SUMMARY

The carcinogenicity of seven metabolites of the essential amino acid tryptophan and nine other nitrogen-containing aromatic compounds was studied by the individual suspension of these chemicals in cholesterol pellets and the surgical implantation of the pellets into the bladders of mice. Pellets consisting of the 8-methyl ether of xanthurenic acid, xanthurenic acid, 8-hydroxyquinaldic acid, 3-hydroxy-L-kynurenine, and 3-hydroxyanthranilic acid—all metabolites of tryptophan—and N-hydroxy-acetylaminofluorene, Ar-acetyl-2-naphthylhydroxylamine, and 2-naphthylhydroxylamine suspended in this medium induced a statistically greater number of bladder carcinomas than did cholesterol pellets alone. Pellets of 4,8-quinolinediol, kynurenic acid, 8-hydroxyquinoline-N-oxide, 2,8-quinolinediol, 2,6-quinolinediol, 2-naphthylacetamide, 2-nitronaphthalene, and 2-amino-1-naphthol hydrochloride mixed with cholesterol were not significantly more active than were the pellets of cholesterol. The significance of the data is discussed and related to the abnormalities of tryptophan metabolism observed in some patients with urinary bladder cancer.

The suggestion (9) that the abnormal metabolism of the essential amino acid tryptophan in some patients with urinary bladder cancer might be of causal significance in this disease has received considerable experimental support. Dunning, Curtis, and Maun (16) demonstrated a high incidence of bladder tumors in rats fed DL-tryptophan and 2-acetylaminofluorene, whereas no bladder tumors were present in those animals fed only supplemental 2-acetylaminofluorene. Brown et al. (8, 9) reported the excretion of significantly elevated quantities of kynurenine, 3-hydroxykynurenine, acetylkynurenine, and kynurenic acid in the urine of about one-half of a group of patients with spontaneous bladder cancer. Boyland and Williams (7) observed abnormally large quantities of 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and anthranilic acid in the urine of ten patients with bladder carcinomas, and Abul-Fadl and Khalafallah (1) found that 3-hydroxyanthranilic acid occurred in the urine of patients with bilharzial bladder cancer in 4 times the concentration that it was present in the urine of control subjects. However, patients with industrial bladder cancer excreted normal quantities of urinary tryptophan metabolites (28).

Allen et al. (2) reported that 3-hydroxy-DL-kynurenine, 3-hydroxyanthranilic acid, and 2-amino-3-hydroxyacetophenone were carcinogenic for the mouse bladder when suspended in cholesterol pellets and surgically implanted into this organ. The combined incidence of benign and malignant tumors present in groups composed of small numbers of animals was used to arrive at these conclusions. Clayson et al. (14) were unable to demonstrate a significant number of malignant bladder tumors following implantation of 3-hydroxyanthranilic acid suspended in cholesterol. Attempts to induce a significant incidence of mouse bladder carcinomas with metabolites of tryptophan suspended in paraffin wax (2, 12) by the pellet implantation technique (23) were not successful. The in vivo evaluation of the rate of elution of several tryptophan metabolites from a cholesterol vehicle (10) demonstrated that these compounds were leached out of this vehicle much more rapidly than they...
were from paraffin wax (12). Because this more rapid rate of exposure of the bladder mucosa to the test chemical might be more favorable to the induction of bladder neoplasm in this test system, and because it is considered essential to ascertain whether these naturally occurring urinary metabolites possess oncogenic activity for the urinary bladder, it was decided to test a series of tryptophan metabolites and several other aromatic nitrogen-containing compounds, for which elution data were available (10), for their mouse bladder carcinogenicity. These experiments are the subject of this report.

MATERIALS AND METHODS

The preparation of the chemicals (10, 12) and the cholesterol pellets containing the test compounds (10) was previously described. The details of the animal selection and care and of the surgical technic (2, 23) were presented (11).

