Sequential Cytological Changes during Development of Respiratory Tract Tumors Induced in Hamsters by Benzo(a)pyrene-Ferric Oxide

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SUMMARY

The exfoliative cytology of the lung was studied during the induction and early development of respiratory tract tumors. Syrian golden hamsters received multiple intratracheal injections of benzo(a)pyrene-Fe₂O₃ for 14 weeks at a cumulative dose of 45 mg benzo(a)pyrene. Shortly after start of the experiment a severe, probably toxic, cytological response to the carcinogen application was observed; this response rapidly diminished during further injections and disappeared after cessation of carcinogen administration.

The average time interval from start of carcinogen application to death was 35.5 weeks. During this time specimens revealed a progression from mild atypia of squamous metaplastic cells, to moderate atypia, to marked atypia, to changes indicative of cancer. These carcinogen-induced progressive cytological changes showed striking morphological similarities to cytological changes described in cigarette smokers prior to the development of lung cancer. By the 25th week of the experiment, specimens of all carcinogen-treated animals had cells suggestive or conclusive of cancer. In animals that died after the 35th week, the diagnosis of cancer had been made at an average time of 19 weeks before death.

Sensitivity and specificity of the cytological method for diagnosing cancer were very high, since no false negative diagnoses were made in tumor-bearing animals nor were false positive diagnoses made in control animals that received Fe₂O₃ alone or in carcinogen-treated animals that did not develop tumors. Cytological typing of malignant tumors was efficient for histologically well-differentiated tumor types.

INTRODUCTION

In spite of advances in the treatment of many malignant diseases, the cure rate for lung cancer has remained dis-paringly low (3). Curative treatment of bronchogenic carcinoma has only been achieved if the disease was detected at a very early, usually roentgenologically negative, stage. Therefore it is to be hoped that the mortality of bronchogenic carcinoma can be reduced through earlier diagnosis (4, 15, 24). Pulmonary cytology is to date the most promising of all diagnostic procedures for early diagnosis of lung cancer (for review see Ref. 7). There are, however, some inherent difficulties in the diagnosis of bronchogenic carcinoma through sputum cytology, which may explain why this method has not yet reached the same degree of reliability and applicability as the Papanicolaou test for diagnosis of cervical cancer. First of all, the surface of the bronchial tree is more than 1000 times larger than that of the cervix. Furthermore, bronchial lesions can be diagnosed only in the sputum if they spontaneously shed a sufficient number of cells. In contrast to this, cytological samples are removed mechanically from the cervix. Another major problem in pulmonary cytology is the difficulty of distinguishing carcinogen-induced irreversible epithelial changes since the human respiratory tract is commonly exposed to various carcinogenic and/or noncarcinogenic insults such as cigarette smoke, infections, and air pollutants in a rather uncontrolled manner. It appeared to us that an experimental animal system would be very suitable for investigation of the latter problem since animals can be protected from uncontrolled respiratory insults and therefore one can study the cytological and histological response to a single factor without interference from other factors. An animal model suitable for inducing bronchogenic carcinomas which closely simulate those found in man is available (20), and we recently described a method for doing repeated pulmonary cytology in small laboratory animals (22).

The objectives of the present study were to establish the sequence of cytological changes occurring after application of a carcinogen up to the time of lung tumor development, to determine how soon before death cytological detection of cancer was possible, and to investigate whether the exfoliated cells indicative of cancer could be used for determining the type of tumor. Since one of us (G. S.) had collected extensive cytological and histological material from cigarette smokers and uranium miners developing bronchogenic carcinoma (15–17), we were able to compare this human material to the cytological and histological changes...
found in hamsters during development of chemically induced lung cancer.

MATERIALS AND METHODS

Animals. Fifty-eight male Syrian golden hamsters (Lakeview golden random bred, LVK/LAK, Lakeview Hamster Colony, Newfield, N. J.) were used for this study. These random-bred animals were 12 weeks old at the start of the experiment and were kept for life-span. They were housed 5 per cage in filtertop plastic cages and had free access to feed (Purina Laboratory Chow 5001C) and water. After cessation of carcinogen application hamsters were checked daily for any symptoms of respiratory tract disease such as irregular breathing, wheezing, or gasping. The body weights of the animals were determined at 2-week intervals. Animals were allowed to die spontaneously or were killed when moribund. The hamsters remaining (8 experimental and 5 controls) were killed 42 weeks after the start of carcinogen application to terminate the experiment.

