The Carcinogenic Activities of \( N \)-Hydroxy-2-acetylaminofluorene and Its Metal Chelates as a Function of Retention at the Injection Site

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(McArdle Laboratory for Cancer Research, Medical Center, University of Wisconsin, Madison, Wisconsin)

SUMMARY

The carcinogenic activities of s.c. administered \( N \)-hydroxy-2-acetylaminofluorene (\( N \)-hydroxy-AAF) and a variety of its metal chelates were investigated with reference to the time of exposure of the tissue to the compounds. No tumors were obtained at the sites of 4 injections of \( N \)-hydroxy-AAF (3.2 mg/injection), but 4 and 16 of 20 rats developed sarcomas at the sites of 8 and 16 injections, respectively. Ear duct carcinomas were also found in about one-half of the rats in each of the latter groups. An approximate correlation was noted between the retention of \( N \)-hydroxy-AAF administered as the potassium salt or as various metal chelates and the incidence of sarcomas at the injection site. The nickelous, cobaltous, ferric, or cupric chelates induced moderate to high incidences of sarcomas with 1 or 4 injections; in these cases one-half of the administered \( N \)-hydroxy-AAF was retained at the injection site for 4—50 days. The manganous and zinc chelates and the potassium salt were less active at the injection site; the half-retention times for these derivatives ranged from 2\( \frac{1}{2} \) hr to 21 days. The comparable half-retention time for \( N \)-hydroxy-AAF was about 2 hr. Injection of the metal derivatives s.c. with short half-retention times resulted in higher incidences of mammary tumors than injection of the derivatives with half-retention times of 4 or more days. When administered p.o., the cupric chelate of \( N \)-hydroxy-AAF induced the same spectrum of tumors as \( N \)-hydroxy-AAF, but the incidences were lower.

\( N \)-Hydroxy-2-acetylaminofluorene (\( N \)-hydroxy-AAF) has proved to be a potent carcinogen on repeated p.o. and i.p. administration to rats and a variety of other animals (17, 18, 20, 31). While \( N \)-hydroxy-AAF had little activity at the site of 6 s.c. injections, its cupric chelate induced sarcomas with high incidence after a single injection (20). The greater activity of the cupric chelate appeared to be related to the retention at the injection site for long periods of \( N \)-hydroxy-AAF administered in this poorly soluble form, as compared to the rapid removal of \( N \)-hydroxy-AAF administered as such. However, since it was possible that the cupric ion played some additional role in augmenting the carcinogenic activity of \( N \)-hydroxy-AAF, the present study was undertaken to investigate the effect of variations in the metal component on the carcinogenicity and retention at the injection site of metal derivatives of \( N \)-hydroxy-AAF.

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1 This investigation was supported by a research training grant, CRTY-5002, and by Program-Project grant CA-07175 of the National Cancer Institute, USPHS; by a grant from the Jane Coffin Childs Memorial Fund for Medical Research; and by the Alexander and Margaret Stewart Trust Fund.

