Respiratory Tract Tumors in Hamsters after Intratracheal Instillations of Benzo(a)pyrene Alone and with Furfural

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SUMMARY

Three groups of young adult Syrian golden hamsters, each consisting of 35 males and 35 females, received 36 weekly intratracheal instillations of furfural, benzo(a)pyrene (BP), and BP + furfural, respectively. The control group, which consisted of 35 males, received 0.9% NaCl solution but was otherwise similarly treated. For some interim information, 3 males from the control group and 3 males and 3 females from each of the other groups were killed and autopsied at Week 30. The experiment was terminated after 78 weeks.

BP alone (suspended in 0.9% NaCl solution) induced respiratory tumors in 41 out of the 62 hamsters examined. Squamous cell carcinoma of the trachea was the most frequent type of tumor observed.

In comparison with treatment with BP alone, intratracheal instillation of BP + furfural resulted in earlier development of metaplastic changes of the tracheobronchial epithelium, a shorter latent period for tracheobronchial tumors, and a few more bronchial and peripheral squamous cell carcinomas. These results suggest a cocarcinogenic effect of furfural on the respiratory tract of hamsters.

Peritracheal sarcomas were observed in 2 hamsters (3%) treated with BP alone. An augmenting effect of furfural on the induction of peritracheal sarcomas was apparent from the fairly high incidence of these tumors (33%) occurring in the group treated with BP + furfural.

There was no indication that furfural possesses carcinogenic activity of its own.

INTRODUCTION

In the course of analytical investigations by the aroma research department of our institute, several reactive dicarbonyl compounds (among others succinic dialdehyde and levulinic aldehyde) have been detected in wood smoke. These compounds are capable of reacting with the NH₂ group of amino acids or proteins, thereby yielding brown pigments as reaction products. This browning reaction was used to detect similar reactive carbonyl compounds in cigarette smoke. Although several gas chromatographic fractions showed a positive browning reaction with glycine, the highly reactive dicarbonyl compounds present in wood smoke could not be detected in condensates of cigarette smoke. Furfural, a monocarbonyl compound, appeared to be the main component responsible for the browning reaction of cigarette smoke (P. J. Groenen, unpublished data). Like acetaldehyde and acrolein, it is present in cigarette smoke in relatively large quantities varying from 45 to 110 µg in the vapor phase of the smoke of 1 cigarette (9). As an aldehyde furfural may remove free tissue thiols, which are known to have a protective function against the lethal effects of certain chemical and physical carcinogens (1, 15) and, moreover, have been tentatively suggested to protect the bronchial epithelium against the attack by carcinogens (4, 10).

BP, which is present in cigarette smoke, has been adequately shown to be carcinogenic for the respiratory tract of several animal species (3, 8, 11, 12, 17, 18, 22). The carcinogenic response, however, is dependent on physical and physicochemical factors such as penetration, retention, and particle size of the carcinogen and also on the characteristics of the experimental animal (7, 16, 20, 22—25). A very high incidence of tracheobronchial tumors was obtained in Syrian golden hamsters by Saffiotti et al. (22) by applying repeated intratracheal instillations of a suspension of fine crystalline BP particles attached to hematite as a carrier dust. Their success was ascribed to adequate penetration of BP into the pulmonary tissue resulting in a sufficiently effective dose at the target site (20, 22). Inadequate penetration or rapid elimination very probably explains the failure of experiments in which BP was administered by either inhalation or intratracheal instillation without the use of a carrier dust (20).

However, intratracheal administration of BP alone (in 0.9% NaCl solution) might be suitable to study cocarcinogenic factors in respiratory tract carcinogenesis, because, theoretically, it would minimize the risk of inducing so many respiratory tumors that augmentation cannot be detected.

The purpose of the experiment described in this report was to examine whether furfural might be of significance for the genesis of lung cancer by acting either as a complete carcinogen or as a cocarcinogenic agent. As a test of the latter possibility, furfural and BP were allowed to act concurrently on the respiratory tract of hamsters for a relatively long period.