Carcinogen Application. BP² (Aldrich Chemical Co., Milwaukee, Wis.) and the carrier dust ferric oxide (Fe₂O₃, Fisher Scientific Co., Fair Lawn, N. J.; count medium diameter, 0.2 μm) were prepared as a 1/1 mixture by precipitation from acetone (19) and suspended in sterile 0.9% NaCl solution, containing 10% steroid suspension medium,³ to give a concentration of 33.3 mg BP and 33.3 mg Fe₂O₃/1.0 ml (count medium diameter, 2.5 μm). For i.t. instillations animals were anesthetized by inhalation narcosis with methoxyflurane (metofane, Pitman-Moore, Fort Washington, Pa.) by a previously described method (22). The same i.t. injection technique described by Saffiotti et al. (20) was used. Forty-eight hamsters were given multiple i.t. injections of the carcinogen-ferric oxide suspension during 14 weeks (total dose 45 mg BP and 45 mg Fe₂O₃). Because of mortality, the initial individual dose of 5 mg BP in 0.15 ml suspension medium was reduced to 3.3 mg BP in 0.1 ml suspension medium and the weekly injections were interrupted (Chart 1). Ten control hamsters received Fe₂O₃ only, using the same total dose (45 mg Fe₂O₃), dose schedule, and suspension medium as for the carcinogen-treated animals.

Cytological and Histological Techniques. Cytological samples were obtained from control and carcinogen-treated animals every 2 to 5 weeks, from 5 weeks after 1st injection until time of death (Chart 1). Cytological specimens were collected by rinsing the tracheal lumen with 0.9% NaCl solution. We recently described this method in detail (22). We found that the procedure can be repeated without harming the animal. Furthermore, it was shown that specimens obtained in this way are representative of the entire lower respiratory tract and contain practically no contamination of squamous cells from the oral cavity or the nasopharynx.

Samples were prefixed for more than 4 hr by adding equal amounts of 95% ethanol containing 5% Carbowax (Carbowax R 1540, polyethylene glycol, Union Carbide Corp., New York, N. Y.) to the samples. After prefixation, cytological slides were prepared from the liquid specimens (22). Then slides were postfixed in 95% ethanol and stained after the method of Papanicolaou (13). Diagnostic screening of cytological slides was performed without knowing animal number or date of sampling. We used commonly accepted general criteria for the cytological diagnosis of atypia and cancer and for cytological typing, as described by Saccomanno et al. (15) and others (6, 13, 23). The efficiency of our cytological method for diagnosing cancer or type of malignant tumor was expressed in “sensitivity,” which is the proportion of individuals classified as positive to those who are positive (i.e., a measure of the false negative rate), and in “specificity,” which is the proportion of individuals classified as negative to those who are negative (i.e., a measure of the false positive rate), according to Wilson and Jungner (25).

Complete necropsies were performed on all animals at time of death. Lung, heart, and trachea were removed en bloc and fixed in 10% buffered paraformaldehyde. After fixation the lung lobes were separated and sectioned along the axis of the main bronchus. To detect all pathological lesions, interrupted serial sections were made at 200-μm intervals throughout larynx, trachea, and lungs and were stained with hematoxylin and eosin. Since hamster respiratory tract tumors closely simulated the histological appearance of human lung cancer, they were classified according to the WHO classification (10). The human material used for morphological comparison to our hamster material has been documented and summarized in a recent publication (15). It consisted of histopathological material and cytology smears of sputum samples from heavy smokers and from uranium miners who were also smokers.

