Effects of Dimethyl Sulfoxide on Dimethylbenzanthracene-induced Carcinogenesis in the Hamster Cheek Pouch

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SUMMARY
The studies demonstrated that the use of dimethyl sulfoxide (DMSO) as a vehicle for dimethylbenzanthracene in the production of experimental tumors in the hamster cheek pouch, significantly reduced the latent period for tumor production when compared to the usual mineral oil vehicle. No systemic or local changes could be attributed to the DMSO itself, and the morphology of induced tumors was not changed. This finding may be of significance for the possible use of DMSO in other carcinogenesis studies and, particularly, for further investigations of the two-phase phenomenon of carcinogenesis in the hamster cheek pouch.

INTRODUCTION
During the twelve years since dimethylbenzanthracene (DMBA)-induced carcinomas were first produced in the hamster cheek pouch (9, 10), efforts have been made to demonstrate in this mucous membrane the two-phase phenomenon of carcinogenesis originally described in skin by Berenblum (1, 2).

The suitability of the hamster cheek pouch as a site for the study of mucous membrane carcinogenesis has been the subject of some controversy. Its differences from the moist environment of the oral cavity itself have been pointed out (14). The lack of a portal of entry through the thickly keratinized pouch epithelium also has been considered a drawback. Another difficulty is the length of the latent period for tumor induction. Despite the fact that, using radiographic and fluorescence microscopy methods (8, 11, 12), carcinogens have been shown to penetrate the epithelium, repeated applications are necessary, even with optimal carcinogenic doses, to obtain a latent period of 8 to 12 weeks. When the suboptimal carcinogenic doses required for the evaluation of various promoting agents are employed, the latent period is further lengthened until almost the entire life span of the animal may be required to obtain useful results. Although carcinogens themselves have been shown to pass through the cheek pouch epithelium, there is no evidence to indicate that other agents, of possible interest as tumor promoters, have similar properties. A number of failures with regard to the penetration of these latter agents have been experienced in our laboratory.

That vehicles used for the application of carcinogens may shorten the latent period for tumor induction was demonstrated in the cheek pouch with a non-ionic surface active agent, polyoxyethylene sorbitan monostearate (3). This material and related sorbitan monostearate derivatives have also been shown to have similar effects in skin carcinogenesis experiments (4, 13). During the past few years, considerable interest has been centered on dimethyl sulfoxide (DMSO) for a large variety of investigational and clinical uses. For the purposes of this study, its principal properties of interest were that it is an excellent solvent of low toxicity (15), that it has great penetrating qualities (7), and that it enhances the absorption of a variety of drugs through skin and mucous membranes without apparently affecting their pharmacologic properties (5, 6).

The objective of this experiment was to evaluate the effectiveness of dimethyl sulfoxide when used as a solvent for dimethylbenzanthracene. Specifically, the aim was to see if DMSO would affect the latent period and character of induced tumors by increasing the penetrability of the carcinogen.

MATERIALS AND METHODS
A total of 112 male golden Syrian hamsters, 9 weeks of age, were divided into seven groups of 16 animals each. Pairs of animals were housed in small mesh wire cages over a bedding of Pellicel. Their diet consisted of laboratory chow and water ad libitum. Three groups received applications of 0.005% (increased to 0.05% after 17 weeks), 0.1%, and 0.5% DMBA dissolved in DMSO, respectively. Three other groups received the same concentrations of carcinogen dissolved in mineral oil. The seventh group served as a control and received applications of DMSO only.

The solutions were applied to the right cheek pouch by means of a No. 4 camel’s hair brush, according to the method described by Morris (9). All animals were weighed and examined weekly, and the cheek pouches were inspected with each application of...
solution. The 0.005% and 0.05% solutions were applied twice weekly, while the 0.1% and 0.5% solutions were administered three times weekly.

Animals in the group receiving 0.05% DMBA in DMSO were sacrificed and autopsied at 2-, 5-, 10-, and 15-week intervals after the appearance of tumors. The group receiving 0.05% DMBA in mineral oil, and the DMSO control group were autopsied at the end of the 52-week experimental period. Animals in the 0.1% and 0.5% groups were sacrificed with complete autopsies when impending death from tumors was obvious.

Data was gathered on the latent period of first tumor formation, the number and size of tumors produced, and the survival time with tumor in the 0.1% and 0.5% carcinogen groups. Histologic examinations consisted of recording the morphologic characteristics of the tumors, the incidence of metastasis and evidence of pathologic changes in body organs. Tissues were studied on routine sections stained with hematoxylin and eosin.

