METHOD OF ESTIMATING SMALL DOSES OF 3,4-BENZPYRENE IN THE SKIN OF MICE

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Among many investigations of the problem of chemical carcinogenesis, a group can be distinguished in which attempts were made to examine the fate and conversion in the organism of the carcinogenic substance itself [2, 4, 5, 8, etc.].

The chance of a successful solution of problems of this type is largely determined by the existence of a method of identification of both the carcinogen and its derivatives. To identify the carcinogenic hydrocarbons, their fluorescence may be used, for its spectrum is reasonably characteristic [6].

The earliest investigators observed changes in the character of fluorescence of the skin and other organs at various times after exposure to one of the most powerful carcinogenic hydrocarbons, namely 3, 4-benzpyrene (3,4-BP). Later, methods were introduced to estimate the quantity of carcinogen in biological material. Frequently the object used in such investigations was the skin of small laboratory animals, in most of which 3,4-BP produces tumors comparatively easily by surface application [7-10].

As a rule the method used to identify the carcinogens was that of analysis of the spectrum of fluorescence. The spectra of fluorescence of skin extracts were studied at various times after painting with the carcinogen [3, 11] or the spectra of fluorescence of microscopic preparations were examined (in fixed and unfixed material) [1, 8]. Comparatively recently results have been obtained showing changes in the spectrum of fluorescence after introduction of 3,4-BP into a special capsule mounted on the rabbit's ear [9].

Attempts by most investigators to identify the spectra not only of 3,4-BP itself, but also of its derivatives, led to the result that the doses used in these investigations were fairly large, and mainly determined by the method itself. Meanwhile, it has now been shown that small doses of a carcinogen, if applied repeatedly, may be used to demonstrate a process of "pure" carcinogenesis without the development of intensive inflammation, ulcers, and so on.

Besides, the investigation of the action of small doses of carcinogens is of special importance because clearly such doses should be used for studying the effect of various carcinogenic factors in the external environment on man and when analyzing the basis for appropriate hygienic measures.

To investigate the dynamics of resorption of small doses of 3,4-BP in the skin of mice, the author has constructed an apparatus and has developed an experimental method.

The apparatus for determining the intensity of fluorescence of 3,4-BP is based on the Soviet ML-2 microscope. Its block diagram is shown in Fig. 1. To excite fluorescence, the region of the spectrum of a type SVDSh-250 mercury lamp transmitted by a type UFS-3 filter (8 mm) was used. The intensity of fluorescence was recorded by a type FEU-35 photomultiplier. Since a maximum of the intensity of fluorescence in the region 400-420 mμ (its precise position depends on the character of the solvent) is characteristic of 3,4-BP, while in the spectra of its metabolites and in the spectrum of the natural fluorescence of the skin the intensity of fluorescence in this particular region is very small, an interference filter with a transmission maximum in the region of 413 mμ was introduced before the photomultiplier instead of the usual ZhS-3 blanking filter. The changes in the intensity of fluorescence falling on the photocathode mainly reflected changes in the concentration of dissolved 3,4-BP and were practically independent of the number of crystals of this substance or the concentration of its metabolite. The signal from the photomultiplier was fed through a correcting amplifier to an ordinary panel galvanometer (50 mA), the readings of which

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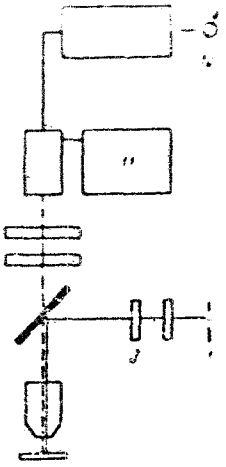

Fig. 1. Block diagram of the apparatus for estimating 3,4-benzpyrene in the skin of mice. 1) Light source; 2) condenser; 3) UFS-3 filter (8 mm); 4) object; 5) objective of microscope; 6) interference plate; 7) ZhS-3 filter; 8) interference filter (413 μ); 9) FEU-35; 10) amplifier; 11) source of supply for FEU-35; 12) galvanometer.