RESULTS

Types of Carcinogen-induced Cytological Changes

Cells of the Early Reaction Type. Some of the abnormal cells appearing shortly after carcinogen application revealed characteristic morphological differences from nor-
Exfoliative Cytology in Respiratory Carcinogenesis

Intratracheal instillation of carcinogen induced a transitory but severe cytological response. Large numbers of polymorphonuclear leukocytes and pulmonary macrophages containing Fe₂O₃ particles were observed. In addition, many cells of the early reaction type were found in pulmonary cytological specimens of all carcinogen-treated animals. Cells of this type were most prominent in the 1st cytological specimens (taken 1 week after the 5th i.t. instillation); during further carcinogen application they rapidly diminished in number and disappeared completely within 1 month after termination of carcinogen instillations. Besides cells of the early reaction type, squamous metaplastic cells were also found early in the experiment; however, this cell type did not disappear after cessation of carcinogen application. Chart 2 shows the sequential cytological changes observed in carcinogen-treated hamsters during development of respiratory tract tumors. Only the highest degree of atypia found in a specimen is recorded. Regardless of the type of respiratory tract tumor which finally developed in an animal, sequence and morphological appearance of the premalignant cytological changes were similar in all carcinogen-treated animals. Regular squamous metaplastic cells and cells showing mild atypia were consistently found throughout the experiment and were the prominent type during the early weeks. Later in the experiment, cells with higher degrees of atypia appeared. In cytological specimens of some individual animals, a temporary decrease in degree of cytological abnormality was observed.

**Sequence of Carcinogen-induced Cytological Changes**

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while in some others a high degree of atypia was noted early in the experiment. However, evaluating the group of animals as a whole there was a statistically significant progression from mild over moderate and marked atypia to cells suggestive and conclusive of cancer (Table I).

There was no indication from this experiment (Chart 2) that animals that developed a severe degree of atypia early in the experiment also developed early respiratory tract neoplasms. A surprising finding was that cells indicative of cancer were found in the tracheal washings of almost all animals at the same time (21 weeks), regardless of whether they died early or late in the experiment. Therefore, the average time interval between earliest cytological detection of cancer and death of the animal was much longer (19 weeks) in animals that survived the 35th week of experiment than it was in animals that died between the 18th and 35th week (9 weeks). At the 21-week time point, 16 of 31 animals showed cells conclusive, and 12 showed cells suggestive, of cancer. Cells indicative of cancer were usually quite scanty at this time and only became more abundant in the last samples before an animal died. Often these cells were camouflaged by marked atypical metaplastic cells, which were more frequent in these samples. Weight loss or clinical symptoms of respiratory disease, such as wheezing or gasping, were not found earlier than 1 or 2 weeks before death.

No cytological abnormalities were observed in cytological specimens of control animals that received i.t. instillation of Fe$_2$O$_3$ alone. Only an increase of pulmonary macrophages, which were frequently multinucleated and contained large amounts of Fe$_2$O$_3$ particles, was found.

Comparing the cytological responses of hamsters and men whose respiratory tracts were exposed to carcinogenic substances, a similar early toxic reaction was observed in men who started working in uranium mines and were exposed to carcinogenic radon daughters for the 1st time. As in hamsters, the cells of the early reaction type disappeared when the exposure to the carcinogen continued. Squamous metaplastic changes with gradually increasing signs of atypia then appeared, which were practically indistinguishable from those observed in hamsters during the development of bronchogenic carcinomas.

Table I

<table>
<thead>
<tr>
<th>Diagnoses in exfoliative cytology of hamster lungs at various times after start of carcinogen application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thirty-seven effective animals surviving the 5th week after start of carcinogen application are included. Only the highest degree of abnormality found in a cytological specimen of an animal at a given time is recorded.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>No. of specimens* at wk:</th>
<th>5</th>
<th>9</th>
<th>15</th>
<th>21</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild atypia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate atypia</td>
<td></td>
<td>26 (70)</td>
<td>12 (34)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marked atypia</td>
<td></td>
<td>10 (27)</td>
<td>19 (54)</td>
<td>9 (27)</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Suggestive of cancer</td>
<td></td>
<td>1 (3)</td>
<td>4 (12)</td>
<td>20 (61)</td>
<td>2 (6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Conclusive of cancer</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2 (6)</td>
<td>12 (39)</td>
<td>10 (36)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>37 (100)</td>
<td>35 (100)</td>
<td>33 (100)</td>
<td>31 (100)</td>
<td>28 (100)</td>
<td>25 (100)</td>
</tr>
</tbody>
</table>

\[
\text{Av. change}^b \text{ between time points} \quad p^2 < 0.001 \quad p < 0.001 \quad p < 0.001 \quad p < 0.1 \quad p < 0.02 
\]

*a Percentage in parentheses.

