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What is This?
Factors Influencing Experimental Carcinogenesis in the Hamster Cheek Pouch

ALVIN L. MORRIS*

Department of Pathology and Division of Dental Research, University of Rochester, School of Medicine and Dentistry, Rochester, New York

Research in the field of oral cancer is the responsibility of the dental profession, whereby knowledge of the neoplastic process can be combined with an appreciation of the local environmental factors peculiar to the oral cavity. Salley1 gave impetus to this important field of investigation when he produced the first experimental oral squamous-cell carcinoma in the cheek pouch of the hamster. The opportunity now exists to investigate experimentally the relationship of many variables in the neoplastic transformation of an oral tissue. It is important to our progress that we be able to correlate the results of successive experiments and of experiments carried out in different laboratories. This can be accomplished only if experimental variables can be controlled so that the experimental lesion can be uniformly reproduced. The need which exists for the standardization of procedures stimulated the studies which are presented here.

Salley's2 work revealed that, of the three most commonly employed carcinogenic hydrocarbons, the application of 9,10-dimethyl-1,2-benzanthracene to the hamster cheek pouch epithelium elicited the greatest response. It was also shown that mineral oil was the vehicle of choice for that carcinogen. In order to study tissue response, it seemed necessary to standardize as much as possible factors related to tissue stimulation.3, 4 Greenstein,5 in discussing extrinsic carcinogenic factors, wrote:

Thus, these and many other observations indicate that the carcinogenic potency of an agent does not reside in the nature of the agent alone but is a function of the following factors: the dosage, the nature of the vehicle, the mode and length of time of administration of the agent; the strain, the species, the sex, and the age of the test animals; the site of application, the presence of concomitant factors such as the level of essential dietary constituents and the number of animals kept in a cage, and perhaps still other as yet unknown conditions.

In an effort to determine the extent to which some of the above-mentioned factors represent variables in the experimental production of cancer in the hamster cheek pouch, three separate experiments were carried out to investigate (1) whether the age of the animal at the time of initial exposure to a carcinogen affects the response of the cheek pouch mucosa (age experiment); (2) the response of the cheek pouch to different concentrations of a carcinogen (concentration experiment); and (3) whether the frequency of application of a carcinogen affects the response of the cheek pouch mucosa (frequency experiment).

* Present address: School of Dentistry, University of Pennsylvania, Philadelphia 4, Pennsylvania.
EXPERIMENTAL PROCEDURES

Age experiment.—Forty-five 3-week-old hamsters of both sexes were divided into three littermated groups. All these animals had been born on the same day. An additional group of fifteen was composed of approximately 18-month-old hamsters. All animals eventually received a 0.5 per cent mineral oil solution of 9,10-dimethyl-1,2-benzanthracene painted on the medial wall of both pouches three times per week. The painting was begun on the first group when they were 3 weeks of age (age at weaning); the second group when 6 weeks old; the third group when 9 weeks old. The 18-month-old animals of the fourth group were painted simultaneously with the first group. The animals were carefully examined at each painting and the date of development of the initial gross lesion in each animal was recorded after the manner previously described. As the lesions progressed in size, the largest tumor in each animal was biopsied. Painting of a given animal continued until a biopsy specimen from that animal was confirmed microscopically to be squamous-cell carcinoma. The date of the biopsy was recorded in the records as the date of malignancy. At this time, application of the carcinogen was stopped, but the animal was continued on the experiment without painting until death, the date of which was also recorded.

Concentration experiment.—Sixty 5-week-old hamsters of both sexes were divided into four littermated groups. Each group received one of the following concentrations (in mineral oil) of 9,10-dimethyl-1,2-benzanthracene: 1.5 per cent, 0.5 per cent, 0.1 per cent, and 0.05 per cent. The carcinogen was applied three times per week to the medial wall of both cheek pouches. After 15 weeks of the experiment, only three animals in the 0.1 per cent group had developed lesions, and the animals of the 0.05 per cent group were entirely free of any gross changes. It was decided at that time to discontinue painting the 0.05 per cent group. Since the two groups of animals receiving the weaker concentration showed no signs that exposure to the carcinogen was going to be responsible for their death, all remaining animals were sacrificed after 20 weeks of the experiment.

