Susceptibility of Guinea Pigs to Chemical Carcinogens: 7,12-Dimethylbenz(a)anthracene and Urethan

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SUMMARY

Single s.c. injections of 25, 20, 15, 10, 5, 1, and 0.1 mg 7,12-dimethylbenz(a)anthracene, administered at birth to randomly bred Hartley albino guinea pigs, resulted in the induction of various types of s.c. tumors at the injection site. In addition, malignant lymphomas, tumors of skin, lungs, uterus, ovaries, breast, etc., were also evoked by the treatment. Three i.v. injections of 3 mg 7,12-dimethylbenz(a)anthracene, given first at birth and on every third day, gave rise to higher incidences of tumors of the breast, lungs, uterus, ovaries, and skin than in the corresponding control groups. Five s.c. injections of 1 mg urethan/g body weight, administered similarly as in the previous group, produced tumors of lungs and ovaries.

In contrast to earlier reports, when the carcinogenic dose is expressed quantitatively on mg/g body weight basis, according to the present finding, the guinea pig was found to be at least as susceptible as the mouse to chemical stimulus.

INTRODUCTION

In the past several decades, chemical carcinogenesis studies were essentially limited to the use of 3 species: mice, rats, and hamsters. Sporadic attempts to introduce guinea pigs were made. These were soon, however, partially forgotten, apparently due to economic factors and because numerous investigators in the field felt that this species was relatively refractory (in comparison to mice and rats) to tumor induction by carcinogenic chemicals (3, 13, 22, 26, 27, 30, 33).

Earlier studies dealing with the induction of tumors in guinea pigs by chemicals and other types of agents have been tabulated (15, 28, 29). Reviews and evaluations on the subject have also been carefully prepared (4, 22). The production of tumors was most successful by chemicals, mainly with polycyclic hydrocarbons. These included DMBA (3, 18–20), urethan (6), 3-methylcholanthrene (2, 4, 8, 16, 18, 23, 24, 27, 30, 31), benzopyrene (presumably 3,4-benzopyrene) (13, 14, 17, 18, 26, 33), and 1,2,5,6-dibenzanthracene (16, 18, 21, 25). The relatively poor response of guinea pigs to the stimuli of carcinogenic chemicals has been attributed to an inherent species resistance. Such claims were primarily based on a number of observations that spontaneous neoplasms were less frequent in guinea pigs than in other laboratory animals. At the beginning, only a few, often single, cases of spontaneous tumors were reported to exist and were described at great length (1, 11, 12). Later, however, the investigators published their findings systemically and found more lesions (5, 9). The literature on this subject has been extensively reviewed from time to time (9, 22, 33).

The present work is part of our integrated program in studying the influence of age in chemical carcinogenesis and is intended to investigate the susceptibility of guinea pigs to the development of tumors by single and repeated injections of DMBA and repeated injections of urethan.

MATERIALS AND METHODS

Hartley albino guinea pigs were obtained from a randomly bred colony of The Institute for Medical Research, The Chicago Medical School, Chicago, Ill., and transferred in June 1968 to the present address. They were housed in stainless steel cages on wire mesh, separated according to sex in groups of up to 6, and given Rockland guinea pig diet in pellets, tap water ad libitum, and once weekly green lettuce.

The chemicals used were DMBA (Eastman Organic Chemicals, Rochester, N. Y.), purified by chromatography on magnesia/Celite, and ethyl carbamate (urethan, Fisher Scientific Company, Fair Lawn, N. J.), reagent grade. The purified DMBA was dissolved in tri-n-capryllin oil (Trioctanoin, Eastman) purified previously by vacuum distillation. The DMBA was also used in unpurified form in a special 15% fat emulsion prepared by the Upjohn Company, Kalamazoo, Mich. The fat emulsion contained: DMBA, 0.5% w/w; poloxalkol, 0.3% w/w; cottonseed oil, 15% w/w; water, sufficient to make 100 ml. Urethan was dissolved in sterile 0.9% NaCl solution. The experimental and control groups and the treatment are described as follows:

Group 1. Three newborn (less than 24 hr old) guinea pigs were given a single s.c. injection on the back of 50 mg DMBA in 0.5 ml tri-n-capryllin oil. The average weight of animals was 82 g (range, 70 to 106). The injections were...
made with a tuberculin syringe equipped with a 25-gauge needle.