MATERIALS AND METHODS

Solutions and Suspensions. Furfural (Schuchardt, Munich, Germany) was purified by distillation, and its purity was

1 This work was supported by a grant from the research fund of the Scientific Advisory Committee Smoking and Health; this fund has been established by the Dutch Cigarette Industry Foundation.

2 The abbreviation used is: BP, benzo(a)pyrene.
checked by gas-liquid chromatography. A 1.5% solution of furfural in 0.9% NaCl solution (v/v) was stored at −20° in quantities sufficient for 1 series of instillations.

BP was used as supplied by Fluka, A. G., Buchs, S. G., Switzerland; its purity was checked by thin-layer chromatography and appeared to be higher than 99%. It was ground in a glazed mortar for about 0.5 hr, after which a 0.5% BP suspension in 0.9% NaCl (w/v) or in 1.5% furfural was prepared by ultrasonic vibration (5 to 10 min at an output frequency of 50 kcqs modulated at 100 cps rate and an output power of 200 watts; Kerry’s ultrasonic generator, Type KS 200, and cleaning bath, Type KS 201 (Kerry Ultrasonics Ltd., Hitchin, Herts., England). The suspensions were stored at −20° in quantities sufficient for 1 group treatment. Just prior to use, they were again vibrated ultrasonically for 2 to 3 min. The suspended BP particles appeared microscopically to vary considerably in size. The majority of the crystals was smaller than 10 μm, but larger particles (up to 80 μm) were also present. The particle size distribution by weight, which was determined by photosedimentation, was 90% < 50 μm, 72% < 30 μm, 53% < 20 μm, 19% < 10 μm, 3.4% < 5 μm.

Attempts to prepare a more concentrated suspension of BP in 0.9% NaCl solution (e.g., 1 or 1.5% BP), without the use of a carrier dust or a surface-active agent, were unsuccessful because the carcinogen was found floating or appeared to precipitate as large aggregates of particles. A suspension considered acceptable was obtained only when the BP concentration did not exceed 0.5% (w/v).

The theoretical weight of BP in each instilled dose was 1 mg (in 0.2 ml of solution). The actual BP content determined in several series of representative samples ranged from 70 to 120%. Observations on the amount of BP retained in the lung after a single instillation of BP suspended in 0.9% NaCl solution showed the following BP recovery percentages: 40 to 50% 1 day after the administration, 10 to 20% after 3 days, and 2 to 4% after 8 days.

The 0.9% NaCl solution (sterile, nonpyrogenic) was obtained from Baxter Laboratories, Inc., A. Christiaens N.V., Brussels, Belgium.

Animals. Randomly bred male and female Syrian golden hamsters were used in this experiment. The animals were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. They were separated by sex and housed in a suspended type of metal screen-bottom cages in sets of 5 in a temperature-controlled room at 24°. The animals were fed a pelleted stock diet and water ad libitum. They were 10 weeks old at the beginning of the experiment.

Procedures. Three test groups of hamsters, each consisting of 35 males and 35 females, and 1 control group consisting of 35 males received a course of 36 weekly intratracheal instillations. For the control animals (Group 1), each instillation consisted of 0.2 ml of 0.9% NaCl solution. The animals of the test groups received weekly either 0.2 ml of 1.5% furfural in 0.9% NaCl solution (Group 2), 0.2 ml of 0.5% BP in 0.9% NaCl solution (Group 3), or 0.2 ml of 0.5% BP in 1.5% furfural in 0.9% NaCl solution (Group 4). Before each treatment, the animals were anesthetized with 0.3 to 0.4 ml of a 1% solution of Brietal sodium [sodium a-dl-1-methyl-5-allyl-5-(1-methyl-2-pentyl) barbiturate, Lilly & Co., Indianapolis, Ind.] injected i.p. After about 4 months, however, it grew progressively more difficult to choose the correct dose of Brietal sodium for each animal, and a few hamsters died during treatment. Thereafter we used a light anesthesia with freshly distilled ether, which was satisfactory and moreover much less time consuming. The instillation procedure was not essentially different from that described in detail by Saffiotti et al. (22).