*b Average change of degree of atypia in samples of 2 consecutive time points. Movement of a diagnosis in an individual animal from 1 degree of atypia to the next higher 1 is scored as + 1; movement to the next lower degree as − 1 (compare with Chart 2).

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**Exfoliative Cytology in Respiratory Carcinogenesis**

**Cytological-Histological Correlation of Respiratory Tract Tumors**

All of the carcinogen-treated animals dying after the 15th week of experiment had developed tumors of the respiratory tract. A total of 57 malignant respiratory tract tumors was found in 33 animals. Several animals therefore carried more than 1 tumor; 14 hamsters had 2 and 5 hamsters had 3 respiratory tract neoplasms. In addition, noninvasive epithelial lesions such as regular squamous metaplasia, pleomorphic proliferations showing various degrees of atypia, and carcinoma in situ were found in the tracheal-bronchial tract of all carcinogen-treated animals. No such changes were found in control animals.

About 60% of the malignant tumors were located in the stem, primary, or secondary bronchi, 25% were located in the trachea, and 15% were in the larynx, usually at or slightly below the vocal cords. The carcinomas ranged from microscopic lesions to large masses that occupied 1 or more lung lobes almost completely. Since most malignant tumors originated from the larynx, trachea, and proximal parts of the bronchial tree, they usually did not become very large before they caused narrowing of the major airways and, subsequently, suffocation resulting in death; the average tumor size was about 1.9 mm. Most carcinomas were highly infiltrative. They invaded blood vessels, penetrated the walls of the trachea and bronchi, and extended into the lung parenchyma, esophagus, and other adjacent tissue, confirming the observations made by Saffiotti et al. (20, 21). Several hamsters showed metastases in the lungs, where they appeared as small scattered nodules. Metastases into regional lymph nodes were found in only 1 animal.

Cytological samples of all tumor-bearing animals showed positive indication of cancer prior to death (Chart 2); therefore, none of these animals had a false negative diagnosis when it died. No false positive diagnoses of cancer were made in carcinogen-treated hamsters which did not develop respiratory tract tumors or in the control group that received i.t. instillations of Fe$_2$O$_3$ alone. Thus, the efficiency of our cytological method in diagnosis of respiratory tract cancers was high.

Respiratory tract tumors of the hamsters were classified into 3 main types of differentiation known from human pathology—epidermoid carcinoma, adenocarcinoma, and anaplastic undifferentiated carcinoma. The small-cell anaplastic carcinoma (or oat-cell carcinoma) was the only common histological type of human lung cancer which was not observed in our hamster material. The number of tumors in the different histopathological classes is given in Table 2. Most adenocarcinomas were found in animals that died late in the experiment, while all animals with sarcomas died rather early (Chart 2). On the other hand, epidermoid carcinomas occurred randomly throughout the experiment.

Well-differentiated epidermoid carcinomas (Fig. 8, a and b) produced squamous-like cells, which often showed a high degree of nuclear and cellular pleomorphism. Because of keratin formation the cytoplasm often assumed a brilliant orange or yellow color in Papanicolaou stain. In contrast to epidermoid carcinomas, adenocarcinomas (Fig. 9, a and b) shed clusters of round or cuboidal cells which usually showed less pleomorphism but had characteristically malignant nuclei. A columnar cell shape and a gland-like pattern of the clumps of cells were occasionally noticeable. The cytoplasm was usually basophilic and had a foamy vacuolated appearance; occasionally larger vacuoli were seen. A reliable cytological differentiation of adenocarcinomas of the acinar from the papillary type was not possible. Large-cell anaplastic carcinomas (Fig. 10, a and b) shed large cells, often with great but varying amounts of eosinophilic or basophilic cytoplasm. Orangophilia was not noted, indicating the absence of keratinization. The nuclei were pleomorphic, often large and sharply outlined, and had prominent single or multiple nucleoli. Sarcomas were all poorly differentiated and shed cells of spindly shape having nuclei with an extremely coarse nuclear material.