Carcinogenicity studies.—Three separate experiments (Table 1) involving different groupings of the sixteen test compounds and cholesterol were initiated. Pellets composed of cholesterol alone and pellets containing 2-nitronaphthalene were included in all three experiments. N-Hydroxy-2-acetylaminofluorene, N-acetyl-2-naphthylhydroxyxylamine, 2-naphthylhydroxylamine, 2-amino-1-naphthol hydrochloride, and 2-naphthylacetamide were tested in Experiments 1 and 2. 2,6-Quinolinediol, 2,8-quinolinediol, 8-hydroxyquinoline-N-oxide, 3-hydroxy-1-kynurenine, 8-hydroxyxanthranilic acid, xanthurenic acid, 8-hydroxyquinalidic acid, the 8-methyl ether of xanthurenic acid, 4,8-quinolinediol, and kynurenic acid were tested in Experiments 2 and 3. A group of animals in which a sham operation was performed, consisting of (a) incision of the bladder, (b) momentary insertion of a pellet of pure cholesterol, (c) removal of the cholesterol pellet, and (d) closure of the bladder and suture of the abdomen, was included in Experiment 1. Six months elapsed between the completion of the surgical procedures on the animals included in Experiment 1 and the initiation of Experiment 2, and 3 months passed between completion of the surgical procedures on the animals included in Experiment 2 and the beginning of Experiment 3.

The following protocol was established prior to the initiation of Experiment 1 and was utilized with the three separate experiments. A sufficiently large number of mice would be subjected to the surgical insertion of pellets containing either cholesterol alone or one additional compound mixed with cholesterol so that a minimum of 30 animals would survive longer than 175 days. The animals whose bladders contained pellets of the same composition would be referred to as a group, and each compound would be tested at least twice in separate experiments. A negative control group of mice, given implants of pellets of pure cholesterol, and a positive control group of mice, given pellets of 2-amino-1-naphthol hydrochloride, a bladder carcinogen for the mouse when implanted in paraffin wax pellets (4), were to be included in every experiment. However, because of the inability to obtain a large enough number of survivors in the group exposed to 2-amino-1-naphthol hydrochloride in Experiment 2 (Table 1), and because of a scarcity of many of the other compounds, 2-nitronaphthalene was selected as the compound to be tested in the three experiments. The animals within a group were allowed to survive until 455–490 days following surgery, or until approximately fifteen animals were alive in any one group. Only animals surviving more than 175 days were to be evaluated for the presence of bladder carcinoma.

Pathological examination.—At autopsy the bladders were distended with Bouin’s fixative inserted through the urethra. The bladders were bisected in the mid-sagittal line and inspected grossly, sectioned at 5 μ, and stained with hematoxylin and eosin. We are indebted to Mrs. Betty Decker and Mr. Jerald Armstrong for the preparation of these sections and to Dr. A. M. Pamukcu, Professor of Pathological Anatomy, Faculty of Veterinary Medicine, University of Ankara, Turkey, for the histological study and review of many of these sections.

The bladders were inspected for the presence of squamous metaplasia, benign tumors, and carcinomas and were evaluated according to the criteria of Bonser and Jull (5). Carcinomas which were not observed to invade muscle were classified as Grade I; those which were seen to invade muscle or which demonstrated other evidence of malignancy such as local metastases were classified as Grade II. Those bladders containing a benign and malignant tumor or harboring two or more carcinomas were counted as having only one malignant tumor. Only the total incidence of carcinomas was used to assess carcinogenicity. Statistical comparison of the incidence of carcinomas related to the introduction of pellets of cholesterol containing a test chemical was made with the cholesterol alone group, and probabilities of statistical significance were computed by the exact method for 2 × 2 tables (19).