The hamsters were checked daily and weighed individually once per week during the first month and at 4-week intervals thereafter. After an experimental period of 30 weeks, 3 males of the control group and 3 males and 3 females randomly taken out of each of the test groups were killed and autopsied to obtain some interim information. The experiment was terminated after 78 weeks when nearly all hamsters treated with BP or BP + furfural (Groups 3 and 4) had died spontaneously or had been killed because of their poor condition. Autopsies were performed on all animals, except a few lost through cannibalism. For killing the animals and removal of the lungs at autopsy, the technique described by Saffiotti et al. (22) was used. Following fixation in 10% neutral buffered formalin, the pulmonary lobes were separated, and dehydration in alcohol was done under vacuum until all air was removed from the specimens. The trachea with stem bronchi, pulmonary lobes, liver, kidneys, and any other organs showing gross pathology were processed through paraffin, sectioned at 5 μm (trachea with bronchi and pulmonary lobes at 3 levels), and routinely stained with hematoxylin and eosin. Special stains applied when indicated included periodic acid-Schiff, Alcian blue, Azan, Van Gieson, Gomori's method for reticulin fibers, and Kreyberg's method for keratin and mucin.

RESULTS

The average body weights and survival rates of the animals are given in Table 1. The average weights of the males in Group 4 (BP + furfural) were lower than those of males in the other groups; the differences with the controls were statistically significant from Week 16 onwards. The hamsters treated with BP + furfural also showed the lowest survival rate, while the life-span of the animals treated with BP alone was shorter than that of the controls and furfural-treated animals. Death in Groups 3 and 4 (BP and BP + furfural, respectively) was most frequently due to asphyxia resulting from the obstruction of the trachea by tumors. Starting at Week 17, rapidly growing s.c. tissue masses became visible in the ventral neck region (peritracheally) of several male and female hamsters treated with BP + furfural. These nodules, which were often very large (measuring up to 6 x 5 x 5 cm) and which caused respiratory distress, turned out to be sarcomas. Death from these peritragheal sarcomas, occurring almost exclusively in the BP + furfural group, contributed considerably to the higher mortality rate in this group compared to that in the group given BP alone.

Pathology

Small foci of hemosiderin-laden macrophages were observed in the lungs of most of the animals treated with furfural, BP,
or BP + furfural; only a few 0.9% NaCl solution-treated controls showed such foci. Acute tracheobronchitis, bronchopneumonia, atelectasis, and interstitial pneumonitis were occasionally seen in animals in which tumors of the tracheobronchial tree obstructed the lumen.

Animals Killed at Week 30. The 6 hamsters of Group 4 (BP + furfural), which were randomly taken and killed at Week 30, all showed hyperplasia and squamous metaplasia with cellular and nuclear atypism of the tracheal epithelium. In 2 of them, the epithelium of the main stem bronchi was also hyperplastic. Only 3 out of the 6 hamsters of Group 3 (BP alone) showed a slight degree of epithelial hyperplasia in the trachea, and in only 1 case a small area of squamous metaplasia was observed in the trachea. In Group 2 (furfural), the trachea of 1 out of the 6 animals killed at Week 30 showed small areas of slightly hyperplastic epithelium. Neither hyperplasia nor metaplasia of the tracheobronchial epithelium was seen in 0.9% NaCl solution-treated controls.

Peribronchial adenomatoid lesions were observed in nearly all test hamsters killed at Week 30 but in none of the controls killed at that time. These proliferations were slightly more pronounced in animals given BP + furfural than in those treated with either BP or furfural.

None of the animals killed after 30 weeks had developed epithelial tumors of the respiratory tract. A large polymorphic sarcoma in the ventral neck region was found in 1 female of Group 4 (BP + furfural).

Animals That Died during or at the End of the Experiment. The main findings were given in Table 2. A high incidence of tracheal tumors occurred in males and females and nuclear atypism of the tracheal epithelium. In 2 of them, which were randomly taken and killed at Week 30, bronchopneumonia, atelectasis, and interstitial pneumonitis in the trachea. In Group 2 (furfural), the trachea of 1 out of the 6 animals killed at Week 30 showed small areas of slightly hyperplastic epithelium. Neither hyperplasia nor metaplasia of the tracheobronchial epithelium was seen in 0.9% NaCl solution-treated controls.