Frequency experiment.—Thirty hamsters of both sexes were divided into two littermated groups of fifteen each. A 0.5 per cent mineral oil solution of 9,10-dimethyl-1,2-benzanthracene was painted three times per week on both cheek pouches of the animals of one group. The animals of the other group were similarly treated twice weekly. The methods of painting and recording of data were identical with those described for the preceding experiments. The emphasis in this experiment was on the latent period required for tumor development. Biopsies were not performed on these animals, and no microscopic study of the tumors was carried out. The experiment was terminated after all animals exhibited tumors and an estimation had been made of the relative growth rate of tumors in the two experimental groups.

The animals used in the first two experiments were Syrian hamsters (Cricetus auratus) representing the eighth generation of a strain of animals developed in the Rochester Inheritance Study. The hamsters for the last experiment were obtained from the Albino Farms of Redbank, New Jersey. Approximately equal numbers of both sexes were used. All animals were separated by sex and kept in groups of three or four in wire-mesh cages over wood shavings. Tap water and Purina Fox Chow pellets were provided ad libitum. To this diet fresh carrots and lettuce were supplemented
weekly. Each animal was weighed once each week throughout the course of an experiment.

Detailed examination and biopsy of the hamster pouch was facilitated by the use of Johansen's immobilizer. A more rapid method for exposing the pouch for application of the carcinogen was devised which utilized the retractor shown in Figure 1.

The carcinogenic hydrocarbon 9,10-dimethyl-1,2-benzanthracene was obtained from the Distillation Products Division of the Eastman Kodak Company, Rochester, New York. It was dissolved in U.S.P. mineral oil and kept at all times in tightly stoppered actinic glass bottles. A No. 4 camel's-hair brush was used in a circular motion to paint the carcinogen on the anterior medial wall of the hamster cheek pouch. It was desired to deliver uniform amounts of carcinogen to each animal and to use a dosage which was large enough to produce maximum carcinogenic response without using great excesses, which might increase toxicity. Two painting techniques were investigated, namely, a "dripping-brush" method and a "wiped-brush" method in which the brush was wiped once against the side of the container before passing it to the hamster.

Fig. 1.—Diagram of hamster cheek pouch retractor. A, light shield; B, electric light bulb; C, magnifying glass; D, ring stand; E, immobilizer; F, retractor for lateral pouch wall. The hamster is held behind the neck and brought into contact with the retractor so that the arm (arrow) is inserted into the opening of the pouch. By moving the hamster horizontally, the lateral wall is retracted, exposing the interior of the pouch. (See insert.)
pouch. When watch glasses were weighed on an analytical balance before and after
painting with each method, it was revealed that reasonable uniformity was afforded by
both methods. Average amounts of solution delivered to the watch glasses were 47.7
and 31.3 mg. by the dripping- and wiped-brush methods, respectively. During an early
experiment in which sixty hamsters received the carcinogen by the different methods
in opposite pouches, no differences could be determined in the responses of the two
pouches. The wiped-brush method was thereafter used exclusively, since it afforded
less likelihood of extra-oral contamination with the carcinogen, was fast, and appar-
ently supplied to the pouch as much as, or more than, the minimum effective dose as
expressed in tumor production.

The latent period for tumor production was measured as the time required, after
the initial application of the carcinogen, for appearance of the first grossly observable
tumor. This period, important in the evaluation of data, depended for its accuracy on
the ability to recognize the very early lesion as soon as it became manifest. The initial
gross alteration of the hamster cheek pouch mucosa, indicating that a tumor would
subsequently occupy a given site, was observed to take one of three forms. It was first
seen as a tiny, pedunculated, reddish papilloma; a small, distinct, rounded raised pink
area; or an irregular whitish raised area. These changes were designated 1, 2, and 3,
respectively. The type of change first observed and its location were recorded in an
animal's individual record. The area of alteration was examined at subsequent paint-
ings, and any changes were recorded. When progressive growth into a frank tumor
was confirmed, the date of the first observed change was entered into the records as the
date of initial lesion.

For histologic examination, tumors were excisionally biopsied from the everted
pouch in animals anesthetized by intraperitoneal injection of sodium pento-barbital
(Veterinary Nembutal). After fixation in Bouin's solution, hematoxylin and eosin-
stained sections were prepared in the usual manner. The histologic appearance of a
normal hamster cheek pouch can be seen in Figure 2. Two early tumors exhibiting
considerable histopathologic variation are shown in Figure 3. Examples of experi-
mentally induced squamous-cell carcinomas arising in the hamster cheek pouch can
be seen in Figures 4 and 5.