RESULTS

The number of animals subjected to the surgical implantation of pellets, the distribution of the deaths of the animals for 75-day periods beginning 175 days after surgery, the total number of animals surviving a minimum of 175 days following surgery, and the average survival of the animals in a group are tabulated in Table 1 for Experiments 1, 2, and 3. Thirty-eight, 33, and 36 per cent of the animals in Experiments 1, 2, and 3 that did not live for 175 days following surgery died within 30 days owing to impaction of the pellets into the urethra. A few animals were lost because of cannibalism. The average survival of the individual groups of animals ranged from 307 days (2-amino-1-naphthol hydrochloride, Experiment 2) to 489 days (cholesterol alone, Experiment 1). Those animals that were exposed to test chemicals and that demonstrated a significantly greater incidence of bladder carcinomas than cholesterol alone (Table 2) lived, on the average, fewer days than did the cholesterol control groups. All animals living longer than 175 days with pellets in their bladders survived an average of 410 days. The total number of animals whose bladders could be evaluated in any one group varied from eight (2-amino-1-naphthol hydrochloride, Experiment 2) to 50 (3-hydroxyxanthranilic acid, Experiment 3). In most groups at least
## Table 1

Survival of Mice Living More Than 175 Days Following Bladder Implantation, and Incidence of Changes in Mouse Bladders with Implants of Compounds Suspended in Cholesterol

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experiment No.</th>
<th>No. Mice Given Implants</th>
<th>No. Mice which Died or were killed (Days)</th>
<th>Average Survival (Days)</th>
<th>Squamous Metaplasia</th>
<th>Benign Tumor</th>
<th>Carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1</td>
<td>38</td>
<td>0 7 3 5 11 26</td>
<td>417 0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cholesterol alone</td>
<td>2</td>
<td>82</td>
<td>1 3 5 1 22 32</td>
<td>489 0</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>72</td>
<td>0 4 1 16 21</td>
<td>411 0</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>N-Hydroxy-2-acetylaminofluorene</td>
<td>1</td>
<td>66</td>
<td>1 1 6 14 22</td>
<td>399 3</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68</td>
<td>2 3 8 19 34</td>
<td>461 2</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>N-Acetyl-2-naphthylhydroxylamine</td>
<td>1</td>
<td>72</td>
<td>0 4 3 9 14 30</td>
<td>461 2</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47</td>
<td>2 5 15 22</td>
<td>330 0</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2-Naphthylhydroxylamine</td>
<td>1</td>
<td>56</td>
<td>4 2 4 15 25</td>
<td>366 2</td>
<td>3</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>112</td>
<td>2 3 4 10 8 27</td>
<td>411 1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2-Naphthylacetamide</td>
<td>1</td>
<td>60</td>
<td>2 4 4 18 30</td>
<td>434 4</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>59</td>
<td>1 0 4 6 14 25</td>
<td>438 2</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2-Amino-1-naphthol-HCl</td>
<td>1</td>
<td>78</td>
<td>3 2 6 14 25</td>
<td>392 5</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>111</td>
<td>4 1 0 3 8</td>
<td>307 0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2-Nitronaphthalene</td>
<td>1</td>
<td>80</td>
<td>0 4 4 5 17 30</td>
<td>467 2</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>76</td>
<td>0 1 9 4 19 33</td>
<td>457 0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>41</td>
<td>0 2 0 13 15</td>
<td>413 0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2,6-Quinolinediol</td>
<td>1</td>
<td>77</td>
<td>1 2 9 12 25</td>
<td>438 0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>72</td>
<td>5 0 2 15 22</td>
<td>373 1</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2,8-Quinolinediol</td>
<td>1</td>
<td>82</td>
<td>2 1 5 13 11 32</td>
<td>420 0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>75</td>
<td>0 0 5 15 17 37</td>
<td>454 0</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8-Hydroxyquinoline-N-oxide</td>
<td>1</td>
<td>75</td>
<td>1 8 5 8 10 32</td>
<td>401 0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>89</td>
<td>2 5 3 9 49 49</td>
<td>428 0</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>3-Hydroxy-L-kynurenine</td>
<td>2</td>
<td>97</td>
<td>1 0 13 23 23</td>
<td>386 1</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>58</td>
<td>1 2 1 17 17</td>
<td>382 2</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3-Hydroxyanthranilic acid</td>
<td>2</td>
<td>59</td>
<td>1 3 3 18 28</td>
<td>454 1</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>89</td>
<td>2 5 3 40 50</td>
<td>412 6</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>2</td>
<td>88</td>
<td>4 3 5 10 32</td>
<td>399 1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>90</td>
<td>2 4 17 23 23</td>
<td>335 1</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8-Hydroxyquinaldic acid</td>
<td>2</td>
<td>87</td>
<td>1 2 10 26 26</td>
<td>443 2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>92</td>
<td>3 2 18 26 26</td>
<td>374 0</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8-Methyl ether of xanthurenic acid</td>
<td>2</td>
<td>91</td>
<td>5 3 4 15 33</td>
<td>398 1</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>92</td>
<td>0 2 5 21 21</td>
<td>417 1</td>
<td>0</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>4,8-Quinolinediol</td>
<td>2</td>
<td>84</td>
<td>0 4 1 29 34</td>
<td>430 1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>94</td>
<td>3 4 13 23 23</td>
<td>367 0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>2</td>
<td>84</td>
<td>2 2 4 16 24</td>
<td>415 0</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>101</td>
<td>4 2 34 44 44</td>
<td>408 0</td>
<td>4</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
twenty animals survived long enough to have their bladders subjected to microscopic inspection.