Peripheral (lung) adenomas were most frequently found in animals given BP alone (Table 2). As most of these tumors occurred late in the experimental period, their relatively high incidence in the BP group compared to the group given BP + furfural may be ascribed to the higher survival rate of the animals of the BP group.

Except for a very small tracheal papilloma in 1 control animal, no respiratory tract tumors or peritracheal sarcomas were observed in 0.9% NaCl solution- or furfural-treated animals.

Morphological Findings

The lesions of the respiratory tract which could be ascribed to the administration of furfural, BP, or both ranged from hyperplasia to extensive malignant neoplasms originating in epithelial or connective tissue. Their incidence in the various groups was presented above, their morphology will be described below.

Larynx. Only 2 tumors were found at this site, 1 papilloma and 1 carcinoma. The papilloma appeared as a small papillomatous growth consisting of many layers of well-differentiated squamous cells. The carcinoma showed nuclear pleomorphism, contained tubular elements, and invaded the adjacent connective tissue.

Table 1

Average weights and survival rates of Syrian golden hamsters treated intratracheally with 0.9% NaCl solution, furfural, BP, or BP + furfural.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>60</th>
<th>72</th>
<th>78</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9% NaCl solution</td>
<td>85/35</td>
<td>102/33</td>
<td>123/32</td>
<td>118/31</td>
<td>112/27</td>
<td>109/27</td>
<td>104/23</td>
<td>111/22</td>
<td>113/22</td>
</tr>
<tr>
<td>2</td>
<td>Furfural</td>
<td>84/35</td>
<td>101/32</td>
<td>115/32</td>
<td>120/29</td>
<td>114/26</td>
<td>112/26</td>
<td>110/20</td>
<td>117/18</td>
<td>120/18</td>
</tr>
<tr>
<td>3</td>
<td>BP</td>
<td>83/35</td>
<td>98/34</td>
<td>115/29</td>
<td>112/29</td>
<td>105/26</td>
<td>112/23</td>
<td>115/12</td>
<td>108/8</td>
<td>110/6</td>
</tr>
<tr>
<td>4</td>
<td>BP + furfural</td>
<td>83/35</td>
<td>95/35</td>
<td>104/29</td>
<td>103/27</td>
<td>100/23</td>
<td>94/15</td>
<td>83/7</td>
<td>92/3</td>
<td>93/2</td>
</tr>
</tbody>
</table>

| Females |
|---------|----------|--------|--------|--------|--------|--------|--------|--------|--------|
| 2       | Furfural | 85/35 | 109/35 | 117/35 | 121/34 | 117/30 | 114/29 | 115/25 | 124/14 | 128/14 |
| 3       | BP       | 86/35 | 112/34 | 117/33 | 122/33 | 116/30 | 109/23 | 97/17 | 102/3 | 106/1 |
| 4       | BP + furfural | 85/35 | 108/34 | 117/34 | 119/31 | 118/23 | 112/10 | 100/5 | – d 0 | – d 0 |

a A course of 36 intratracheal instillations were given once weekly. Each instillation consisted of 0.2 ml of 0.9% NaCl solution, 0.2 ml of 1.5% furfural in 0.9% NaCl solution, 0.2 ml of 0.5% BP in 0.9% NaCl solution, and 0.2 ml of 0.5% BP in 1.5% furfural in 0.9% NaCl solution for Groups 1, 2, 3, and 4, respectively.

b In Week 30, 3 males of Group 1 and 3 males and 3 females out of each of the other 3 groups were killed and autopsied to obtain some interim information.

c Difference with the controls (Group 1) is statistically significant, according to Student's t test.

d No animals left.
Table 2

Respiratory tract lesions in Syrian golden hamsters given a course of 36 weekly intratracheal instillations of 0.9% NaCl solution, furfural, BP, or BP + furfural

The animals killed at Week 30 are included in this table.