RESULTS

The data for the age experiment are summarized in Table 1, which reveals that
whether the hamster is 3, 6, or 9 weeks of age at initial exposure to the carcinogen, it
has little effect on the length of time required for initial lesion formation. A significant-
ly greater length of time, however, is required for lesions to develop in the cheek pouch
of animals which are 18 months of age when first exposed.

Determination of time required for the tumors to become malignant depended on an
estimation of when to biopsy, based largely on the size of the tumors. From the data
obtained it is possible to say that most of the animals had malignant tumors after ap-
proximately 35 painting (12 weeks), but this cannot be interpreted to mean that many
of the biopsied tumors were not malignant much sooner.

The animals in the concentration experiment receiving the 1.5 per cent solution ex-
hibited an extreme reaction to the carcinogen after the second application. The mild
erythema and necrosis of the cheek pouch mucosa, commonly seen during the first
Fig. 2.—Histology of normal hamster cheek pouch. Hematoxylin and eosin. Medium power.

Fig. 3.—Early tumors arising in the cheek pouch of the hamster in response to repeated applications of a chemical carcinogen. Note the histopathologic variation in the two lesions. Hematoxylin and eosin. Low power.
Fig. 4.—Squamous-cell carcinoma arising from the cheek pouch of the hamster. Hematoxylin and eosin. Low power.

Fig. 5.—Section taken from within a papillomatous squamous-cell carcinoma arising in the hamster cheek pouch. Hematoxylin and eosin. Low power.
week of painting in animals receiving the 0.5 per cent concentration of the carcinogen, were very severe in the 1.5 per cent group. In some of these animals the pouches never healed, and eight animals of this group died before development of initial lesions. In contrast to this observation, the animals in the 0.1 per cent group exhibited only very mild erythema of the cheek pouch mucosa. The animals receiving 0.05 per cent were without observable changes. The animals in these last two groups appeared to be in conspicuously better condition throughout the course of the experiment.

Table 2 summarizes the data of this experiment. It reveals that those hamsters which survived the early applications of the 1.5 per cent carcinogen required practically the same length of time to develop initial tumors as did the animals of the 0.5 per cent group. The hamsters in the 0.1 per cent group all developed lesions by the end of the experiment, but the latent period was markedly increased. When these animals were sacrificed after 20 weeks, it was found that the tumors in nine of the group were malignant. It was noted that the animals of the 0.05 per cent group, which had no gross evidence of tumors when the painting was stopped after 15 weeks, apparently went on to develop lesions. When these animals were sacrificed 5 weeks later, six were found to have tumors, two of which were malignant.

During the early weeks of the experiment on frequency of application of the car-

### TABLE 1

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Initial</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Animals</td>
<td>Mean No. of Paintings</td>
</tr>
<tr>
<td>3 week</td>
<td>15</td>
<td>16.3±2.1</td>
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<tr>
<td>6 week</td>
<td>15</td>
<td>15.8±3.0</td>
</tr>
<tr>
<td>9 week</td>
<td>15</td>
<td>16.9±4.3</td>
</tr>
<tr>
<td>1½ year</td>
<td>11</td>
<td>20.8±3.9</td>
</tr>
</tbody>
</table>

* Compared with 3-week group.

### TABLE 2

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Initial</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Hamsters</td>
<td>Mean No. of Paintings</td>
</tr>
<tr>
<td>1.5 per cent DMBA</td>
<td>7</td>
<td>14.8±3.8</td>
</tr>
<tr>
<td>0.5 per cent DMBA</td>
<td>15</td>
<td>16.2±1.9</td>
</tr>
<tr>
<td>0.1 per cent DMBA</td>
<td>14</td>
<td>51.6±8.4</td>
</tr>
</tbody>
</table>

* Compared with 0.5 per cent group.
cinogen it was noted that the inflammatory response of the animals receiving the carcinogen only two times per week was conspicuously milder and somewhat later in developing than the inflammation seen in the other group. Eight and one-third weeks after the initial painting, tumors were present in all animals in the group painted three times per week. (Mean number of paintings required for initial lesions was 22.) Only four animals of the group painted twice weekly had developed tumors at this time. Twelve weeks after initial painting, one animal in the three-times-per-week group exhibited tumors less than 3 mm. in diameter, ten had 3–10-mm. lesions, and three had lesions larger than 10 mm. At the same time, in the group painted twice weekly, one animal was without a tumor, three had questionable initial lesions, seven exhibited tumors smaller than 3 mm., and two had lesions larger than 3 mm. With continued painting, all animals eventually developed tumors. It was thus evident that a shorter latent period was required for tumor development in the animals exposed to a carcinogen three times per week than in those receiving the carcinogen twice weekly.