Groups 2 to 8. The additional groups, Groups 2 to 8, were given similar injections of 25, 20, 15, 10, 5, 1, and 0.1 mg DMBA. Their average weights were 80, 95, 93, 93, 96, 100, and 94 g, respectively.

Group 9. Forty guinea pigs were given 3 i.v. injections in the brachial or femoral veins of 3 mg DMBA in 0.6 ml fat emulsion, starting at birth on every 3rd day (total, 9 mg). Their average weight at birth was 92 g (range, 70 to 121).

Group 10. Thirty-eight guinea pigs were given 5 s.c. injections, in the back, of 1 mg urethan/g body weight starting at birth on every 3rd day. Their average weight at birth was 91 g (range, 64 to 115).

Group 11. Twenty-one newborn guinea pigs were treated like Group 1, by an injection of 0.5 ml tri-n-capryllin oil. Their average weight was 96 g (range, 74 to 116).

Group 12. Twenty-one guinea pigs were observed from birth and kept as untreated controls. Their average weight at birth was 99 g (range, 72 to 134).

All the animals were carefully checked and weighed at weekly intervals, and the gross pathological changes were routinely recorded. The animals were allowed to die or were killed with ether when found to be in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and fixed in 10% buffered formalin. Histological studies were done on the liver, spleen, kidneys, adrenals, and at least 4 lobes of the lungs of each animal as well as on those organs which showed gross pathological changes. Sections from these tissues were stained routinely with hematoxylin and eosin and with additional special methods when necessary.

In tabulating the results, the latent periods of visceral tumors and malignant lymphomas were determined from the birth of the animal to the time of death. The latent periods of skin and s.c. tumors were based on the time at which they were first recognized grossly in the live animal.

RESULTS

Survival Rates

The survival rates at weaning (8 weeks of age) in the treated and control guinea pigs are recorded in Tables 1 and 2. At weaning time, the incidences of survivors were the following in the various groups: 1, 0; 2, 14%; 3, 13%; 4, 29%; 5, 70%; 6, 58%; 7, 100%; 8, 96%; 9, 52%; 10, 76%; 11, 100%; 12, 90%. The survival rates after weaning were recorded through the entire experiment. It became evident from the data that a single injection of 25, 20, 15, 10, and 5 mg and the repeated i.v. injections of DMBA reduced the survival. The lower doses of DMBA and the urethan treatment had no apparent effect on it.