The incidence of microscopic changes in the mouse bladders implanted with compounds suspended in cholesterol is listed in Table 1. No bladder carcinomas were observed in the group of animals in which a sham operation was performed. The incidence of carcinomas noted for the replicate groups of animals was in some cases strikingly uniform—e.g., N-acetyl-2-naphthylhydroxylamine (6/30—20 per cent, 6/22—27 per cent), 2-naphthylacetamide (5/30—17 per cent, 4/25—16 per cent), 3-hydroxy-L-kynurenine (6/23—26 per cent, 6/17—35 per cent). However, in other cases the duplication of tumor incidences among replicate groups of animals was poor—e.g., 3-hydroxyanthranilic acid (2/28—7 per cent, 15/50—30 per cent), 2,8-quinolinediol (0/32—0 per cent, 8/37—22 per cent), 2-nitronaphthalene (7/30—23 per cent, 2/33—6 per cent, 2/15—13 per cent). Since most of the bladders of the mice were inspected after the mice had survived a definite period of time, no data were collected to determine the average time at which carcinomas were noted. Some carcinomas were observed in the bladders of animals that died prior to the termination of the experiment and those compounds that were associated with a significant incidence of carcinomas (Table 2) were also associated with the early appearance of carcinomas—e.g., 3-hydroxy-L-kynurenine, 185 days; 2-naphthylhydroxylamine, 214 days; N-acetyl-2-naphthylhydroxylamine, 301 days; and the 8-methyl ether of xanthurenic acid, 313 days following surgery.

The group demonstrating the greatest number of benign tumors was the cholesterol alone group in Experiment 1. However, this group on the average survived longer than any other group, and most of the benign lesions were observed in the bladders of the oldest mice in this group. Other groups showed a comparably low incidence of benign tumors which did not parallel the distribution of carcinomas. A small number of bladders showed mucosal squamous metaplasia.

The incidence of carcinomas and the probabilities of statistical significance are summarized for the combined, replicate groups of animals in Table 2. Only animals surviving more than 175 days have been included in this evaluation in order that the results obtained may be compared to the incidence of these lesions reported by other workers (2-4, 12, 14). Seven carcinomas were found in the bladders of 87 mice exposed to the presence of cholesterol alone. This represents the first demonstration of the bladder carcinogenicity of the 8-methyl ether of xanththurenic acid, the natural L-isomer of 3-hydroxykynurenine, xanthurenic acid, and 8-hydroxyquinclidic acid. 3-Hydroxy-DL-kynurenine and 3-hydroxyanthranilic acid were found to produce a highly significant incidence, whereas xanthurenic acid and 8-hydroxyquinclidic acid were noted to induce a significant incidence of bladder carcinomas when compared with cholesterol alone. This represents the first demonstration of the bladder carcinogenicity of the 8-methyl ether of xanththurenic acid, the natural L-isomer of 3-hydroxykynurenine, xanthurenic acid, and 8-hydroxyquinclidic acid. 3-Hydroxy-DL-kynurenine and 3-hydroxyanthranilic acid were reported by Allen et al. (2) to be active as bladder carcinogens on the basis of the number of both benign and malignant lesions observed. 4,8-Quinolinediol and kynurenine acid were inactive as mouse bladder carcinogens in the present studies.