2 Chemical Abstracts nomenclature, \( N \)-2-fluorenylacetohydroxamic acid.

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MATERIALS AND METHODS

Compounds.—\( N \)-Hydroxy-AAF, m.p. 144°—146°C, was synthesized as described previously (21). For the preparation of the potassium salt 1.0 gm of \( N \)-hydroxy-AAF was dissolved in 75 ml of 1 \( N \) potassium hydroxide, and 100 ml of 5 \( N \) potassium hydroxide was added. The white precipitate which formed within 5 min was filtered by suction and washed quickly with 100 ml of a 3:1 mixture of absolute ethyl ether and 95% ethanol. The salt was then washed with 15 ml of ethyl ether and air dried on the filter. The potassium salt was stored at 0°C; even at this temperature it was stable for only 3—4 days. The cupric, manganous, zinc, nickelous, and cobaltous chelates were synthesized in a manner analogous to that described previously for the cupric chelate (20) by combining ethanolic solutions of \( N \)-hydroxy-AAF and the appropriate metal acetate (reagent grade cupric acetate monohydrate, manganous acetate tetrahydrate, cobaltous acetate tetrahydrate, zinc acetate dihydrate, or nickelous acetate tetrahydrate). The precipitates were collected by suction on sintered-glass filter funnels and washed repeatedly with ethanol and ethyl ether. For the ferric chelate 2.1 mmoles...
of reagent-grade anhydrous ferric chloride in 20 ml of absolute ethanol was mixed with 6.3 mmol of the dry potassium salt of N-hydroxy-AAF in 75 ml of absolute ethanol. The resulting deep red solution was taken to dryness in a rotary-flash evaporator at 37°C, and the chelate, collected from the sides of the flask with a rubber policeman, was washed with distilled water until the test for chloride was negative. The chelates were dried in vacuo over calcium chloride and stored in the refrigerator. The chelates were always used within 1 month after preparation.

On titration with titanium trichloride (10, 21) each compound gave values between 97 and 105 % of theoretical. The procedure was exactly as described (21) except that 1 hr, instead of 15 min, was allowed for reduction with titanium trichloride; an asbestos pad was inserted between magnetic stirrer and the flask to prevent heating. The titration values and the elementary analyses for the 5 new chelates are listed in Table 1. These data show that the ferric chelate is a 3:1 complex and the other new chelates are 2:1 complexes between metal ion and N-hydroxy-AAF.

Carcinogenicity studies. —In each of the experiments, albino rats were housed in groups of 4 in raised, screen-bottomed cages and were fed a grain diet and water ad libitum. The composition of the diet per kilogram was as follows: ground yellow corn, 680 gm; linseed oil meal, 160 gm; powdered skim milk, 120 gm; alfalfa leaf meal, 20 gm; corn oil, 10 gm; NaCl (iodized), 5 gm; Ca₃(PO₄)₂, 5 gm; vitamin A acetate 50,000 units, α-tocopherol acetate, 3 mg; vitamin D₃, 20 µg. Male rats from the Holtzman Breeding Laboratories, North Wilmington, Mass., were used for all other experiments.

In the 1st experiment, groups of 20 male rats with initial weights of 90–105 gm received s.c. injections of 3.2 mg of N-hydroxy-AAF or 3.0 mg of AAF in the right hind leg twice weekly for 4 or 8 weeks. In the 2nd and 3rd experiments, groups of 12 female rats with initial weights of 100–120 gm received 3.2 mg of N-hydroxy-AAF s.c. or an equivalent amount of this compound as one of the metal chelates or as the potassium salt. These rats were given 1 injection of a compound into the right hind leg and 4 injections of the same compound into the left hind leg at weekly intervals, except that the N-hydroxy-AAF was injected at only 1 site (4 times). In the 1st experiment the rats which received 16 injections of N-hydroxy-AAF, and in the 2nd experiment the rats which received 4 injections of N-hydroxy-AAF, also received s.c. injections of tricaprylin ( trioctanoin, Eastman Organic Chemicals) in the other hind leg on the same schedule. In the 1st and 3rd experiments groups of control rats received injections of tricaprylin on the same schedule as the other animals in these experiments. The compounds to be injected were ground with a mullite mortar and pestle and suspended without heat in tricaprylin; 0.2 ml was injected per dose. In the 3rd experiment the suspension of the compounds was facilitated by continuous stirring of the suspensions with magnetic stirrers; in the other experiments the suspensions were maintained by forcing the material in and out of the syringe just prior to each injection.

In a 4th experiment, groups of 20 weanling female rats were fed either the regular grain diet, or the same diet with 0.025 % of N-hydroxy-AAF or with an equivalent amount of N-hydroxy-AAF as the cupric chelate. The diets were mixed weekly and stored in the refrigerator; the carcinogens were fed for 4 months.