<table>
<thead>
<tr>
<th>Sites and types of lesions</th>
<th>Initial no. of animals</th>
<th>No. of animals examined&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
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<tr>
<td>Larynx</td>
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<td>30</td>
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<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
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<tr>
<td>Papilloma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trachea Hyperplasia alone</td>
<td>0</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Squamous metaplasia alone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Papilloma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>16</td>
<td>12</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peritracheal sarcoma&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Bronchi Hyperplasia alone</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Squamous metaplasia alone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Adenocarcinoma</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Lung Slight adenomatoid lesion</td>
<td>5</td>
<td>21</td>
<td>14</td>
<td>18</td>
<td>9</td>
<td>12</td>
<td>16</td>
<td>0</td>
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<tr>
<td>Moderate adenomatoid lesion</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Squamous alveolar metaplasia</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total no. of animals bearing tumors of the respiratory tract&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>22</td>
<td>15</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> A number of hamsters were lost because of advanced postmortem changes or cannibalism.

<sup>b</sup> Peritracheal sarcomas are not considered respiratory tract tumors.

<sup>c</sup> Several animals had developed a tumor in more than 1 segment of the respiratory tract.

Tracheobronchial Tree. Hyperplasia and metaplasia of the tracheal and bronchial epithelium were found to various degrees (Figs. 1 and 2). In a few animals of the furfural group, a slight to moderate degree of hyperplastic changes occurred in the tracheobronchial tree, but neither squamous metaplasia nor atypical features were seen in the hyperplastic areas. Patches of hyperplasia and stratified squamous metaplasia showing nuclear and cellular atypism were exclusively found in hamsters treated with BP or BP + furfural. Keratinization was a common finding. As appeared from the examination of the animals randomly killed at the 30th week, these changes developed earliest in animals treated with BP + furfural. In cases where tracheal or bronchial carcinomas were present, hyper- and metaplastic epithelium was often found to cover extensive areas of the tracheobronchial tree.

Squamous cell carcinoma was the most frequent type of tumor induced (Figs. 3 to 5). They were most frequently found in the trachea but occurred also in bronchi (Fig. 5). Occasionally, the tracheal tumors were multiple. They varied considerably in size and showed different degrees of squamous differentiation and keratinization, but invariably they...
infiltrated the underlying tissues (Fig. 4). In some of the
tumors, tubular structures were present, indicating an
adenocarcinomatous type of differentiation. The first
squamous cell carcinoma was seen at Week 25 in a female
treated with BP + furfural.

Two tracheal carcinomas were so poorly differentiated that
they were classified as anaplastic carcinomas.

Two adenomas and 1 adenocarcinoma of the bronchial
epithelium were found in the BP-treated group. Both
adenomas consisted of acini or tubules mainly lined with
stratified squamous epithelium, but mucus-containing cells
were also recognizable. The malignant tumor was a typical
papillary adenocarcinoma.

The tracheal papillomas appeared as well-differentiated,
exophytic papillomatous growths without definite signs of
cancers.

Peritracheal sarcomas were the first tumors to be observed,
starting at Week 15. They grew rapidly and usually attained
large size. Some were highly necrotic. Microscopically, they
appeared to range from poorly differentiated fibrosarcomas to
extremely pleomorphic, undifferentiated sarcomas (Fig. 6).
Mono- and multinuclear giant cells with bizarre,
hyperchromatic nuclei were common. In most of the
neoplasms, many mitotic figures were seen. The amount of
reticulin fibers varied considerably. A variety of histological
patterns were often recognizable within the same tumor.
Pulmonary metastases were found in several hamsters.

**Lung.** The peripheral adenomatoid lesions appeared as
proliferations of bronchioloalveolar epithelium which spread
along the alveoli. They were often multiple and varied
considerably in size; they showed an adenomatous pattern and
consisted of cuboidal or columnar epithelium. Macrophages
laden with iron-positive pigment granules were occasionally
interspersed between the glandlike spaces.

Alveolar metaplasia was invariably seen as a few alveoli lined
by either stratified squamous epithelium or mucus-producing
cells. In case of mucous cell metaplasia, distended alveolar
spaces filled with mucus were occasionally encountered
indicating a retention of mucus.

Peripheral adenomas usually appeared as small nodules
consisting of tubular and papillary structures lined by cuboidal
epithelium. In a few instances, the tumors consisted of acini
lined by stratified epithelium, which was suggestive of
squamous epithelium (Fig. 7), although an occasional
superficial cell appeared to contain Alcian green-positive
material indicating mucus formation. These nodules were
circumscribed and had developed their own supporting tissue.