Three substituted quinoline compounds were included in this study for comparison with the naturally occurring quinoline metabolites of tryptophan. 2,6-Quinolinediol, 2,8-quinolinediol, and 8-hydroxyquinoline-N-oxide were not found to be active as bladder carcinogens.

**DISCUSSION**

A low incidence of 8 per cent carcinomas was found when pellets of cholesterol alone were implanted into mouse bladders. This incidence is comparable to the 6.5 (3) and...
9.0 (14) per cent of bladder carcinomas observed with this
vehicle by other workers. Hieger and Orr (21) demon-
strated the weak sarcomagenic properties of cholesterol
following repeated subcutaneous injections into mice.
Thus, though cholesterol possesses the advantages of being
readily compressed into pellets without heating and of
allowing the test compounds to diffuse into the urine at a
fairly rapid rate (10), the low but constant incidence of
bladder carcinomas induced by this vehicle precludes the
choice of this substance as an ideal suspending media.
However, no known vehicle is free from this objection, since
pellets made of molten (4) or crushed paraffin (3) wax or
stearic acid (3) alone, inserted into mouse bladders, also
induced a small number of vesical carcinomas. The mech-
anism of the induction of bladder carcinomas by these
three vehicles alone, paraffin, stearic acid, and cholesterol,
is unknown.

It is possible that chemical decomposition of the chole-
sterol in the pellets occurs in situ in the bladder, giving rise
to small amounts of relatively strong carcinogens or large
quantities of relatively weak carcinogens. If this decom-
position were effected only by the urine—i.e., if the added
presence of the test chemical did not influence this hypo-
thetical alteration of the cholesterol, then one would expect
to observe the same incidence of tumors from group to
group. Clearly, this is not the case, since some groups of
animals had a significantly greater number of bladder car-
cinomas than the cholesterol alone group (Table 2). Fur-
thermore, no spectrophotometric or chromatographic
evidence of the interaction of organic urinary constituents
with cholesterol pellets could be detected (10). Alter-
natively, it is possible that the presence of high concen-
trations of test chemicals in the cholesterol might facilitate
the chemical decomposition of this substance. Under
these circumstances it might be anticipated that the
chemical alteration of the cholesterol would be associated
with a simultaneous chemical alteration of the test chemi-
cal, because few organic chemical reactions are known
where the interacting chemical species do not both
undergo chemical alteration, with the possible exception of
organic catalysts. It appeared probable that some of the
more reactive and labile test compounds would demon-
strate evidence of decomposition that might be detected
with the methods used. Yet for most of the compounds
no evidence could be found, by chromatographic or spec-
trophotometric methods of analysis, for the chemical al-
teration of the test chemicals either before or after their
implantation into the mouse bladders (10). On the other
hand, the obviously extensive decomposition of 2-amino-1-
naphthol hydrochloride in the cholesterol pellets implanted
into the bladders of mice (10) was not associated with a
significant incidence of neoplasms. Thus, there does not
appear to be good evidence for a relationship between de-
composition of the chemicals in the pellets and the inci-
dence of carcinomas obtained.