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TABLE 1
ANALYTICAL VALUES FOR THE METAL DERIVATIVES OF N-HYDROXY-2-ACETYLAMINOPHLORENE (N-HYDROXY-AAF)

<table>
<thead>
<tr>
<th>Compound</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>TITRATION WITH TICL (%)</th>
<th>MELTING (DECOMPOSITION) POINT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(C₁₀H₁₂NO₃)₂</td>
<td>67.42</td>
<td>67.72</td>
<td>4.56</td>
<td>4.83</td>
<td>5.14</td>
</tr>
<tr>
<td>Zn(C₁₀H₁₂NO₃)₂</td>
<td>66.27</td>
<td>66.64</td>
<td>4.25</td>
<td>4.61</td>
<td>5.20</td>
</tr>
<tr>
<td>Ni(C₁₀H₁₂NO₃)₂</td>
<td>67.17</td>
<td>66.47</td>
<td>4.28</td>
<td>4.54</td>
<td>5.13</td>
</tr>
<tr>
<td>Co(C₁₀H₁₂NO₃)₂</td>
<td>67.16</td>
<td>67.19</td>
<td>4.28</td>
<td>4.40</td>
<td>5.12</td>
</tr>
<tr>
<td>Fe(C₁₀H₁₂NO₃)₂</td>
<td>70.10</td>
<td>70.21</td>
<td>4.55</td>
<td>4.89</td>
<td>5.35</td>
</tr>
<tr>
<td>Cu(C₁₀H₁₂NO₃)₂</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KC₁₀H₁₂NO₃</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Where more than one value is given, each figure is for a different preparation.
  b The metal derivatives of N-hydroxy-AAF did not give good melting points. At temperatures above 150°C, each compound charred and finally melted at a temperature which depended on the time the compound had been heated. To obtain minimum decomposition under standard conditions the melting point was determined as the temperature at which the compound melted 30 sec after insertion into a melting-point block which was heated to give a temperature rise of 1°C/min. For this purpose replicate samples of the chelate in melting point capillaries were inserted into the melting-point block at 1°C intervals in the range of the previously determined approximate melting point.
  c Elementary analyses were not made on the cupric chelate, which was characterized earlier (20), or on the potassium salt, which is stable for only a few days.

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* Added as a concentrate which contained 1,000,000 I.U./gm of vitamin A acetate, a-tocopherol acetate, 3 mg; vitamin D₃, 20 µg. Male rats from the Holtzman Rat Company, Madison, Wis., were used for the 1st experiment; female CD non-inbred rats from the Charles River Breeding Laboratories, North Wilmington, Mass., were used for all other experiments.

In the 1st experiment, groups of 20 male rats with initial weights of 90–105 gm received s.c. injections of 3.2 mg of N-hydroxy-AAF or 3.0 mg of AAF in the right hind leg twice weekly for 4 or 8 weeks. In the 2nd and 3rd experiments, groups of 12 female rats with initial weights of 100–120 gm received 3.2 mg of N-hydroxy-AAF s.c. or an equivalent amount of this compound as one of the metal chelates or as the potassium salt. These rats were given 1 injection of a compound into the right hind leg and 4 injections of the same compound into the left hind leg at weekly intervals, except that the N-hydroxy-AAF was injected at only 1 site (4 times). In the 1st experiment the rats which received 16 injections of N-hydroxy-AAF, and in the 2nd experiment the rats which received 4 injections of N-hydroxy-AAF, also received s.c. injections of tricaprylin (trioctanoin, Eastman Organic Chemicals) in the other hind leg on the same schedule. In the 1st and 3rd experiments groups of control rats received injections of tricaprylin on the same schedule as the other animals in these experiments. The compounds to be injected were ground with a mullite mortar and pestle and suspended without heat in tricaprylin; 0.2 ml was injected per dose. In the 3rd experiment the suspension of the compounds was facilitated by continuous stirring of the suspensions with magnetic stirrers; in the other experiments the suspensions were maintained by forcing the material in and out of the syringe just prior to each injection.