Tumor Incidences

The number and latent periods of the different types of tumors are given in Tables 1 and 2. It is obvious that the treatments significantly enhanced the formation of various neoplasms. In the following, a brief description of the more important lesions is presented.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of injected animals</th>
<th>No. of survivors at weaning</th>
<th>No. of tumor-bearing animals</th>
<th>Total no. of tumors</th>
<th>No. of animals with tumors at injection site</th>
<th>[latent period: age (wk)]</th>
<th>No. of animals with other tumors</th>
<th>[latent period: age (wk)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25 mg DMBA</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1 papilloma of skin (35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20 mg DMBA</td>
<td>30</td>
<td>19</td>
<td>3</td>
<td>2</td>
<td>1 malignant lymphoma (7)</td>
<td></td>
<td></td>
<td>1 malignant lymphoma (11)</td>
</tr>
<tr>
<td>4</td>
<td>15 mg DMBA</td>
<td>34</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2 malignant lymphoma (5, 6)</td>
<td></td>
<td></td>
<td>1 malignant lymphoma (16)</td>
</tr>
<tr>
<td>5</td>
<td>10 mg DMBA</td>
<td>31</td>
<td>11</td>
<td>6</td>
<td>9</td>
<td>2 adenocarcinomas of stomach (137, 153)</td>
<td></td>
<td>1 adenocarcinoma of stomach (153)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5 mg DMBA</td>
<td>31</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>2 adenocarcinomas of breast (12, 80)</td>
<td>1 adenocarcinoma of breast (153)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 mg DMBA</td>
<td>26</td>
<td>18</td>
<td>14</td>
<td>26</td>
<td>5 adenocarcinomas of stomach (139, 149, 164, 199, 251)</td>
<td></td>
<td>2 adenocarcinomas of thyroid (251, 255)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>100 µg DMBA</td>
<td>29</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td>3 adenocarcinomas of breast (141, 164, 171)</td>
<td></td>
<td>1 adenocarcinoma of breast (141)</td>
<td></td>
</tr>
</tbody>
</table>

*a*Single s.c. injection in tri-n-capryllin oil.

*b*Latent period: age in weeks.
### Table 2

#### Tumor distribution in DMBA- and urethan-treated and control guinea pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial no. of guinea pigs at birth</th>
<th>No. of tumorbearing animals</th>
<th>No. of animals with tumors [latent period: age (wk)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 DMBAIjjections</td>
<td>40</td>
<td>10 9</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>11 d 8 11</td>
<td>11 d 6 10</td>
<td>11 d 8 11</td>
<td>18 d 6 7</td>
<td>11 d 6 10</td>
</tr>
<tr>
<td>10 Urethan injections</td>
<td>38</td>
<td>12 9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11 Control, oil injected</td>
<td>21</td>
<td>9 d</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>12 Control, uninjected</td>
<td>21</td>
<td>9 d</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

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**Subcutaneous Tumors at the Site of Injection**

A total of 33 treated animals developed 37 tumors at the injection site. Histologically, they were classified as follows: fibrosarcomas, 16; rhabdomyosarcomas, 3; myxosarcomas, 3; hemangiosarcomas, 3; fibromas, 3; chondrosarcomas, 2; hemangiomas, 2; liposarcoma, 1.

Cytologically, the fibrosarcomas were composed of spindle-shaped fibroblasts arranged in an orderly fashion and exhibited a fairly uniform degree of malignancy (Fig. 1). In myxosarcomas, the stellate-shaped cells had multiple cytoplasmic processes and were located in loose stroma of fine fibrils (Fig. 2). In chondrosarcomas, the large atypical hyaline cartilage cells were interwoven with the neoplastic fibroblasts (Fig. 3). The hemangiosarcomas consisted of fibrosarcomatous elements interspersed with angioma-like sinuses (Fig. 4). The cells of liposarcomas were of the giant, ring-shaped variety, most of them with a single large vacuole containing lipid (Fig. 5). The fibromas showed a scarlike tissue mass with darkly stained, oval nuclei and abundant halo-like cytoplasm.

**Other Tumors**

**Adenocarcinomas of Breast.** As can be seen from the tables, 19 animals developed 23 adenocarcinomas of the breasts. Interestingly, in addition to those in the 15 females, such lesions were also observed in 4 males. Histologically, the cuboidal epithelial cells of the glands formed acinar structures. Their sizes and shapes were rather uniform, and they invaded the surrounding parenchyma.

**Tumors of Ovaries.** Altogether 8 animals developed such lesions. In 6, the tumors were classified as adenomas (Fig. 6); out of these, in 3 instances, tumors were found only in 1 ovary, while in the other 3 females, adenomas were observed in both organs. Furthermore, in 2 females, adenocarcinomas were seen, one unilateral and the other bilateral.