It was felt that some insight into the effect of the carcinogen on the growth and development of the hamster could be gained by observation of changes in body weight throughout the course of the experiments. That the carcinogen itself had a detrimental effect, apparently exclusive of its carcinogenic action, is supported by the following observations: (1) the older the animals were upon first receiving the carcinogen, the greater was the weight which they ultimately attained; (2) an inverse relationship was observed between the weight ultimately attained and the concentration of the carcinogen received; (3) animals receiving the carcinogen only two times per week ultimately attained greater weights than those receiving the carcinogen three times per week.

Comparison of the total days of survival of the animals revealed that age at initial exposure had little effect on the number of days an animal survived. All animals followed a similar terminal course. The size and apparently the number of the tumors in the pouches continued to increase until there was gross evidence of swelling externally. In some cases erosion of tumors through the lateral wall of the pouch occurred. The animals became increasingly emaciated until the time of death. Although a consideration of the metastatic spread of these tumors was not included in this study, it has been observed that metastasis does occur, and one can readily demonstrate the presence of tumor in the submandibular lymph nodes.

During the course of the experiments described, a total of 150 hamsters was subjected to experimental carcinogenesis. The investigator, animal facilities, experimental procedures, and recording methods were the same in all experiments. Therefore, it seemed permissible to correlate all the results in an attempt to determine the extent to which sex, heredity, and caging conditions represented variables in this experimental production of oral cancer in hamsters.

In the experiments, sixty hamsters were studied which varied in age from 3 to 9 weeks on initial exposure to a 0.5 per cent concentration of the carcinogen. Thirty-two of these animals were males; twenty-eight were females. Since age variation between 3 and 9 weeks was shown to have no effect on carcinogenesis, the variable of sex alone could be studied in this group. No difference in the response of the two sexes to carcinogenic stimulation of the cheek pouch was observed. One sex might still be more suitable for this type of experimentation if it was found to be more
resistant to the toxic effects of the carcinogen, irrespective of tumor production. No difference could be observed, however, in the survival of the sixty hamsters in question, grouped according to sex. The females were observed to attain heavier weights than the males; however, this is a routine finding in normal hamsters.

The data were reviewed on the thirty animals which received weaker concentrations of the carcinogen and on the fifteen hamsters which received the carcinogen less frequently. Here again it was not possible to demonstrate a sex difference.

The majority of hamsters used in the experiments represented the eighth generation of animals developed in the Johansen and Hodge Rochester Inheritance Study. The possibility exists that a difference in cancer susceptibility has resulted from inbreeding of these animals. Such a difference was apparent when the response of these hamsters to carcinogenic stimuli was compared with that of a group of hamsters obtained from a commercial breeding farm. In this latter group the average latent period for tumor development was slightly longer. When the response of animals from different litters within either strain was studied, however, no variations could be demonstrated. It is concluded that hereditary susceptibility will not represent an experimental variable when doing cancer research on hamsters, as long as all animals on a given experiment are derived from the same strain.

In setting up experiments with hamsters, it is convenient to keep either three or four animals in each cage. It seemed desirable to rule out the possibility that conditions of crowding within a cage or relative availability of food and water could in some way affect animals being used for experimental cancer research. Individual records kept on animals used in the experiments presented here made it possible to compare the tumor production and weights of thirty hamsters, which difference could be demonstrated between the two groups.

**DISCUSSION**

It is of interest and importance to those working in the field of experimental oral cancer to be aware of the differences and similarities between those factors which influence the induction of neoplasia of oral tissues and those which influence neoplasia of non-oral tissues. Because of the limited amount of work to date in experimental oral cancer per se, it is necessary to relate the results of the work reported here to the findings of those working in experimental cancer in general.