In a 4th experiment, groups of 20 weanling female rats were fed either the regular grain diet, or the same diet with 0.025 % of N-hydroxy-AAF or with an equivalent amount of N-hydroxy-AAF as the cupric chelate. The diets were mixed weekly and stored in the refrigerator; the carcinogens were fed for 4 months.
Tumor incidences in male rats given multiple s.c. injections of
N-hydroxy-2-acetylaminofluorene (N-hydroxy-AAF) or AAF

Each rat received twice-weekly s.c. injections of 0.2 ml of tricaprylin alone, or containing 3 mg of AAF or 3.2 mg of N-hydroxy-AAF, in the right hind leg. The group which received 16 injections of N-hydroxy-AAF also received 16 injections of tricaprylin in the left hind leg. The initial weights of the rats were 90-105 gm; the average weights of the rats in the 4 groups were 437-469 gm at 6 months. There were 20 rats/group.

The tumors were recorded every 2-4 weeks, and at autopsy each tumor and any other gross lesion were fixed in neutral 10% formalin, sectioned at 6 µ, and stained with hematoxylin and eosin.

Retention studies.—The compounds to be injected were ground with a mullite mortar and pestle, suspended in tricaprylin with a magnetic stirrer, and injected s.c. into 120-gm female rats in the same manner as for the carcinogenicity studies. Representative animals were killed immediately after the injection and at various intervals thereafter. The skin and fat around the injection site were discarded, the leg was amputated, and the tissue surrounding the injection site (about 5 gm) was excised. The tissue was minced in 5-10 ml of chloroform in the 50 ml stainless steel container of a Lourdes model UM homogenizer and was minced in 5-10 ml of chloroform in the 50 ml stainless steel container of a Lourdes model UM homogenizer and immersed in ice. After removal of the suspension, 5 ml each of methanol and chloroform were added to the container, and the homogenizer was run for an additional 5 min. This material and additional rinsings of the apparatus were added to the original suspension, so that the final preparation contained 35 ml each of methanol and chloroform. After storage overnight at 5°C, hydrogen sulfide was bubbled through the suspension for 10 min to decompose the chelate; the nickelous chelate seemed to be more resistant and was treated for 20 min. Nitrogen was then bubbled through the mixture for 10-15 min to remove much of the hydrogen sulfide, and the suspension was filtered with suction through a sintered glass plate covered with a layer of glass wool. The filtrate and washings were adjusted to a standard volume and stored at -5°C for 24-48 hr; during this time a soft white precipitate formed in extracts from both control animals and those receiving injections. N-Hydroxy-AAF was determined from the ultraviolet absorption of the clear supernatant solution in a Beckman DB spectrophotometer.

Results

Tumor induction by N-hydroxy-AAF with repeated s.c. injections.—While sarcomas have been found only rarely at the sites of 1-6 s.c. injections of 3.2 mg of N-hydroxy-AAF (20), 16 of 20 rats which received 16 such injections over an eight-week period developed sarcomas at the injection site with an average latent period of 6 months (Table 2). With 8 such injections, only 4 of 20 rats developed sarcomas, and the average latent time was 10 months. Approximately one-half of the rats in either group developed ear duct carcinomas, and 3 rats from each of these groups had a mammary carcinoma.

The rats which received 16 injections of N-hydroxy-AAF also received on the same schedule 16 injections of tricaprylin alone in the opposite hind leg. No tumors developed at these injection sites. The injections of tricaprylin were made because Walpole (28) reported the development of sarcomas in rats fed AAF at the sites of injection of carageenin, carboxymethylcellulose, or Tween 60, and Huggins and Grand (12) found sarcomas at the sites of injection of sesame oil in rats fed 3-methylcholanthrene.

No tumors developed in the rats which received 16 injections of tricaprylin alone or 16 injections of AAF in tricaprylin.

