The fluorescence microscopic study (5) of the absorption by mouse skin of methylcholanthrene in benzene has shown that a large portion of the carcinogen goes immediately to the sebaceous glands, where it is found dissolved in the lipid droplets within the gland cells. Another portion is absorbed by the keratinized layer of the epidermal epithelium, where it is dissolved in the free intracellular lipids of that layer. There is no evidence from these fluorescence studies that any of the carcinogen is absorbed directly by the living cells of the epidermis.

After a single application of methylcholanthrene the sebaceous glands degenerate rapidly and usually disappear completely by the fourth day. As the glands degenerate, sebum with its dissolved carcinogen is pushed into the hair follicles and, through them, onto the surface of the skin. Here it redissolves a part of the dry carcinogen remaining from the time of the painting. This layer of carcinogen-containing lipid bathes the surface of the skin until the keratin layer flakes off, usually at the sixth to eighth day. During this 6 to 8 day period localized areas of the epidermis and of the hair follicles become hyperplastic and show a tendency toward differentiation. The epithelial cells assume irregular sizes and shapes and often contain abnormal nuclei. Earlier work (1, 2, 3) had shown that these changes are often progressive, leading, in a considerable fraction of susceptible strain animals, to the development of carcinomas. In order to understand better the significance of the selective distribution and retention of methylcholanthrene, it was desirable to determine the reaction of skin to the carcinogen dissolved in a medium that resembles sebum.

Anhydrous wool fat (anhydrous lanolin) was chosen as a vehicle for this purpose since it presumably represents chiefly the secretion of the sebaceous glands of the sheep. We have no data on the composition of sebum from the mouse, but have assumed that it is similar to that of other species. 20-Methylcholanthrene was dissolved in the melted lanolin to a concentration of 0.3 per cent. This solution was melted (40–45°C at the time of use) and applied by means of a brush to a large area of the back of each mouse.

**Single Application**

In the first experiment, 15 male and 15 female mice, laboratory bred from Swiss strain mice purchased of Tumblebrook Farm, were shaved over a large part of the back and to each was applied, after an interval of several days, a quantity of the carcinogen solution sufficient to contain approximately 0.3 mgm. of methylcholanthrene. This is the same amount of the carcinogen as was used in benzene solution by Cramer and Stowell (3) to produce malignant neoplasms in this strain of mice. Regrowth of hair occurred in these mice just as in untreated shaved mice. There were subsequently no skin changes to indicate that the mice had received treatment with a carcinogenic chemical. After 8 months the 26 survivors appeared to be perfectly healthy, normal animals.

**Repeated Applications**

The failure of methylcholanthrene in lanolin to induce any macroscopically visible change in the skin of the mice in the first experiment led us to consider the effects of continuous application of such a solution. Previously reported experiments (6) had shown that in Swiss mice thrice weekly applications of a 0.3 per cent solution of methylcholanthrene in benzene for 14 weeks caused the development of malignant tumors in 33 per cent of the mice by the end of the period of painting, in 50 per cent at the end of the 17th week, and in all the surviving animals at the end of the 26th week.

In the present experiment 50 mice of the Swiss strain were used. Since mice were not available from the source previously used (Tumblebrook Farm) these animals were purchased from the Albino Farms, Red Bank, New Jersey. Experiments with a benzene solution carried out at the same time indicate that the Albino Farm Swiss strain mice readily developed skin cancer. The carcinogen in lanolin was applied 3 times weekly for 14 weeks to the unepilated skin with an
average dose per application calculated to be approximately 0.2 mgm. This is from 2 to 4 times the dose that was given when thrice weekly paintings with benzene solutions were employed. Macroscopic examinations in ultraviolet light show that such a lanolin solution spreads uniformly over a large area of the back of the mouse, remaining semifluid from the heat of the animal's body. Fluorescence microscopic studies of skin painted with the methylcholanthrene-lanolin solution show that the absorption of the carcinogen in this vehicle is similar to the absorption of a benzene solution. Since the solvent, lanolin, is not volatile, there is no crust of dry, crystalline methylcholanthrene left on the surface of the skin and the only fluorescence observed is the blue-violet characteristic of the dissolved carcinogen. This appears in the sebaceous glands and in the keratin layer. After a single painting this fluorescence disappears in 2 to 3 days, which is much quicker than with a benzene solution. Even so it may be assumed that with the 3 paintings weekly the skin was exposed continuously to the carcinogen in lanolin for the entire 14 week period.