There is a lack of agreement in the literature in regard to the relationship of age of animal to susceptibility or resistance to the development of experimental cancer. Some workers have concluded that age is of no importance. In only one of a series of experiments by Dunning and co-workers did age of animals present a variable in tumor production. The increased resistance of younger animals which they observed was attributed to the fact that it required more time to break down or wear out some defense mechanism in younger cells.

Frequent claims have been made for an increased susceptibility of younger animals to tumor development. Lemon and Smakula observed that sarcomas transplanted to the cheek pouch of 2-3-week-old hamsters grew at a faster rate than if the hamsters were 6-8 weeks of age at transplantation. Cowdry, Cowdry, Carruthers, and Suntzeff, and Cowdry and Suntzeff produced epidermal cancer in mice by applica-
tion of methylcholanthrene and observed young animals to be more susceptible. They suggested a difference in the sebaceous glands or in the chemical constituents of the skin as a possible explanation for their findings. Blum and co-workers\textsuperscript{15} induced tumors in mice by ultraviolet radiation. The induction time for old mice was longer than for younger animals receiving the same penetrability of younger skin. Strong, Smith, and Gardner\textsuperscript{16} observed that experimental sarcomas as well as carcinomas could be more easily produced in young animals. Shimkin\textsuperscript{17} has expressed the opinion that the greater susceptibility of young animals can be explained merely on the basis that they receive a larger carcinogenic dose per gram of body weight.

The results of the experiment reported here reveal that younger hamsters are more susceptible to experimental oral cancer. The cheek pouch does not contain sebaceous glands or other accessory structures, and so variations in these components cannot explain the age difference noted. It is doubtful that Shimkin's theory of increased dosage per gram of body weight can be applied, inasmuch as the animals in the 9-week-old group had attained the greater part of their growth before exposure to the carcinogen. It seems logical, however, that, in a tissue undergoing constructive metabolism, biochemical changes and abnormal cells would be produced earlier in response to carcinogenic stimulation.

In spite of the fact that the animals ultimately died at approximately the same age, a consideration of the weight curves suggests that the carcinogen was more toxic to the 3-week-old hamsters than to the older animals. This observation, coupled with the fact that the 3-week-old weanlings were too small to handle conveniently, makes it seem unwise to use animals of this age in future experiments. Approximately 5 weeks appears to be the age of choice from the standpoint of both ease of manipulation and tumor production.

Most reports in the literature which have dealt with variations in concentration of carcinogen have been concerned with subcutaneous injection of the carcinogen, a procedure which has doubtful application to the present experiment. Berenblum\textsuperscript{18} and Poel\textsuperscript{19} however, applied various concentrations of carcinogens to the skin of several species of laboratory animals with results similar to those presented here.

The early death of eight animals in the 1.5 per cent concentration group, even before the development of initial lesions, was felt to be due to the extremely severe inflammation seen in these animals. The direct relationship observed between degree of inflammation and concentration of the carcinogen, coupled with the inverse relationship between ultimate weight attained and carcinogen concentration, was taken as evidence of the toxic nature of the carcinogen. A concentration of 0.5 per cent of the carcinogen appears to be the one of choice for the rapid development of malignant tumors, from the standpoint of both a short latent period and maximum survival of the animals.

Many systemic and local factors are believed to have an effect on the metabolism of the oral tissues. It is interesting to speculate as to how these effects may be related to cancer of the mouth. It appears that it would be difficult to demonstrate an effect of subtle changes upon the carcinogenic process by using an experimental technique which elicits a tissue response of 100 per cent tumors in 12 weeks. It seems desirable, therefore, to have a weaker concentration which can be used to produce a submaximal response that might lend itself to alterations in tumor production as a result of subtle
alterations in the host tissues. The 0.1 per cent concentration apparently qualified in this regard. The 0.05 per cent concentration did ultimately develop tumors.

It is recognized that one cannot divorce concentration from dosage. Dosage, on the other hand, is closely related to frequency of application.\textsuperscript{20} Blum and co-workers,\textsuperscript{15} using ultraviolet light on the skin of mice, found that the induction time was shorter when the dosage was given in more frequent intervals. Saffiotti and Shubik\textsuperscript{21} observed that small repeated doses of carcinogen to the skin of Swiss mice were more effective than large single applications, irrespective of total quantity. The work of Cramer and Stowell\textsuperscript{20} and Salaman and Roe\textsuperscript{22} indicated that skin cancer can be induced in mice by smaller doses if the applications are made at longer intervals.