Tumor induction by s.c. injection of metal derivatives of N-hydroxy-AAF.—Of the compounds studied, the cobaltous, cupric, ferric, and nickelous chelates of N-hydroxy-AAF induced the highest incidences of sarcomas at the sites of injection (Table 3). In Experiment 2, sarcomas developed at each site which received 4 injections at weekly intervals, and at 42-83% of the sites which received only 1 injection. The average latent time before the sarcomas were first palpable ranged from 3.3 to 5.7 months and was shortest for the cobaltous and cupric chelates. On the other hand, sarcomas developed at only 25-50% of the sites

<table>
<thead>
<tr>
<th>GROUP NO.</th>
<th>COMPOUND</th>
<th>NO. OF INJECTIONS</th>
<th>SARCOMAS AT INJECTION SITE</th>
<th>Mammary carcinomas*</th>
<th>Ear duct carcinomas (12 mo.)</th>
<th>Other tumors</th>
<th>NEGATIVE SURVIVORS (12 mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N-Hydroxy-AAF</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>N-Hydroxy-AAF</td>
<td>16</td>
<td>6</td>
<td>13</td>
<td>16</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>AAF</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Solvent only</td>
<td>16</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

* In addition 1 rat in Group 1 with a mammary carcinoma and 2 rats in Group 2 without mammary carcinomas each bore a single mammary adenoma.

The sarcomas all developed at the sites of injection of N-hydroxy-AAF; no tumors developed at the opposite site which received injections of tricaprylin.

<p>| TUMOR INCIDENCES IN MALE RATS GIVEN MULTIPLE S.C. INJECTIONS OF N-HYDROXY-2-ACETYLAMINOFLUORENE (N-HYDROXY-AAF) OR AAF |
|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|</p>
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</thead>
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<td>8</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<tr>
<td>2</td>
<td>N-Hydroxy-AAF</td>
<td>16</td>
<td>6</td>
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<td>16</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>4</td>
<td>Solvent only</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tbody>
</table>

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TABLE 3
INCIDENCES OF TUMORS IN RATS WHICH RECEIVED S.C. INJECTIONS OF N-HYDROXY-2-ACETYLAMINOFUORENE (N-HYDROXY-AAF) OR ITS METAL DERIVATIVES

Except for Group 8, each rat received s.c. injections once in the right hind leg and 4 times at weekly intervals in the left hind leg of 0.2 ml of tricaprylin alone or containing 3.2 mg of N-hydroxy-AAF as one of the metal derivatives. The rats of Group 8 received 4 injections of tricaprylin in one hind leg and 4 injections of tricaprylin containing 3.2 mg of N-hydroxy-AAF in the other hind leg. There were 12 female rats, initial weight of 100-120 gm, per group. Experiment 2 was terminated at 12 months; Experiment 3 was terminated at 8 months.

<table>
<thead>
<tr>
<th>GROUP NO.</th>
<th>COMPOUND</th>
<th>CUMULATIVE NO. OF RATS WITH SARCOMAS AT SITES OF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 INJECTION</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 MO. 4 MO. 5 MO. 6 MO. 8 MO. 12 MO. 3 MO. 4 MO. 5 MO. 6 MO. 8 MO. 12 MO.</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td>1 Injection</td>
</tr>
<tr>
<td>1</td>
<td>Cobaltous chelate</td>
<td>1 7 8 8 8 8 8 4 12 12 12 12 12 0 0</td>
</tr>
<tr>
<td>2</td>
<td>Cupric chelate</td>
<td>0 4 7 8 10 10 2 7 10 11 12 12 3 (4) 0</td>
</tr>
<tr>
<td>3</td>
<td>Ferric chelate</td>
<td>1 3 4 5 5 5 2 5 7 11 12 12 2 (3) 0</td>
</tr>
<tr>
<td>4</td>
<td>Nickelous chelate</td>
<td>0 0 0 5 5 5 0 0 1 7 12 12 1 1 0</td>
</tr>
<tr>
<td>5</td>
<td>Manganous chelate</td>
<td>1 1 1 1 2 2 2 2 4 5 6 7 (11) 0</td>
</tr>
<tr>
<td>6</td>
<td>Zinc chelate</td>
<td>0 0 1 2 2 2 0 1 2 3 3 4 6 (16) 1</td>
</tr>
<tr>
<td>7</td>
<td>Potassium salt</td>
<td>0 0 0 0 0 0 2 2 2 3 3 3 5 (10) 3</td>
</tr>
<tr>
<td>8</td>
<td>N-Hydroxy-AAF</td>
<td>— — — — — — — 0 0 0 0 0 0 3 (3) 7</td>
</tr>
<tr>
<td>11</td>
<td>Solvent only</td>
<td>— — — — — — — 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td>1 Injection</td>
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<tr>
<td>9</td>
<td>Cobaltous chelate</td>
<td>0 7 8 8 8 — 0 8 11 11 11 — 0 0</td>
</tr>
<tr>
<td>10</td>
<td>Cupric chelate</td>
<td>0 0 2 6 6 — 0 5 10 12 12 — 0 0</td>
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<tr>
<td>11</td>
<td>Nickelous chelate</td>
<td>0 0 0 0 1 — 0 3 7 10 12 — 0 0</td>
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<td>12</td>
<td>Nickelous chelate</td>
<td>0 0 1 2 2 — 0 4 7 11 11 — 1 (1) 0</td>
</tr>
<tr>
<td>13</td>
<td>Solvent only</td>
<td>0 0 0 0 0 — 0 0 0 0 0 0 11</td>
</tr>
</tbody>
</table>

