The Effect of Solvents in Methylcholanthrene Epidermal Carcinogenesis*

A Comparison of Benzene and Acetone

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The application of carcinogenic hydrocarbons to the skin of experimental animals has been the subject of numerous investigations since Cook and Kennaway and their associates first used dibenzanthracene in 1930. However, few observations have been published on the effect of the method of application or of the solvent for the carcinogen, upon carcinogenesis. In other publications (6-8) we have emphasized the importance of variations in the technic of the application of methylcholanthrene, such as the frequency of painting, the size of the exposed area, and the concentration of the carcinogen. In this paper, which is a contribution to a group investigation directed by Dr. E. V. Cowdry, benzene and acetone are compared as solvents for 20-methylcholanthrene in epidermal carcinogenesis.

The importance of solvents in the production of sarcoma by the subcutaneous injection of methylcholanthrene has been discussed by others, including Shimkin and Andervont (12).

Benzene and chloroform have been the most commonly used solvents for the topical application of carcinogens. In 1937, Taschner, Gottlieb, and Spritzer (14) used acetone as a solvent for methylcholanthrene. They found it less toxic than benzene for the mice. Benzene and acetone were compared by Taschner and Spritzer (15). In 1938, Taschner and Gottlieb (16) compared acetone and chloroform. They found acetone to be as effective as chloroform and less toxic than benzene.

In 1939, Shimkin and Andervont (12) used acetone as the solvent for various hydrocarbons applied to the skin of mice. For his study of changes antecedent to tumor formation, Orr (9) preferred acetone to benzene as a solvent for carcinogenic hydrocarbons because acetone had less effect upon the skin. Pullinger (10) also stated a preference for acetone as a solvent, but none of these investigators published comparative observations on the effect of solvents upon carcinogenesis.

When Crabtree (5), to retain the carcinogen in a film at the site of application, added 2 per cent liquid paraffin to acetone, ether, or benzene, the production of benign tumors in the skin of mice was accelerated. He used several different carcinogens in his series and did not directly compare the solvents acetone and benzene. But, as Watson (15) and subsequently Bonser (4) have shown, the more rapid production of benign epidermal tumors under one set of conditions does not necessarily indicate an earlier production of malignant tumors. Quite recently Bradbury, Bachmann, and Lewisohn (3), using chiefly small groups of 15 or less mice, reported that acetone had a definite accelerating effect on the action of certain carcinogenic agents when applied to the skin. They attribute this to the water-miscible property of acetone.

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MATERIALS, METHODS, AND RESULTS

Female Swiss mice 3 months of age were kept under similar environmental conditions in three series which were run simultaneously. In the "benzene series" (series I) 39 mice were painted thrice weekly with a 0.3 per cent solution of methylcholanthrene in benzene. In the "acetone series" (series II) a 0.3 per cent solution of methylcholanthrene in acetone was applied to 39 mice 3 times a week. The 45 mice in series III—the "acetone-benzene series"—were painted thrice weekly with the same 0.3 per cent solution of methylcholanthrene in acetone on the same days as the animals in series I and II and, in addition, on alternate days, 3 times a week, they were painted with a solution of the pure benzene solvent. The benzene used was of Analytical Reagent grade and the acetone of U.S.P. quality. Because of its low solubility it is not feasible to use a solution of a higher concentration than 0.3 per cent of methylcholanthrene in acetone. When not in use all solutions were kept tightly stoppered in the icebox to reduce evaporation of the solvent and changes in concentration of the solute.

Using a standard technic described in a previous paper (6, 7), the various solutions were applied by the same worker to a moderately large area of the back of each mouse with a single stroke of a No. 4 camel's hair brush. All mice received 42 applications in a period of 100 days, after which time no additional treatment was given. The mice gained in weight, suggesting that the painted solutions were well tolerated. Macroscopic observations on the presence of epilation, ulceration, swellings, papillomas, and malignant neoplasms were recorded each week. In each mouse the presence of a malignant neoplasm was confirmed by microscopic examination of several sections taken at different angles through the skin of the mouse's back.

At the end of the 1st week of the experiment 85 per cent of the mice in the benzene series, 78 per cent in acetone-benzene series, and 64 per cent of the animals in the acetone series showed some degree of epilation.
By the 3rd week the hair had started to regrow in many of the animals so that fewer mice showed complete epilation; however, practically all of the mice in each series showed some epilation.

On the basis of our previous experiments, tumors were classified as elongated swellings, warty swellings, papillomas, or malignant tumors. The elongated swellings appeared near the midline of the back as one or two ridges in the skin parallel to the long axis of the mouse and to the direction in which the brush with its solution was applied. These elongated, edematous-appearing swellings often disappeared rather suddenly or they were sometimes transformed into warty swellings or into true papillomas. The warty swellings were more discrete, semiglobular elevations in the skin, and they either regressed or developed into papillomas.

The incidence of elongated swellings, which was similar in each series, increased rapidly up to the 3rd week after which it began to decrease. The warty swellings, a nonneoplastic type of tumor which often developed eventually into papillomas, increased steadily in incidence after the 2nd week for several weeks. At the 3rd week 77 per cent of the mice in the acetone series, 67 per cent of the mice in the acetone-benzene, and 56 per cent of those in the benzene series had either elongated or warty swellings on the painted skin area. The production of swellings was slightly slower in the benzene series which did not reach its maximum of 67 per cent until the 4th week of the experiment.