Two adenocarcinomas were found, 1 in Week 72 and the
other in Week 78. They showed a pattern typical for a
papillary adenocarcinoma (Fig. 8).

Three squamous cell carcinomas were located peripherally.
They were small in size but showed the definite characteristics of
invasive squamous cell carcinoma. No metastases of any of
the respiratory tract tumors were observed.

**Lesions in Other Organs.** Only 4 tumors were found at other
sites than the respiratory tract, namely, 1 lymphoreticular
cancer of the mesenteric lymph nodes in a male control
hamster, 1 malignant pheochromocytoma in a male treated
with furfural, and 1 thyroid carcinoma and 1 mesenchymal
type of tumor in the abdominal cavity, each in a female
treated with BP alone. They are considered spontaneous
tumors.

In the liver, multiple cystic structures (measuring up to 25
mm) lined by flattened bile duct epithelium and filled with a
greenish fluid were frequently observed in test and control
hamsters over 1 year of age. These livers also showed
destruction of parenchyma and pericystic hemorrhages and
fibrosis. Many hepatocytes and Kupffer cells contained
iron-negative, lipofuscin-like pigment granules.

Amyloidosis of the kidneys, which was most marked in
females, occurred in many hamsters of the control group and
the various test groups. The severity of the amyloid deposits
increased with increasing age.

There was no indication that the hepatic and renal changes
were related to treatment.

**DISCUSSION**

The results of this study did not disclose any evidence that
furfural is a tumorigen for the respiratory tract of hamsters.
The slight hyperplasia of the tracheobronchial epithelium, as
well as the alveolar metaplasia and peribronchiolar
adenomatoid proliferations of the bronchioloalveolar
epithelium noticed in animals which were treated with furfural
alone, point to an irritating action of furfural.

The peripheral adenomatoid lesions have been seen in
hamsters by many investigators (5, 6, 11, 12, 22, 24) either
after the administration of a carcinogenic or a noncarcinogenic
substance and even in untreated control animals (5).
Occasionally, they have been presumed preneoplastic (5).
Saffioti et al. (24) even reported a few cases of direct
extension of peripheral proliferative lesions into neoplastic
areas. Although in our study, peribronchiolar proliferations
also occurred to a higher degree in animals treated with BP
than in furfural-treated hamsters, the transition of such a
lesion into a neoplastic growth was never observed. Our
findings, therefore, support the view that they are nonspecific
lesions, very probably associated with irritation or
inflammation. Of course, once present, they may be more
susceptible for the attack by a carcinogen than the normal
bronchioloalveolar epithelium.

An unexpected finding was the high incidence of respiratory
tract tumors in hamsters given BP alone. This was surprising
because, so far, a high number of similar tumors has not been
reported after intratracheal instillation of BP, unless a carrier
dust or at least a surface-active agent had been administered
simultaneously (12, 22, 24). The frequent and prolonged
intratracheal administration of a relatively high amount of BP
(1 mg of BP weekly for 36 weeks) explains, very probably, the
high tumor yield in this study. The scarcity of reports on the
administration of BP suspensions without carriers may
possibly be ascribed to the technical difficulties met when
preparing such a suspension. The suspension which we used
was not ideal because of the presence of a number of relatively
large BP particles, but it was obviously satisfactory for our
purpose to expose the respiratory tract to a threshold dose of
BP administered once a week. Neither the presence of large BP
particles nor the lack of information about the exact
distribution of BP crystals over the respiratory tract was
considered an important disadvantage for our study. The use
of a carrier dust has been shown to warrant a high tumor yield (22). Our results have clearly demonstrated that neither a carrier substance nor a surface-active agent are indispensable for the induction of respiratory tract tumors by BP particles administered intratracheally.