* The numbers in parentheses indicate the total number of mammary carcinomas in the group. In addition 1 rat with 1 mammary adenoma was observed in Groups 1, 3, and 8 and 1 rat with 2 mammary adenomas was found in Group 6.
† The negative survivors (rats without tumors) are tabulated at 12 months for Experiment 2 and at 8 months for Experiment 3.

which received 4 injections of the manganous or zinc chelates or of the potassium salt of N-hydroxy-AAF; no sarcomas developed at the sites injected with N-hydroxy-AAF.

The induction of mammary tumors was generally inversely related to the incidence of sarcomas. Only a few of the rats given the cobaltous, cupric, ferric, or nickelous chelates developed mammary tumors, whereas 5-7 of the 12 rats in each of the groups which received the manganous or zinc chelates or the potassium salt had 1 or more mammary carcinomas at death or by 12 months. Three rats which received 4 injections of N-hydroxy-AAF developed mammary carcinomas; these rats received only 80% as much carcinogen as the rats which received 5 injections of the metal derivatives (4 injections at one site and 1 injection at another).

In Experiment 3, the cobaltous and cupric chelates again induced sarcomas more rapidly than the nickelous chelate, although the difference in latent periods was less marked than in the previous experiment. It was thought that the slower rate of tumor induction by the nickelous chelate in Experiment 2 might have been related to particle size, since this compound forms hard crystals which are rather difficult to powder. However, in Experiment 3 there was no appreciable difference in tumor induction by particles of nickelous chelate similar to those used in Experiment 2 and the same material which had been ground to a finer powder in a mullite mortar.

Relative retention of N-hydroxy-AAF and its metal derivatives at the site of injection.—In confirmation of earlier studies (20), N-hydroxy-AAF was rapidly removed from the injection site, and the potassium salt was removed almost as quickly (Chart 1). By 14 hr, N-hydroxy-AAF injected in either form could not be detected, and the half-retention time of N-hydroxy-AAF injected as such or as the potassium salt had 2-2½ hr. On the other hand, the half-retention time of N-hydroxy-AAF injected as the manganous or zinc chelate was 2 days, and that of N-hydroxy-AAF injected as the ferric or cobaltous chelate was 4-6 days. The cupric and nickelous chelates were mobilized very slowly; 70-80% of the N-hydroxy-AAF was recovered at the injection site after 2 weeks. Extrapolation of the curves gave half-retention times of 35-50 days, and green color, presumably due to these chelates, was sometimes visible at the injection sites on autopsy of the rats several months after the last injection.

