MORPHOLOGICAL CHANGES IN THE SKIN OF ANIMALS AFTER A SINGLE APPLICATION OF CARCINOGENIC HYDROCARBONS

N. N. Vasil'eva

UDC 612.79.014.46 : 615.277.3 : 547.21

After a single application of 9,10-dimethyl-1,2-benzanthracene (DMBA) signs of polymorphism and atypia of the epidermis against the background of diffuse and irregular hyperplasia were visible on the 9th day, while foci of proliferation were observed on the 11th day. As a rule the precancerous changes in the epidermis were accompanied by changes in the connective tissue of the sclerosing dermatopathia type after application of 9,10-dimethyl-1,2-benzanthracene, 20-methylcholanthrenen, and benzpyrene, the number of sebaceous glands in the skin of mice falls rapidly and sometimes the glands disappear altogether. Regeneration of the sebaceous glands after application of methylcholanthrene and benzpyrene is observed on the 7th-9th day, and after application of dimethylbenzanthracene on the 15th-17th day.

An important aspect of the study of the theoretical basis of cancer prophylaxis is the discovery of the ways by which carcinogenic substances enter the animal body and their ultimate fate.

Since carcinogenic hydrocarbons are readily soluble in fats, after application to the skin they are distributed more or less uniformly on its surface, they dissolve in the sebum, and accumulate selectively at the mouths of the hair follicles. They penetrate rapidly into the sebaceous glands through their efferent ducts, undergo absorption there, and can then be carried by the blood stream to organs and tissues, as is proved by the development of "distant" tumors (of the mammary glands, lungs, forestomach, an so on) after application to the skin.

Investigations have shown that the earliest morphological manifestations of carcinogenesis develop, after the epidermis, in the sebaceous glands and hair folicles [1, 3, 6]. Even a single application of carcinogenic hydrocarbons leads to disappearance of the sebaceous glands in mice very early (5-7 days after application). Attempts have been made to use this phenomenon as a rapid test for evaluation of the carcinogenicity of compounds [2, 4, 5, 7].

The object of the present investigation was to compare the morphological reactions in the skin of mice, rats, and guinea pigs, animals which differ in their sensitivity to carcinogenic agents, to a single application of carcinogenic and noncarcinogenic hydrocarbons: 9,10-dimethyl-1,2-benzanthracene (DMBA), 20-methylcholanthrene (MC), benz(α)pyrene (BP), pyrene (P), and anthracene (A).

EXPERIMENTAL METHOD

Experiments were carried out on 514 animals (Table 1): 35 guinea pigs, 192 rats (noninbred and Wistar), and 287 mice (lines A and C57BL).

Solutions of the test substances (1% BP and P solutions; 0.5% MC and A solutions; 0.25-0.5% DMBA solution) in benzene were applied to the previously shaved skin of the interscapular region: 2 drops to each mouse and 5 drops to each rat and guinea pig. Applications to the skin of the mice and rats were made in

Department of Carcinogenic Agents, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 3, pp. 116-119, March, 1971. Original article submitted December 22, 1969.

TABLE 1. Scheme of Experiment with Single Application of Solutions of Carcinogenic and Noncarcinogenic Hydrocarbons in Benzene to the Skin

Species of animals	No. of animals	Longest period (in days)	Dose (in μg)				
			DMBA	A	BP	P	MC
Guinea pigs	35	27	400	400	800	800	—
Rats	192	37	400	400	800	800	400
Mice	287	42	40	80	160	160	80