**Tumors of Lungs.** In 14 animals, 22 lung tumors were observed. Out of these, in 11 animals the lesions were classified as adenomas, and in the other 3 they were found to be adenocarcinomas.

**Tumors of Thyroid.** Only 2 animals developed unilateral lesions and both were classified as adenomas.

**Skin Tumors.** Eleven animals had 11 lesions. All were benign and were classified as papillomas.

**Tumors of Uterus.** Seventeen animals developed 21 tumors of this organ. Out of these, 9 animals had 12 adenocarcinomas; most of them were serous type (Fig. 7), and a few were mucous type (Fig. 8). Three had 3 squamous cell carcinomas, 2 had 2 adenomas, 1 had 2 hemangiosarcomas, and in the remainder in each a myxosarcoma and a fibroma have been observed.
In addition, in a number of animals other types of neoplasms, including 14 malignant lymphomas (3 lymphocytic type and 11 histiocytic type), were found. They are neoplasms, including 14 malignant lymphomas (3 lymphocytic type and 11 histiocytic type), were found. They are listed in the tables.

DISCUSSION

The present results demonstrate that single s.c. injections of 25, 20, 15, 10, 5, 1, and 0.1 mg DMBA administered at birth to guinea pigs resulted in the induction of various types of s.c. tumors at the injection site. In addition, malignant lymphomas, tumors of skin, lungs, uterus, ovaries, breast, etc., also were evoked by the treatment. Three i.v. injections of 3 mg DMBA, given at birth and on every 3rd day, gave rise to higher incidences of tumors of the breast, lungs, uterus, ovaries, and skin than were observed in the corresponding control groups. The 5 s.c. injections of 1 mg urethan/g body weight administered as in the previous group also produced tumors of the lungs and ovaries.

There are only 4 previous reports on the production of tumors in adult guinea pigs following the administration of DMBA. In the first study, repeated s.c. injections totaling 3 to 5 mg induced local sarcomas in one-half of the treated animals (19). In the second investigation, single s.c. injections of 20, 5, and 1 mg were administered, and sarcomas at the site of injection were obtained only with the first 2 doses. One mg had no apparent carcinogenic effect during 15 months of observation. In a parallel group, skin painting with 2 to 3 drops once weekly for 78 weeks produced skin carcinomas (3). In a subsequent work, when 6 guinea pigs each received a single s.c. implantation of 100, 200, 300, 400, or 500 mg, sarcomas were developed only in the 2nd and 4th dose levels (20). Finally, in a more recent study, single s.c. injections of 5 mg DMBA evoked rhabdomyosarcomas in two-thirds of the animals (18). Urethan, on the other hand, did not induce tumors. Only one attempt has been made, however, and in this, 0.1% solution in the drinking water was given for life (6). Other types of polycyclic hydrocarbons have also been effective in inducing tumors in guinea pigs. One of the most widely used was 3-methylcholanthrene (2, 4, 8, 16, 18, 23, 24, 27, 30, 31), but others such as benzopyrene (13, 14, 17, 18, 26, 33) and 1,2,5,6-dibenzanthracene (16, 18, 21, 25) were also used successfully. They were usually administered by s.c. injections, and their tumor yields were essentially confined to the s.c. tissue.

It may be of interest to reexamine the generally accepted view that the guinea pig is less susceptible than other species to tumor development by carcinogenic chemicals. In a previous study in this laboratory, 100, 75, 50, 25, 10, and 1 µg DMBA as single s.c. injections were administered to newborn Swiss mice weighing an average of 1.4 g (32). In the present experiment, DMBA was given to guinea pigs under identical conditions as in the Swiss mice in doses of 25, 20, 15, 10, 5, 1, and 0.1 mg, although the average weight of the guinea pigs was 94 g. Doses as low as 1000 µg in guinea pigs and 50 µg in mice were effective in inducing tumors. This means, in fact, that the guinea pigs received the carcinogen at 10 µg/g body weight while the mice were treated on a 35 µg/g basis. It appears, therefore, that when the comparison is expressed quantitatively according to the present calculation, the guinea pig is at least as susceptible as the mouse to this carcinogenic stimulus.