The biological data indicate that the test compound
plays a major role in the induction of the bladder carcino-
mas observed and that the cholesterol vehicle plays a
minor, if any, part in this process. 20-Methylchol-
anthrene administered locally to the skin (30), subcutaneous
tissues, mammary gland, striated muscle, peritoneum,
spleen, lung, uterus, thyroid gland, kidney, liver (18),
prostate gland (22), and a number of other organs in a
variety of animals (20) induced a high incidence of malig-
nant tumors. Chapman (13) reported that this compound
suspended in mineral oil induced 70–80 per cent bladder
carcinomas when the bladder was implanted as a subcuta-
neous bladder cyst. The additional presence of a paraffin
foreign body within the cyst did not have any effect on the
incidence or the time of appearance of these transplantable
tumors. Bonser and co-workers (3) demonstrated a 49
and 58 per cent incidence of bladder carcinomas following
the introduction of paraffin wax pellets containing 20-
methylenecholanthrene into mouse bladders. Though this
experiment was terminated after 40 weeks, it appears that
the criticism (13, 26) that the yield of carcinomas obtained
by this technic is low with potent carcinogens is no longer
a serious one.

2-Naphthylhydroxylamine was demonstrated by Boy-
land et al. (6) to induce a high incidence of peritoneal
tsarcomas following repeated intraperitoneal injections in
rats. This compound has been shown concurrently and
independently by three different groups of workers to
induce a significant incidence of 21 (3), 29, and 33 (3) per
cent bladder carcinomas when suspended in pellets of
crushed paraffin wax (3), cholesterol (reported here), and
stearic acid (3), respectively. It was reported (3) that the
stearic acid pellets containing this compound disintegrated
after 2–3 weeks in situ. Thus, the period of exposure of
the bladder to either the test chemical or the vehicle was
less than 10 per cent of the time that the test animals were
observed. One-half of the 2-naphthylhydroxylamine
suspended in the cholesterol pellets disappeared in situ
after 54 days (10), and, therefore, the exposure of the
bladder mucosa to this compound was longer when sus-
pended in cholesterol than when suspended in stearic acid.
It is not known how long this compound remained in the
crushed paraffin vehicle. It is difficult to conceive of a
common carcinogenic decomposition product resulting
from the interaction of 2-naphthylhydroxylamine with
cholesterol, paraffin, and stearic acid. The only common
denominator in the three independent experiments was
the presence in pellets of 2-naphthylhydroxylamine, and
one must conclude that this compound played a most im-
portant role in the genesis of the bladder carcinomas that
were observed in significant numbers. 2-Naphthylhy-
droxylamine has been isolated as a urinary metabolite of
2-naphthylamine in man and the dog (32), and it is possible
that it may be a proximate carcinogen of this industrially
hazardous compound.

N-Hydroxy-2-acetylaminofluorene was reported by
Miller, Miller, and Hartmann (25) as capable of inducing
a wide variety of malignant tumors in the rat when admin-
istered by injection or in the diet, and was suggested to be
a proximate carcinogen of 2-acetylaminofluorene. N-Hy-
droxyl-2-acetylaminofluorene also induced a significant
number of bladder carcinomas when tested in cholesterol.
2-Aminofluorene, 2-amino-1-fluorenol, 2-amino-3-fluorenol,
and 2-amino-7-fluorenol were inactive as bladder carcino-
gens when implanted in cholesterol (3, 14) and paraffin
(3, 24), whereas 2-amino-5-fluorenol was inactive when
suspended in paraffin (24). All these fluorene derivatives
have been found to be urinary metabolites of 2-acetylaminofluorene when this carcinogen is administered systemically to the rat (25, 33, 34). N-Acetyl-2-naphthylhydroxylamine was the third N-hydroxy derivative to demonstrate bladder carcinogenicity when suspended in cholesterol. This compound was demonstrated as a urinary metabolite of 2-naphthylamine in man (31). It is possible that the N-hydroxy amine metabolites of aromatic amines may be potent bladder carcinogens if presented to the bladder mucosa in sufficiently high concentration.

