Proximate Carcinogens in the Rat

Elizabeth C. Miller, Prabhakar D. Lotlikar, Henry C. Pitot, et al.

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