Papillomas are usually larger than the warty swellings and, in contrast to the swellings, represent a type of tumor which very rarely regresses while an animal is being painted with the carcinogen. The first papillomas in the benzene and in the acetone-benzene series appeared after 6 weeks in the acetone series, 67 per cent of the mice in the acetone-benzene series, 67 per cent until the 4th week of the experiment. The next highest incidence was obtained in the benzene series using methylcholanthrene in acetone and on alternate days with methylcholanthrene. For instance, by the 16th week of the experiment, had heterotopic bone in the skin adjacent to an anaplastic carcinoma. A detailed report (13) has been made of this and of three other cases of osteosis cutis which occurred in another experiment.

Several experiments were performed to study the early changes produced in the mouse's skin by solutions of methylcholanthrene and of acetone and benzene. Details of observations on the effects of a 0.6 per cent solution of methylcholanthrene in benzene after single and repeated applications to the skin of the backs of mice have already been reported (7, 8). We found the initial effect of the carcinogen upon the skin to be a toxic one reaching its maximum at about the 3rd day after the first painting, at which time there was a destruction of the epithelial cells in the central portion of the painted area surrounded by a peripheral zone of differentiated epidermis of increased thickness. In contrast to the normal mouse epidermis which is about two cells thick, this differentiated epidermis more clearly resembles normal human epidermis and contains basal, spinous, and granular layers and an increased amount of keratin. By the end of the first week the destructive effect was diminished and the peripheral area of skin had increased further in thickness. By the end of the second week the central portion of the painted epidermis also became differentiated.
Similar results were observed in groups of mice painted with a 0.3 per cent solution of methylcholanthrene in either acetone or benzene. Following a single application of this solution the destructive effect was similar but somewhat less pronounced, and the epithelium showed a similar degree of differentiation. With repeated paintings of the acetone solution of the carcinogen, there was less dilation of the blood vessels in the early stages and the lymphatics, especially those around the hair follicles, appeared to be more dilated.

The effect of the pure benzene and acetone solvents upon the mouse skin was also studied. Two mice were killed on both the 3rd and 5th days following a single application of solutions of benzene and of acetone. Although with benzene little change was visible in the microscopic appearance of the skin on the 3rd day, by the 5th day the epidermis had increased in thickness and become differentiated. In some areas there was also a rather diffuse infiltration of the dermis by inflammatory cells, chiefly polymorphonuclear neutrophils and macrophages. On the other hand, following a single painting with the acetone solvent no evident changes were seen in the skin after 3 or 5 days.

To study the vasodilatory effect of methylcholanthrene and of the acetone and benzene solvents, various solutions were painted on localized areas of the ears of a rabbit. The results after a single application are summarized in Table I, in which the intensity of the hyperemia is indicated by the number of plus signs.

The single application of a 0.6 per cent solution of methylcholanthrene in benzene produced an intense hyperemia of the skin extending slightly beyond the periphery of the painted area which lasted 6 days. The hyperemia from pure benzene alone was less pronounced and of briefer duration. The vasodilation in the rabbit's ear produced by a single application of a 0.3 per cent solution of methylcholanthrene in acetone, which represents virtually a saturated solution, is slower in onset, and of about 5 days' duration. Pure acetone, on the other hand, did not produce any appreciable hyperemia of the skin. With several repeated applications of 0.3 per cent solutions of methylcholanthrene in benzene and in acetone to the rabbit's ear, the hyperemia after the second application persisted several days longer than after a single painting. After the 3rd and 4th paintings the vasodilation was less pronounced and of briefer duration.
DISCUSSION

The results shown in Fig. 1 indicate that the carcinogenic response to a 0.3 per cent solution of methylcholanthrene is better in acetone than in benzene solvent. These observations are in agreement with those of Bradbury, Bachmann, and Lewishohn whose series of mice were much smaller than ours.

The efficacy of the carcinogenic response of epidermis to methylcholanthrene is usually analyzed by criteria relating to time, such as the incidence of cancer in the first animal, in 50 per cent of the group, and in 100 per cent of the animals. Differences of only a few weeks in these criteria, especially when small numbers of experimental animals are involved, may not be significant. In experiments designed to achieve a maximum carcinogenic response in a relatively short period of time, a more significant criterion may be the percentage of malignant tumors occurring during a period of several weeks of the experiment.

In a group of 27 Swiss mice painted with a 0.6 per cent solution of methylcholanthrene in benzene, in which the carcinogen is much more soluble, the first carcinoma appeared in 6 weeks and by the 24th week all the animals had developed malignant tumors (7). Although the 0.6 per cent solution of methylcholanthrene in benzene produced carcinoma earlier in both the most susceptible and in the most resistant animals of the group, the incidence of malignant tumors during selected periods of several weeks was higher with the 0.3 per cent solution in acetone. Between the 14th and 17th week of the experiments 67 per cent of the mice painted with 0.3 per cent methylcholanthrene in acetone, 46 per cent of the mice painted with 0.3 per cent methylcholanthrene in benzene, and 41 per cent of the mice painted with the 0.6 per cent methylcholanthrene in benzene developed carcinomas. Actually, a better carcinogenic response to the 0.6 per cent solution was obtained at a later period, between the 14th and 19th weeks of the experiment, when 59 per cent of the mice developed malignant neoplasms.

SUMMARY AND CONCLUSIONS

Epidermal carcinogenesis was obtained somewhat more rapidly with acetone than with benzene as a solvent for 0.4 per cent methylcholanthrene. Mice painted with a 0.3 per cent solution of methylcholanthrene in acetone 3 times a week and with pure benzene on alternate days developed cancer even more rapidly. Fifty-six per cent of the 123 mice painted with methylcholanthrene in these experiments had multiple malignant neoplasms of the skin.

The hyperemia of the skin of a rabbit’s ear after the application of methylcholanthrene in benzene was a combined effect of the carcinogen and of its solvent. The less pronounced hyperemia after the application of methylcholanthrene in acetone was caused by the carcinogen alone, since pure acetone did not produce appreciable hyperemia.

REFERENCES


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