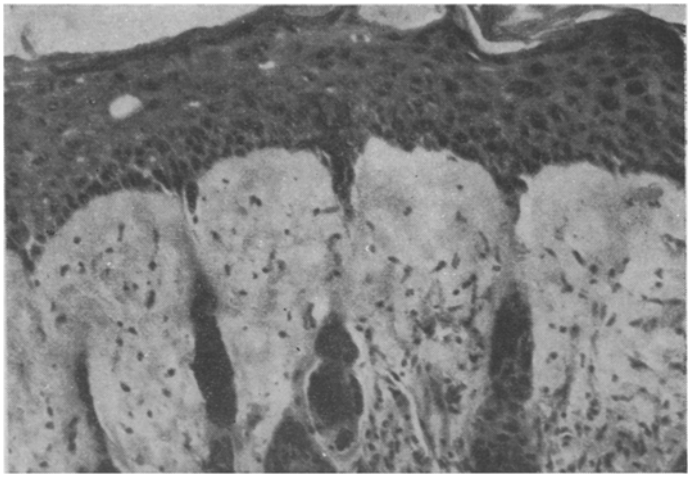

Fig. 1. Skin of C57BL mouse 9 days after single application of DMAB (No. 48). Diffuse, irregular hyperplasia of epidermis and epithelium of hair follicles. Absence of sebaceous glands. Sclerosis of upper part of dermis. Hematoxylin-eosin, 112.5×.

the resting phase of the hair cycle. This was achieved by plucking the hair from the area of skin to be treated 19-20 days before application. In guinea pigs the hair is shed continuously and in no definite order, so that when the tests were performed on these animals it did not matter when the carcinogenic agent was applied.

The animals were killed between 1 and 42 days after application at intervals of 1-2 days. Skin for histological examination was fixed in 10% acid formalin and Carnoy's fluid. Paraffin and celloidin sections were stained by the ordinary methods. Fluorescence of the carcinogenic hydrocarbons was also investigated in unstained fresh frozen sections of the skin, and further sections were studied by the same method after staining with a caffeine solution of benz(α)pyrene by Berg's method to show the sebaceous glands.

EXPERIMENTAL RESULTS

Microscopic examination of the material showed that the most marked hyperplastic changes in the epidermis and inflammatory reaction of the dermis was produced in all animals by DMBA, followed by MC and BP, in agreement with the relative carcinogenic activities of these hydrocarbons. The strongest reaction was found in the skin of the mice. Morphological changes in the skin of the rats and guinea pigs were much less pronounced and were almost identical in type.

Sebaceous glands could not be found in the skin of the rats or guinea pigs at any stage of the experiment. In mice, after a single application of DMBA, MC, and BP, there was a marked decrease in the number of sebaceous glands on the 3rd-5th days, accompanied by hyperplastic changes in the epidermis, and not always corresponding to the severity of the inflammatory reaction of the dermis. Sebaceous glands were found in the skin of the mice at all stages of the experiment after application of the noncarcinogenic hydrocarbons anthracene and pyrene.