Table 2 compares cytological and histological typing of respiratory tract tumors. As mentioned above, a given animal often had more than 1 tumor. This gave rise to diagnostic problems in hamsters with both anaplastic carcinoma and a well-differentiated tumor, i.e., an epidermoid or adenocarcinoma. Usually, in these animals, only the associated well-differentiated tumor type was cytologically detected, and only 5 of 12 anaplastic carcinomas and 2 of 4 sarcomas were accurately typed by cytological techniques. Thus the efficiency of cytological typing was low for anaplastic tumors of the respiratory tract. In cases with combined epidermoid adenocarcinoma, the 2 components were always diagnosed as 2 different tumors. Sensitivity and specificity of cytological typing were high for histologically well-differentiated tumors. Eighteen of 19 epidermoid carcinomas and 13 of 14 adenocarcinomas were correctly diagnosed.

**DISCUSSION**

Histological studies by Auerbach et al. (1) have demonstrated a high correlation between amounts of cigarette smoking and the frequency of certain metaplastic changes in the bronchial epithelium. Saccomanno et al. (15–17) observed, cytologically, the appearance of squamous metaplasia and its progressive development to invasive bronchogenic carcinoma over a long time period in sputum specimens of cigarette smokers and smoking uranium miners. Since cigarette smoke and many other inhalants are mixtures containing only traces of carcinogetic substances but large amounts of toxic noncarcinogenic substances, it is difficult to decide how much of the morphological change observed in these studies is due to toxic noncarcinogenic components of the inhalants. Our study demonstrates that cytological abnormalities indistinguishable from those described in cigarette smokers and uranium miners (15–17) can be induced by application of defined respiratory carcinogens under controlled experimental conditions; as in man, a sequence from mild atypia to moderate and marked atypia to cancer was observed. A future serial sacrifice study will have to show whether the atypical cytological changes found in our carcinogen-treated animals can be
correlated with corresponding noninvasive lesions of the bronchial epithelium. Furthermore, it needs to be clarified whether any (and if so, which) of the atypical metaplastic changes can also be induced by using noncarcinogenic, toxic, or infectious agents.

The severe cytological reaction to carcinogen application found at the start of the experiment appeared to be of toxic and rather nonspecific nature, since cells of the early reaction type rapidly diminished in number during further carcinogen application and disappeared completely after cessation of the carcinogen administration. Cytological changes quite similar to those of the early reaction type have been described by Koss (8), and Koss and Richardson (9) after acute radiation damage of the human respiratory tract. It is noticeable that these cells may simulate bizarre giant cancer cells to perfection, and only very careful application of all cytological criteria prevents the false diagnosis of cancer.

In hamsters that lived longer than 8 months after the 1st application of carcinogen, respiratory tract neoplasms could be detected cytologically at an average time interval of 4.5 months before death. Since lung tumors of human beings usually develop after a long latency period and since several months of hamster life compare to several years of human life, the experiment suggests that using adequate cytological methods, bronchogenic carcinoma of men may be detectable long before loss of weight or clinical symptoms of respiratory tract disease develop. Our experiment was not designed as a serial sacrifice study. Therefore we do not know whether, at the time of 1st cytological indication of cancer, lesions had already progressed to an invasive malignant stage or if they were still in a carcinoma in situ stage. Our experiment suggests that all our animals develop carcinoma in situ or small invasive lesions at about the same time after carcinogen application. However, the time interval at which these lesions progressed to a tumor that killed the animals was quite variable. This would be consistent with studies which have demonstrated a large variation of the time interval with which carcinoma in situ of the cervix of women progresses to cancer (5) and with a recent study by Saccomanno et al. (15) which suggests that the variable time interval is also characteristic for carcinoma in situ of the lung.

Sensitivity and specificity of our cytological method of diagnosing respiratory tract cancer were excellent, since none of the tumor-bearing animals was classified as negative nor were any control animals or carcinogen-treated animals that did not develop lung cancer diagnosed as positive. The efficiency of this method may lie in the fact that cytological samples were collected near or at the site of tumor development and that the tracheobronchial changes were quite extensive because of the high carcinogen dose used. Nevertheless, even in our experiment, only metaplastic cells with a lower degree of atypia were usually abundant in cytological specimens at all times. Higher degrees of atypia were less frequently observed, while cells indicative of cancer were usually scanty and often gave only suggestive evidence when they were first observed. However, they became abundant in the last weeks prior to death of the animals. Since, in all our tumor-bearing hamsters, marked atypia squamous cells were much more frequent than cells indicative of cancer, the danger of missing an occult carcinoma under the diagnosis of marked atypia is quite possible. Therefore, the diagnosis of these cells in human specimens where the sample size is probably relatively smaller becomes very important, and other specialized diagnostic techniques, e.g., fiberoptic bronchoscopy (12), may be useful to exclude the presence of an occult cancer in these cases. Consistent with the results of a recent clinical study (11), cytological typing of the malignant tumors was very efficient for histologically well-differentiated tumor types, while it was much less efficient for poorly differentiated tumors.