Of course it is intriguing to speculate whether or not the susceptibilities of various species to tumor development should be expressed on a mg/g body weight basis. Unfortunately, this subject has not received much attention in the past; only a few unconnected reports have appeared thus far. In this regard, it may be pertinent to point to the findings of drug toxicity studies conducted in various species, including man. By comparison of the different dose levels, it was established that, on a mg/sq m body surface area basis, the maximum tolerated dose in man is about the same as that in each animal species. On a mg/kg body weight basis, however, the maximum tolerated dose in man is much less than in the various lower species (10). The toxic and carcinogenic properties of a given chemical should not be confused. Although the exact nature and the relationship of these 2 phenomena are still basically unknown, it is claimed more recently that, in the case of diethylnitrosamine (7), each has its distinct mechanism of action.

In conclusion, it appears from the present results that it may be profitable to study the susceptibilities of higher species to tumor development, because valuable information concerning the effective carcinogenic dose could be learned. This could very well be useful in helping to figure out the situation in man instead of guessing without experimental evidence.

ACKNOWLEDGMENTS

I thank Dr. Philippe Shubik and Dr. Umberto Saffiotti for their constant interests and encouragements, Mrs. Irene Boreisha for her technical assistance in the experimental work, and Miss Prudence Blackman for the histological preparations.

REFERENCES

Fig. 1. Fibrosarcoma, s.c. The spindle-shaped neoplastic fibroblasts are arranged in an orderly fashion. Note the moderate amount of intercellular connective tissue fibers. The cells exhibit a rather uniform degree of malignancy throughout the entire tumor. Male guinea pig, 29 weeks old, given 1 injection of 5 mg DMBA. H & E, X 32.

Fig. 2. Myxosarcoma, s.c. The stellate-shaped cells are located in a loose stroma in which numerous fine reticulin fibrils occur. Observe the dendritic cytoplasmic processes of the tumor cells. A 16-week-old female guinea pig given 1 injection of 15 mg DMBA. H & E, X 450.

Fig. 3. Chondrosarcoma, s.c. Observe the chondroblastic and fibroblastic elements side by side. Note the atypical hyaline cartilage cells which are interwoven with the spindle-shaped fibroblasts. A 34-week-old male guinea pig given 1 injection of 25 mg DMBA. H & E, X 450.

Fig. 4. Hemangiosarcoma, s.c. The tumor consists of vascular and sarcomatous patterns. Observe the spindle-shaped fibrocytes interspersed with angioma-like sinuses. A 78-week-old male guinea pig given 1 injection of 5 mg DMBA. H & E, X 450.

Fig. 5. Liposarcoma, s.c. The entire area is composed of neoplastic lipoblasts. Few vessels are also visible. Note the distinct, usually unicellular giant signet cells often with a single large vacuole containing lipid. A 22-week-old male guinea pig given 1 injection of 10 mg DMBA. H & E, X 32.

Fig. 6. Adenoma of ovary. Note the papillary pattern of the epithelium. The supporting stroma is markedly evident. A 125-week-old female guinea pig given 9 mg DMBA. H & E, X 56.

Fig. 7. Adenocarcinoma of uterus. The serous type, with the characteristic glandular pattern. A 137-week-old female guinea pig given 1 injection of 20 mg DMBA. H & E, X 32.

Fig. 8. Adenocarcinoma of uterus. Composed of the mucous epithelium with substantial amount of connective tissue stroma. A 141-week-old female guinea pig given 1 injection of 100 µg DMBA. H & E, X 115.
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Figure 1: [Image of cellular tissue]

Figure 2: [Image of cellular tissue]

Figure 3: [Image of cellular tissue]

Figure 4: [Image of cellular tissue]
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