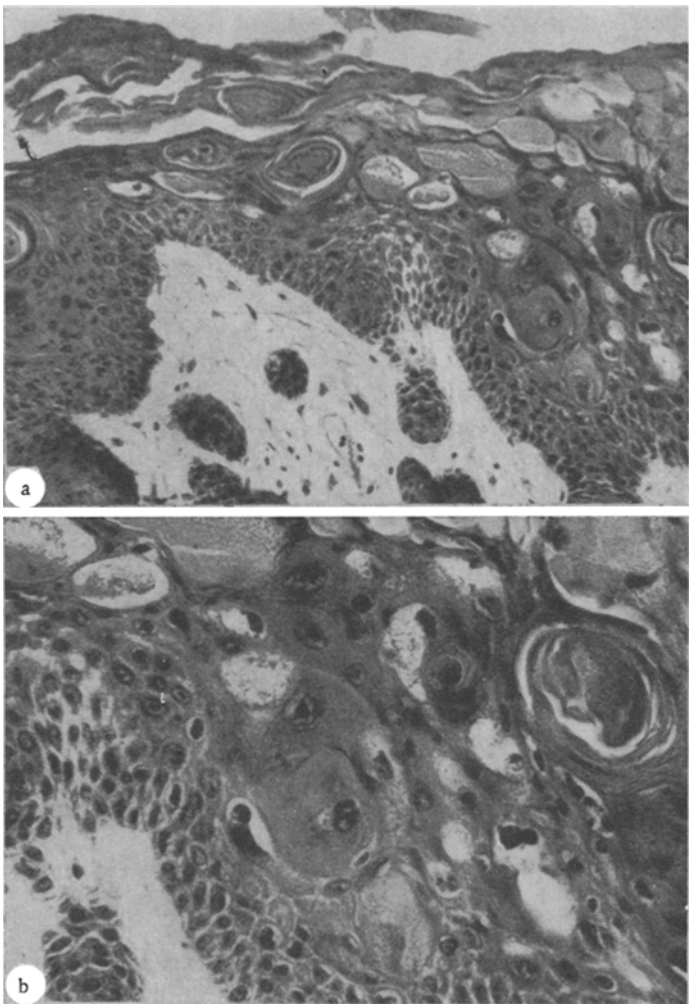

Fig. 2. Skin of C57BL mouse 11 days after single application of DMBA (No. 60): a) focal proliferation of epidermis; sclerosing dermatopathia; inflammation; absence of sebaceous glands. Hematoxylin-eosin, 112.5×; b) polymorphism and atypia of epidermal cells. Hematoxylin-eosin, 200×.

After application of BP to mice, degenerative and necrobiotic changes were found in the cells of the sebaceous glands starting from the 1st day after application. After a single application of DMBA on the 9th day, against the background of a well-marked diffuse, irregular hyperplasia of the epidermis, evidence of atypia and polymorphism of the epithelium was observed (Fig. 1), and on the 11th day foci of proliferation began to appear (Fig. 2a, b). Restoration (regeneration) of the sebaceous glands was observed on the 7th-9th day after application of MC and BP, but not until the 15th-17th day after application of DMBA.

An inflammatory reaction of the dermis was observed in mice after application of all 3 compounds, but it was strongest in the case of DMBA. In the early stages of the experiment (1st-3rd days) the inflammatory lesions in the dermis were mainly focal in character, with infiltration by lymphocytes, histiocytes, and neutrophils. From the 5th to the 7th day, marked edema was observed in the surface layers of the dermis. The process became diffuse. Neutrophils began to predominate among the infiltrating cells. Microabscesses were formed on the sites of the destroyed sebaceous glands (around the hair follicles).

On the 9th-11th day, the foci of inflammatory infiltration contained many lymphocytes, histiocytes, and fibroblasts; very few neutrophils were present. Later (starting from the 15th day) the foci of dermal sclerosis were more extensive; the intensity of the inflammatory reaction was considerably reduced.

In the late stages of the experiment (21st-39th day) only a few focal collections of lymphocytes and histiocytes were present around the blood vessels and skin appendages.

Most commonly the severity of the inflammation (the degree of spread of the process, the character of arrangement of the foci of infiltration, the number of polymorphs present) corresponded to the degree of the hyperplastic changes in the epidermis. In cases of diffuse, irregular hyperplasia of the epidermis with evidence of atypia and polymorphism, however, and also in the presence of foci of proliferation, as a rule the inflammatory changes in the dermis were minimal and the most predominant features were distinctive changes in the superficial parts of the connective-tissue bases of the skin typical of sclerosing dermatopathia.

A study of fluoresence of fresh frozen sections through the mouse skin showed that BP was retained longest (up to 7 days) in the sebaceous glands. Its fluorescence on the skin surface could be detected up to 5 days, and at the mouths of the hair follicles up to 9 days. Fluorescence of DMBA on the skin surface and in the sebaceous glands was observed only during the 1st day, and at the mouths of the hair follicles for up to 3 days. MC was detected in the sebaceous glands only until the 3rd day, but otherwise the duration of its fluorescence was the same as for BP.

LITERATURE CITED

1. W. C. Hueper, Nat. Cancer Inst. Monogr., No. 10, 577 (1963).
2. J. Kracht, G. Grimmer, and E. Hübner, Proceedings of the 8th International Cancer Congress [in Russian], Vol. 2, Moscow-Leningrad (1963), p. 530.
3. W. Montagna and H. B. Chase, Anat. Rec., 107, 82 (1950).
4. W. E. Smith, Bull. New York Acad. Med., 32, 77 (1956).
5. W. E. Smith, Acta Union. Internat. Canc., 13, 77 (1957).
6. V. Suntzeff, C. Carruthers, and E. V. Cowdry, Cancer Res., 7, 439 (1947).
7. V. Suntzeff, A. B. Crominges, E. L. Wyndes, et al., Cancer (Philadelphia), 10, 250 (1957).