There is indication from clinical cytological (14, 18) and histological (2) studies that, when exposure to cigarette smoke is stopped at some time prior to the actual develop-

### Table 2

**Comparison of cytological and histological typing of lung tumors**

Thirty-three hamsters with a total of 57 tumors are included. Fourteen animals had 2 tumors and 5 animals had 3 tumors of the lower respiratory tract. Three animals had 2 separate tumors of the same histological type.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>No. of animals</th>
<th>Epidermoid carcinoma</th>
<th>Adeno-carcinoma</th>
<th>Anaplastic carcinoma</th>
<th>Sarcoma</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermoid carcinoma (I)</td>
<td>19</td>
<td>18</td>
<td></td>
<td>1</td>
<td>18/19 (95)</td>
<td>14/14 (100)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (III la, III 1b)</td>
<td>14</td>
<td></td>
<td>13</td>
<td>1</td>
<td>13/14 (93)</td>
<td>19/19 (100)</td>
<td></td>
</tr>
<tr>
<td>Combined epidermoid-adeno-carcinoma (V)</td>
<td>4</td>
<td></td>
<td>4*</td>
<td>4*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large-cell anaplastic carcinoma (IV 2, IV 3, IV 4)</td>
<td>12</td>
<td></td>
<td>6*</td>
<td>1*</td>
<td>5</td>
<td>5/12 (42)</td>
<td>17/21 (81)</td>
</tr>
<tr>
<td>Sarcoma (X)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Carcinosarcoma (IX)</td>
<td>1</td>
<td></td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Proportion of true positives in cytological typing to all animals with this tumor type. Percentage in parentheses.

* Proportion of true negatives in cytological typing to all animals without this tumor type. Percentage in parentheses.

* Codings used in the WHO classification are given in parentheses.

* Diagnosis of both epidermoid carcinoma and adenocarcinoma was made in these animals.

* Only the associated well-differentiated tumor type was recognized.
ment of cancer, atypical changes of the bronchial tract gradually decrease in number and may actually disappear. It is presently not known, however, at which stage of atypia premalignant changes can still regress easily and revert to normal. The dose of carcinogen used in our experimental system was such that all of the animals surviving the carcinogen administration developed tumors, and no regression of atypical changes was observed. Therefore, we initiated another study using a lower carcinogen dose, which induced respiratory tract tumors in only 30 to 40% of the hamsters and had little effect on their life-span. Thus, in a significant number of animals, atypical changes might become stationary or regress or even revert to normal.

In this paper, results indicate that hamsters that show a severe degree of atypia early in the experiment do not necessarily also develop early respiratory tract neoplasms. It will be important to determine in the experiment in progress, in which only part of the animals will develop tumors, whether early cytological findings during and shortly after carcinogen application can predict whether an individual animal is going to develop respiratory tract neoplasms during its lifetime. If this prediction is possible, it may have some implication to the human situation and suggest that smokers with a high susceptibility to the carcinogenicity of cigarette smoke could be identified by differences in their cytological responses during smoking. Convincing them to cease smoking in time may prevent a significant percentage of lung cancer.

ACKNOWLEDGMENTS

We thank Dr. Paul Nettesheim, in whose laboratory this work was carried out, for encouraging us to pursue these studies and for support and critical discussions throughout the experiments. Thanks are also given to Dr. Toby J. Mitchell for statistical analysis, and we are grateful to Dr. J. W. Reagan, Institute of Pathology, Case Western University, for review of this paper.

REFERENCES

Fig. 1. Exfoliated cells found in tracheal washings of normal hamster: a, ciliated cell; b, goblet cell; c, pulmonary macrophage. Papanicolaou, x 1080.

Fig. 2. Exfoliated abnormal cells of the early reaction type in the tracheal washing of a hamster 5 weeks after start of carcinogen application (25 mg BP with Fe$_2$O$_3$). Cells have large dimensions (compared with neutrophils in same picture) and show striking nuclear and cellular pleomorphism. Nuclear cytoplasmic ratio is in normal range. Note the numerous polymorphonuclear leukocytes (p) and pulmonary macrophages (m) containing Fe$_2$O$_3$ particles in a. Papanicolaou, x 1080.