These results with the lanolin solution of methylcholanthrene are in striking contrast to those obtained with benzene solutions. Epilation, which is an early and regular occurrence with the latter method of application, does not occur. At the end of the 14 week period of painting, 47 mice survived. No tumors had appeared. A week after the painting was stopped a few mice were sacrificed and their skins were examined microscopically (Fig. 1). No hyperplasia was observed, although a slight increase in the amount of keratin was noted. Sebaceous glands were still present but much reduced in size and less numerous than in normal mice. Some increase was noted in the amount of fat within and beneath the dermis. None of the surviving mice had developed a malignant tumor at the end of 26 weeks. One mouse that died in the 23rd
week had a small, warty growth. Microscopic examination showed it to be a keratinizing papillomatous wart of precancerous character. Two other mice were found to have each one minute papilloma at the end of the 26th week. At this time, 12 weeks after the last application, a few more mice were killed for microscopic examination of the skin (Fig. 2). There was no hyperplasia of the epidermal epithelium. Sebaceous glands, having undergone some degree of restoration, were, if anything, larger than normal. There were no changes to indicate that the skin had been considered. Fluorescence spectra were made of methylcholanthrene dissolved in benzene and in anhydrous lanolin. These were compared only with the naked eye, a method believed by Hieger (4) to be more satisfactory than the use of a microphotometer. The position and relative intensity of the three characteristic bands in the blue-violet end of the spectrum appeared to be identical in spectra of the two solutions. Though this is but a crude check, it appears unlikely that a chemical alteration of the carcinogen has resulted from its solution in lanolin.

![Fig. 2. Axial section of mouse skin 12 weeks after the last painting with lanolin solution of methylcholanthrene. The amount of subcutaneous fat is approximately that of normal skin. The sebaceous glands, indicated by arrows, are restored. They are somewhat less frequent but are larger than the sebaceous glands of normal skin. There are no changes in the epidermis or hair follicles to show that the skin had been treated for 14 weeks with methylcholanthrene. Schmidt preparation, unstained. Mag. X 140.](image)

exposed to a potent carcinogen. With the exception of the 3 mice referred to above, the lanolin had completely protected the skin against the carcinogenic and other effects of methylcholanthrene. Throughout the experiment the animals were in good condition, the mortality was low, and many mice were heavier and more obese than mice treated with a benzene solution of methylcholanthrene.

The experiment has been repeated with 60 more mice and at the time of writing—18 weeks after the first application of the lanolin solution of methylcholanthrene—the results are again completely negative.

**Fluorescence Spectra**

The possibility of a chemical combination between the carcinogen and anhydrous lanolin has been considered.

**DISCUSSION**

It is clear that, under the conditions of our experiments, solution of the carcinogen in anhydrous lanolin deprived the hydrocarbon almost completely of its carcinogenic property. Even after 42 applications of such a solution the sebaceous glands persist, though they are smaller and less numerous than in normal skin; the skin is not epilated; and there is no hyperplasia of the epidermis or of the hair follicles. This finding is in itself of interest, but it has, in addition, a special bearing on the fact that the carcinogen, when first applied in benzene solution to mouse skin, is selectively absorbed by the sebaceous glands and rapidly destroys them. Subsequent paintings with methylcholanthrene act on a skin area devoid of sebaceous glands.
Thus we find that methylcholanthrene in benzene, which is effective as a carcinogenic agent, first destroys the sebaceous glands, and that the same substance, in a vehicle resembling sebum, neither destroys sebaceous glands nor induces other changes leading to the development of cancer. Unless the sebum of mice differs essentially from that of sheep it would follow that the sebaceous glands provide a protective mechanism against chemical carcinogens.

Previous observations on the effect of a combination of lanolin with chemical carcinogens on experimental carcinogenesis have been made by C. C. Twort and J. M. Twort (7), but their results were contradictory. In 1930, when working with relatively weak carcinogenic agents such as shale oils and tars, they found that "the addition of animal and vegetable oil to the cancer producing material diminishes the capacity [to form cancer] far more than the dilution would lead one to suspect." Of the various oils used by the Tworts, lanolin had the most pronounced effect in diminishing carcinogenic potency. Later, in 1939, they (8) confirmed this statement with the qualification that it holds true only for dilute solutions or small quantities of carcinogenic gas tars. If, however, relatively large quantities or strong solutions of gas tars are applied, addition of lanolin to the tar will result in a greater yield of tumors than that observed among the controls treated with tar only. In experiments with dibenzanthacene the addition of "a small quantity of lanolin" to the chloroform solution increased "somewhat" the potency of the carcinogen.

The technic of our experiments with methylcholanthrene differs from that used by Twort and Twort in their later experiments. They dissolved the carcinogen in chloroform and added a small quantity of lanolin to this solution, while in our experiments the carcinogen was dissolved in melted lanolin and no other solvent was used.

**SUMMARY AND CONCLUSION**

The carcinogenic activity of methylcholanthrene is almost completely suppressed when it is dissolved in melted anhydrous lanolin and the melted solution is applied to the skin of mice 3 times weekly for 14 weeks. Lanolin represents the sebum of sheep. Unless this sebum differs essentially from that of mice, our results suggest that the sebaceous glands act as a protective mechanism against this chemical carcinogen.

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Sebaceous Glands and Experimental Skin Carcinogenesis in Mice

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