3-Hydroxyxanthanilic acid, a urinary metabolite of tryptophan, was shown capable of inducing a high incidence of myelogenous leukemia and one bladder carcinoma when administered by repeated injection to mice (17). The data obtained in the present experiments indicate that certain metabolites of tryptophan including the 8-methyl ether of xanthurenic acid, 3-hydroxy-L-kynurenine, 3-hydroxyxanthanilic acid, xanthurenic acid, and 8-hydroxyquinaldic acid are as potent as mouse bladder carcinogens in this test system as are some of the carcinogenic metabolites of 2-naphthylamine and 2-acetylaminofluorene. When considered in connection with the reports of altered excretion patterns of tryptophan metabolites observed to occur in about one-half of a group of patients with spontaneous bladder cancer (8, 9, 28, 29) and the elevated levels of excretion of 3-hydroxyxanthanilic acid by patients with bilharzial bladder cancer (1), the data on the incidence of carcinomas induced in mouse bladders with these tryptophan metabolites take on added interest. Furthermore, patients with industrial bladder tumors excreted normal quantities of these metabolites (28). If the increased level of urinary tryptophan metabolites excreted by patients with spontaneous bladder tumors (7–9, 29) was the result of the disease, patients with the clinically and histologically similar industrial lesions might be expected to excrete similar quantities of these same metabolites (28). In addition, data have been presented (11, 27) suggesting that Turkish cows with spontaneous bladder carcinomas have quantitative abnormalities in the urinary excretion of tryptophan metabolites. The observations reported in this study provide supporting circumstantial evidence suggesting that the urinary excretion of elevated quantities of some of these, or possibly related, metabolites may be of causal significance in the etiology of human and bovine bladder cancer.

A comparison of the 50 per cent elution time (T1/2) (10) and the incidence observed for the carcinogenicity of the significantly active and inactive compounds is presented in Table 2. The most active compound, the 8-methyl ether of xanthurenic acid, was also the one most rapidly eluted from the cholesterol pellets. On the other hand, N-hydroxy-2-acetylaminofluorene was eluted from the pellets more slowly than any other compound; yet it too induced a significant incidence of bladder carcinomas. 3-Hydroxy-L-kynurenine, 3-hydroxyxanthanilic acid, and 8-hydroxyquinaldic acid were active carcinogens and were eluted with about the same T1/2 value as 4,5-quinolinediol and kynurenic acid, which were not significantly active. In a similar manner, N-acetyl-2-naphthylhydroxylamine and 8-hydroxyquinoline-N-oxide were eluted at a comparable rate, yet the former was active as a carcinogen whereas the latter was not. It appears, contrary to an earlier suggestion (23), that prolonged, uniform exposure of the bladder to the test compound is not a prerequisite for the induction of a significant number of bladder carcinomas, at least when cholesterol and stearic acid (3) are used as the vehicles. It is possible that the tumor incidence might be significantly altered by a change of vehicle with an associated alteration of the elution half-life.

2-Amino-1-naphthol hydrochloride failed to induce bladder tumors when suspended in cholesterol. The apparent decomposition of this compound when suspended in cholesterol and exposed to urine has been described (10) and may have influenced the low incidence of tumors observed. The carcinogenicity (4) of this compound for the urinary bladder of the mouse has again been reported (3) when suspended in crushed paraffin pellets made by compression.

The technic of implantation into mouse bladders of vehicles containing test compounds as an assay system for the carcinogenicity of urinary substances is of value. It requires the evaluation of large numbers of animals that are exposed to the same compound and are observed for prolonged periods of time. Statistical methods of analysis must be used to assess results, and it seems most reasonable to use the number of carcinomas as the sole criterion of activity of a compound. Little is known about the many factors that must play a role in the induction of tumors by this method. However, some estimate of the probable dose of compound and the duration of exposure of the bladder to the chemical can be obtained by a study of the in vivo elution of chemicals from the pellets.

ACKNOWLEDGMENTS

The assistance of Mr. Carl Morris, Mr. John Drye, Miss Norma Yess, Mrs. Betty Decker, Mrs. Paula White, and Mrs. Mary Bryan with the surgical procedure was greatly appreciated. Mr. Anthony Fuss and Miss Sharon Boehm provided valuable help with the inspection, care, and maintenance of the animals.

REFERENCES

Mouse Bladder Carcinogenicity of Certain Tryptophan Metabolites and Other Aromatic Nitrogen Compounds Suspended in Cholesterol

George T. Bryan, R. R. Brown and J. M. Price


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/24/4_Part_1/596

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.