Tumor induction by N-hydroxy-AAF and its cupric chelate administered in the diet.—The feeding of N-hydroxy-AAF or its cupric chelate produced the same broad spectrum of tumors as observed earlier for N-hydroxy-AAF under similar conditions (18). However, the incidences were generally lower and the latent periods somewhat longer for the tumors induced by administration of the cupric chelate (Table 4). It was thought that the cupric chelate might be absorbed less readily and that sufficient amounts might pass into the lower gastrointestinal tract to induce tumors.
of the large intestine. No tumors of this type were found. Analysis (2, 19) of pools of urine excreted by groups of 4 rats fed either N-hydroxy-AAF or the cupric chelate during the 10th week showed excretions of 5 and 2.5% respectively, of the dose as N-hydroxy-AAF.

**DISCUSSION**

The greater carcinogenic activities of the metal chelates of N-hydroxy-AAF at the s.c. injection site as compared to that of the parent hydroxamic acid appear to be explained to a large extent by the longer retention of the chelates at the site with a slow release of N-hydroxy-AAF to the surrounding tissue. The compounds which induced the highest incidence of sarcomas at the injection site were maintained for a sufficient time, either continuously, as with certain chelates, or periodically, as with N-hydroxy-AAF. This situation appears to be analogous to recent observations with the polycyclic hydrocarbons. Thus, Shabad et al. (26, 27) found a greatly increased carcinogenic activity of 9,10-dimethyl-1,2-benzanthracene in the rat lung if the hydrocarbon was first immobilized by adsorption on black ink particles; under these conditions the hydrocarbon was retained in the lungs for a longer time. Similarly, Saffiotti et al. (23, 24) found that adsorption of 3,4-benzpyrene and 9,10-dimethyl-1,2-benzanthracene to iron oxide particles greatly increased the persistence and carcinogenicity of these hydrocarbons in the hamster lung after intratracheal injection.

While a possible direct contribution of the metals in the chelates to the observed sarcomagenic activity must be considered, an analysis of the available data on the carcinogenic activities of these metals in various forms does not encourage this viewpoint. Injections (s.c.) of metallic nickel (9, 11), metallic cobalt (7, 8), Ni3S2, NiS, CoO and CoS (5), in doses larger than those used here, have given rise to low to high incidences of sarcomas in the rat. While very large repeated doses of iron dextran are sarcomagenic in the rat (6), metallic sponge iron, dialyzed ferric oxide, and various ferric and ferrous salts have little carcinogenic activity (6). No cupric salt or complex, other than the cupric chelate of N-hydroxy-AAF, has been reported to be sarcomagenic. Injections of CuO, CuS, CuS (5) and cupric acetate (20) have failed to produce tumors in the rat. Large doses of copper dextran, copper porphyrin, and copper phthalocyanine were administered s.c. to mice with negative results (6). No sarcomas have resulted from s.c. injections of zinc dust in the rat (7) or from similar injections of zinc and manganese porphyrin derivatives in the mouse (6).

An obvious explanation for the greater retention of the chelates of N-hydroxy-AAF at the injection site as compared to the parent hydroxamic acid is the lower solubilities of the chelates in lipid and aqueous phases. However, as noted by others (1, 4, 30) serious consideration must also be given to the possible importance of chelation of carcinogens or their metabolites to cellular constituents. Possible structures through which N-hydroxy-AAF could be bound to proteins and nucleic acids through metals are as follows:

Studies with AAF-9,14C, AAF-15N, and N-hydroxy-AAF-9,14C have shown that these carcinogens or their metabolic
derivatives are tightly bound in vivo to these cellular constituents in liver and other tissues. In an analogous fashion certain metal powders, oxides, and salts could concentrate unidentified endogenous exogenous carcinogens on these cellular constituents. These bindings could cleanly interfere with the various mechanisms for the replication, transcription, translation, and control of information from the genome and thus lead to the initiation of neoplasia.

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The Carcinogenic Activities of N-Hydroxy-2-acetylamino-2-fluorene and Its Metal Chelates as a Function of Retention at the Injection Site

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