RESULTS

The results of the experiment with regard to latent periods for tumor formation are summarized in Table 1. None of the animals receiving 0.05% DMBA in mineral oil or those receiving DMSO alone developed tumors. By contrast, the incidence of neoplasms in all of the other groups was 100%. All of these tumors were diagnosed microscopically, as squamous cell carcinomas. Of particular interest is that although the solution employed in the first group mentioned was too weak to produce tumors with a mineral oil vehicle, the incidence in the DMSO control group was too weak to produce tumors with a mineral oil vehicle. These differences are statistically significant at the 0.1% level of confidence.

A comparison of mean latent periods in the 0.1% and 0.5% groups revealed that the DMBA-DMSO groups developed tumors 113 and 22 days earlier, respectively, than the corresponding DMBA-mineral oil groups. These differences are statistically significant at the 0.1% level of confidence.

An unexpected finding occurred in the group receiving 0.5% DMBA in DMSO to the right cheek pouch. In this group, 8 of the 10 surviving animals also developed tumors in the left cheek pouch. One additional animal, which did not survive long enough to develop a lesion on the right side did, however, have a tumor on the left side prior to death. Of the 9 tumors in the contralateral pouch, 7 were diagnosed histologically as squamous-cell carcinomas and the other 2 as papillomas. No tumors of the left pouch were observed in any of the other six groups of this experiment.

Although close to 2,000 animals have been used in our laboratory over the past eight years in a variety of carcinogenesis studies, such a finding has never been noted previously. No reference to this phenomenon was found in reviewing the literature. Although local invasion of pouch carcinomas is very common and most animals also develop skin lesions of the right side of the face, presumably due to "spillover" of solutions, metastasis to distant organs is rarely seen. Mucoal lesions due to "spillover" occur occasionally but are usually confined to the lips. In contrast to the presence of contralateral pouch tumors, another, and in the light of the previous finding, unexpected, observation was made in the groups receiving 0.5% and 0.1% DMBA with DMSO. The skin lesions usually seen on the side ipsilateral to the painting when mineral oil was the solvent for DMBA did not occur in the groups in which DMSO was used.

Data on mean survival days with tumor is summarized in Table 2. Although some differences between groups were noted, these did not prove to be statistically significant.

On histologic examination, no morphologic differences were noted in the DMBA-produced squamous-cell carcinomas regardless of which vehicle was used, nor did the tumors on the contralateral side appear different from those on the experimental side. The lesions were either moderately or well differentiated and were primarily exophytic in nature. In the group receiving 0.05% DMBA in mineral oil in which no grossly visible tumors were found, acanthosis, hyperkeratosis, and some mild cellular atypia were the only changes noted.

Examination of other tissues obtained at autopsy failed to reveal any other pathologic changes. No metastatic lesions were found. In view of the findings in the contralateral cheek pouches of one group, sections of tongue, buccal mucosa, and esophagus were viewed with particular attention. No evidence of epithelial changes was noted in any of the sections.

DISCUSSION

The results of these experiments indicate that the use of dimethyl sulfoxide as a vehicle or adjuvant may have considerable potential in experimental carcinogenesis by shortening the latent period needed for tumor formation with suboptimal carcinogenic doses. With regard to the hamster cheek pouch, it should be very helpful in future studies designed to investigate the relationship

![Table 1: Latent Periods for Tumor Production with Dimethylbenzanthracene (DMBA) in Mineral Oil and Dimethyl Sulfoxide (DMSO) Solvents](image1.png)

![Table 2: Mean Survival Days with Tumor with Dimethylbenzanthracene (DMBA) in Mineral Oil and Dimethyl Sulfoxide (DMSO) Solvents](image2.png)
between systemic and local factors in the production of mucous membrane tumors and in the study of the two-phase phenomenon of carcinogenesis. Due to the lack of accessory skin structures in the cheek pouch, it has been difficult to obtain penetration of solutions. Therefore, the latent period for tumor production, particularly with the suboptimal doses of carcinogens necessary to study initiation-promotion, has been excessively long.

The occurrence of contralateral cheek pouch tumors in animals receiving a high dose of DMBA in DMSO requires additional investigation. The histologic pattern of these tumors and the behavior of DMBA-induced cheek pouch tumors in general make it unlikely that these lesions were metastatic. The absence of surrounding skin and intraoral lesions and the failure of this phenomenon to occur in other groups make an explanation based on "spillover" appear unlikely. One possible explanation is that the carcinogen may have been introduced with food pellets which the hamsters store in their pouches and may shift from one side to the other. In that case, the phenomenon further attests to the powerful potentiating action of DMSO.

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REFERENCES

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