Evaluation of the data presented here reveals that a significantly extended latent period is required for tumor development in hamsters receiving the carcinogen only two times per week. This finding is consistent with that of Berenblum,\textsuperscript{18} who compared the results of one and two applications per week on the skin of mice. Thus, from the standpoint of rapid development of malignancies, it is preferable to paint the animals three times per week. From the standpoint of total dosage required to produce tumors, it can be calculated (from the average mg. of carcinogen delivered per painting) that it required a smaller total dose to produce tumors in all animals when the carcinogen was applied at longer intervals. This observation is similar to those made by other workers.\textsuperscript{20, 22}

There is a lack of agreement in the literature in regard to the effect of sex on cancer susceptibility. Dunning,\textsuperscript{10} Branch,\textsuperscript{23} Boyland and Warren,\textsuperscript{24} and Kreshover\textsuperscript{25} have reported that tissue response to chemical carcinogens is not influenced by sex. Leiter and Shear\textsuperscript{26} and Andervont\textsuperscript{27} found in different strains of mice that the males were more responsive. However, the former authors observed the difference only when marginal doses of the carcinogen were applied. Reinhard and Candee\textsuperscript{28} and Kreyberg,\textsuperscript{29} on the other hand, observed earlier tumor formation in female mice following the application of tar to the skin.

Various explanations for a sex difference have been proposed. Bullough\textsuperscript{30} pointed out that the skin of female mice experiences a twofold increase in thickness of the epidermis and frequency of mitoses in the pro-estrus period. Wicks\textsuperscript{31} has observed that males of several strains of mice ordinarily exhibit proteinuria, whereas females excrete much less and frequently none at all. This finding suggests that a difference may exist in the elimination of carcinogens and other materials from the body during carcinogenesis. Cowdry\textsuperscript{12} proposes that perhaps sex difference is accomplished by a difference in the disposal within the body of the carcinogen and/or its products. Using the hamster cheek pouch under the conditions of the experiments discussed here, there was no difference in the response of the males and females to carcinogenic stimuli.

Although some workers have doubted that malignant change is influenced by the genetic constitution of the host,\textsuperscript{10} the majority of evidence supports those who recognize strain variations in response to chemical carcinogens.\textsuperscript{13} Numerous pure strains of mice have been developed in which spontaneous tumors are common, suggesting a hereditary influence on cancer susceptibility.\textsuperscript{10, 12} Some of these inbred strains of mice have also displayed a greater tendency to develop tumors in response to locally applied chemical carcinogens.\textsuperscript{23, 24, 28, 29} From the results obtained in the experiments
reported here, it was apparent that, while hereditary susceptibility may vary between strains of hamsters, it does not represent an experimental variable when all animals on a given experiment are derived from the same strain.

SUMMARY

1. Standardized experimental techniques for chemical carcinogenesis in the hamster cheek pouch are presented.

2. Under the conditions of the experiments described here, the tissues of the cheek pouch of old hamsters are more resistant to carcinogenic stimuli than those of young hamsters. No difference in rate of carcinogenic response is seen between the ages of 3 and 9 weeks. Five weeks appears to be the ideal age for hamsters used for experimental oral carcinogenesis from the standpoint of ease of manipulation and tumor production.

3. Five-tenths per cent concentration of 9,10-dimethyl-1,2-benzanthracene is the optimal concentration for the rapid production of malignant tumors in the hamster cheek pouch. This concentration produces maximum tumor response with minimum latent period, with no loss of animals due to toxicity; 0.1 per cent and 0.05 per cent concentrations will produce graded submaximal responses in tumor production.

4. A shorter latent period is required for tumor development in animals exposed to a carcinogen three times per week than in those receiving the carcinogen twice weekly. A smaller total dose is required to produce tumors in all animals when the carcinogen is applied twice each week than when given three times weekly.

5. The response of the hamsters to the repeated application of the carcinogenic hydrocarbon 9,10-dimethyl-1,2-benzanthracene is not related to the sex of the animals under the conditions of the experiments presented here.

6. Conditions of caging (three or four hamsters per cage) had no apparent effect on the experimental results obtained in the experiments reviewed here.

REFERENCES