Fig. 3. Regular squamous metaplastic cells in the tracheal washing of a carcinogen-treated hamster 7 weeks after start of carcinogen application (25 mg BP with Fe$_2$O$_3$). There is no nuclear or cellular pleomorphism. The cytoplasm is basophilic. Cells are much smaller than those of the early reaction type in Fig. 2 at same magnification. Papanicolaou, x 1080.

Fig. 4. Squamous metaplastic cells showing a mild degree of atypia in the tracheal washing of a carcinogen-treated hamster 7 weeks after start of carcinogen application (25 mg BP with Fe$_2$O$_3$). There is mild variation in nuclear size and shape and slight cellular pleomorphism. The cytoplasm is eosinophilic or organophilic. Papanicolaou, x 1080.

Fig. 5. Squamous metaplastic cells showing a moderate degree of atypia in the tracheal washing 12 weeks after start of carcinogen application (35 mg BP with Fe$_2$O$_3$). The nuclear and cellular pleomorphism has increased compared with Fig. 4. The cytoplasm is orangophilic. Papanicolaou, x 1080.

Fig. 6. Squamous metaplastic cells showing marked degree of atypia in the tracheal washing of a hamster 18 weeks after start of carcinogen application (45 mg BP with Fe$_2$O$_3$). There is a large variation in nuclear size. The nuclear material is condensed and is coal black. The cytoplasm is strongly orangophilic. Papanicolaou, x 1080.

Fig. 7. Cells suggestive of cancer in the tracheal washing of a hamster 15 weeks after start of carcinogen application (45 mg BP with Fe$_2$O$_3$). The nuclear cytoplasmic ratio is greatly enlarged. The cytoplasm is very dense. One of the nuclei is angulated and reveals coal black nuclear material. The other nuclei show a coarse nuclear material. Nucleoli are not observed. Papanicolaou, x 1080.

Fig. 8. Epidermoid carcinoma of a hamster in cytology and histology. a, exfoliated cells found in the tracheal washing 30 weeks after start of carcinogen application (45 mg BP with Fe$_2$O$_3$). Cells show little pleomorphism and are round or cuboidal, occasionally with beaded arrangement. The cytoplasm is basophilic and has a foamy appearance. The hyperchromatic nuclei show coarse chromatin material and multiple enlarged nucleoli. Papanicolaou, x 1320. b, well-differentiated epidermoid carcinoma found in the same hamster at necropsy 41 weeks after start of carcinogen application. The tumor was located at the carina of the trachea. H & E, x 540.

Fig. 9. Adenocarcinoma of a hamster in cytology and histology. a, exfoliated cells found in the tracheal washing 30 weeks after start of carcinogen application (45 mg BP with Fe$_2$O$_3$). Cells show little pleomorphism and are round or cuboidal, occasionally with beaded arrangement. The cytoplasm is basophilic and has a foamy appearance. The hyperchromatic nuclei show coarse chromatin material and multiple enlarged nucleoli. Papanicolaou, x 1320. b, bronchiogenic adenocarcinoma of the papillary type found in the same hamster at necropsy 35 weeks after start of carcinogen application. The tumor was located at the entrance of the left primary bronchus. H & E, x 540.

Fig. 10. Anaplastic large cell carcinoma of a hamster in cytology and histology. a, exfoliated cells found in the tracheal washing 30 weeks after start of carcinogen application (45 mg BP with Fe$_2$O$_3$). Cells are very large and have varying amounts of cytoplasm. Nucleoli are prominent and enlarged. There is a marked variation in nuclear size. Note pulmonary macrophages containing Fe$_2$O$_3$ particles (m). Papanicolaou, x 1320. b, anaplastic large-cell carcinoma found in the same hamster at necropsy 39 weeks after start of carcinogen application. The tumor was located in the left lung lobe. H & E, x 540.
Exfoliative Cytology in Respiratory Carcinogenesis

1a  1b  1c

2a  2b  2c  2d

3  4  5  6  7

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Hans Schreiber, Geno Saccomanno, Donald H. Martin, et al.

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