In comparison with BP alone, the combined intratracheal instillation of BP and furfural showed several interesting differences: (a) earlier development of hyper- and metaplastic changes of the tracheobronchial epithelium; (b) a shorter latent period for tracheobronchial tumors; (c) the induction of a few more bronchial and peripheral squamous cell carcinomas; and (d) the induction of considerably more peritracheal sarcomas. The first 3 differences are indicative of a cocarcinogenic effect of furfural on the respiratory tract of hamsters, but further experimental work will be necessary to substantiate this finding.

Thus far, little attention has been paid to cocarcinogenic factors in respiratory tract carcinogenesis (2, 20, 21). The present results suggest that studies on the biological effects of cigarette smoke components should be focused not merely on the detection of new initiators or complete carcinogens but also on the detection of factors increasing the carcinogenic response of the respiratory tract.

Subcutaneous sarcomas are readily induced in hamsters by s.c. injection of BP or other carcinogenic polynuclear hydrocarbons (13, 14, 19). Saftiotti et al. (22) reported tracheal sarcomas in a few hamsters after intratracheal instillation of BP particles attached to hematite. It seems justified, therefore, to consider BP the factor responsible for the induction of the peritracheal sarcomas observed in our hamsters after treatment with BP alone or BP + furfural. The high incidence of these tumors in the group given BP + furfural points to an augmenting effect of furfural. This augmentation might be based on a facilitating effect of furfural on the deep penetration of (the larger) BP particles into the tracheal submucosa. There were, however, no pathological findings to substantiate such a speculation.

The morphology of the respiratory tract lesions observed in this study was essentially that described previously by other investigators (8, 11, 12, 22, 24) and needs, therefore, no further comment.

Although a cocarcinogenic effect of furfural on the respiratory tract of hamsters may be deduced from the results of the present experiment, the number of tumor-bearing hamsters treated with BP alone was too high to consider 1 mg of BP in 0.9% NaCl solution (0.2 ml of 0.5% BP) administered weekly a satisfactory threshold dose for studies on augmenting factors (cocarcinogenesis) in lung carcinogenesis. Therefore, lower levels of BP are being used in similar subsequent experiments, and moreover experiments have been started meanwhile to determine the dose of BP which evokes a threshold carcinogenic response in the lung of the Syrian golden hamster.

ACKNOWLEDGMENTS

Dr. P. J. Groenen carried out the analytical investigations on cigarette smoke condensates. His knowledge about the chemical and biological aspects of cigarette smoking were of major importance in my decision to examine furfural. I thank Professor Dr. T. Vossenaar for his help in classifying the tumors, his constant interest, and his help with histological techniques. I am indebted to Professor Dr. P. Emmelot for critically reviewing the manuscript. Helpful advice and criticism from Dr. A. P. de Groot are greatly appreciated. The able technical assistance of Mr. D. de Jong, Miss Ank Wierda, Miss Sylvia van Loenen, Mr. H. Immel, and Mr. M. Rijk is gratefully acknowledged. I am indebted to Mr. H. Koor for the photographic work.

REFERENCES


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Figs. 1 to 8. All sections were stained with H & E, except Fig. 7. The time of death is given in weeks after the beginning of the experiment.
Fig. 1. Hyper- and metaplastic tracheal epithelium (female, BP + furfural, 30 weeks). X 370.
Fig. 2. Atypical metaplastic, keratinizing, squamous, tracheal epithelium (female, BP + furfural, 36 weeks). X 370.
Fig. 3. Tracheal squamous cell carcinoma (male, BP, 73 weeks). X 37.
Fig. 4. Detail of Fig. 3 showing the invasive pattern of neoplastic squamous cells. X 140.
Fig. 5. Squamous cell carcinoma arising from a main bronchus (male, BP + furfural, 49 weeks). X 140.
Fig. 6. Polymorphic sarcoma (female, BP + furfural, 32 weeks). X 560.
Fig. 7. Peripheral adenoma consisting of acini lined by stratified epithelium, which is highly suggestive of squamous epithelium (female, BP, 60 weeks). Kreyberg's stain, X 140.
Fig. 8. Peripheral papillary adenocarcinoma (female, BP, 78 weeks). X 37.

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BP-induced Respiratory Tract Tumors

JANUARY 1972

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Respiratory Tract Tumors in Hamsters after Intratracheal Instillations of Benzo(a)pyrene Alone and with Furfural

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