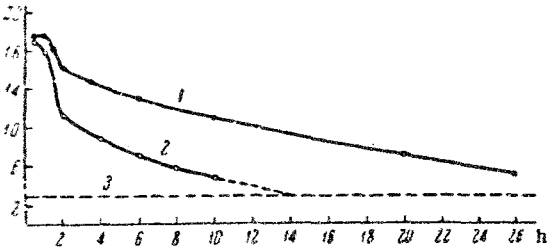

Fig. 2. Dynamics of changes in the intensity of fluorescence after application of 0.5 μg 3,4-benzpyrene. 1) After 20 applications; 2) application to intact skin; 3) background before application.

corresponded to the intensity of fluorescence (413 mμ). The recording circuit was supplied from an electronic stabilizer. The sensitivity of the circuit was determined and periodically checked by relation to a standard solution of 3,4-BP Experiments were carried out on male C57BL and (C57Bl×CBA)F_1 mice. The 3,4-BP was applied in drops to a preliminarily shaved area of the animal's back.

Histological sections cut on a freezing microtome with a cooled knife from unfixed material and also the fluorescence of the skin in vivo were investigated. For this purpose the mice were fixed by their four limbs to a flat plate of organic glass resting on the stage of the microscope. The mice remained alive after such an investigation lasting 7-9 h, and could be used again in the experiments. In this way the dynamics of the resorption of 3,4-BP could be studied in the same animal for several days after application of the substance to the skin.

As a result of the study of the histological sections the following findings were obtained.

Following application of a large dose of carcinogens (100 μg in 1 or 2 drops of solvent) large quantities of 3, 4-BP remained as crystals on the skin surface sometimes up to 24 h. The fluorescence of the various structures of the hair follicles, the surface layers of the epidermis, and the sebaceous glands in this case could be observed for up to 48 h. The intensity of fluorescence reached its maximum after 3-4 h; thereafter it remained practically unchanged so long as yellowish-green crystals of undissolved 3,4-BP were observed on the surface. As soon as the crystals disappeared the intensity of fluorescence (413 mμ) diminished and after 24 h reached the background level.

After the application of a small dose of 3,4-BP (10 μg) no yellowish-green crystals were found on the skin surface after 20 min. The intensity of fluorescence (413 mμ) reached a maximum at 1-2 h, counting from the moment of application of the 3,4-BP. After 24 h the intensity of fluorescence fell to the background level.

The results obtained from the study of the dynamics of the intensity of fluorescence (413 mμ) in vivo after application of different doses of 3,4-BP are summarized in the table.

It is clear from the table that the general principles revealed by the investigation carried out on living mice repeated those observed during the study of the sexes. The presence of a spread-out maximum could be explained by solution of the crystals left on the surface. With small doses, after 24 h no fluorescence (413 mμ) could be found.

Use of the opportunity of repeating the investigation on the same animals, the effect of repeated application of 3,4-BP on the dynamics of its resorption in the skin could be studied (Fig. 2). Analysis of the curves in Fig. 2 revealed a difference in the rate of resorption of 3,4-BP in the intact skin even after prolonged application (for 20 weeks), i.e., toward the time of appearance of primary tumors.

Dynamics of Intensity of Fluorescence (413 mμ) in Vivo after Application of Various Doses of 3,4-Benzpyrene (3,4-BP) to Mice

Dose per animal (in μg)	Number of animals	Position of maximum of fluorescence during period after application (in h)	Time of decrease of fluorescence to background levels (in h)	Remarks
40	6	3-5	30-48	Presence of 3,4-BP crystals in the first few hours
4	13	1.5-2	24-30	
0.4	18	0.5-1	13-20	

The proposed method of recording the intensity of fluorescence (413 mμ) can be used to study the dynamics of resorption of 3,4-BP in the skin of living mice. The method can be used to determine the effective dose of a carcinogen, i.e., to take into account not only the substance applied, but also the time during which a given dose is present at the site of application. It is also possible that certain modifying factors influencing the appearance of tumors (age and sex and species of the animals, the phases of the hair cycle, physical and chemical influences) may in fact not only affect tumor growth by their influence on the rate of resorption of carcinogens. As an example of such influences [illegible] may be considered, because on the one hand they increase the incidence of appearance of tumors with the same total dose administered and on the other hand they lead to the phenomenon of deposition of the carcinogen at the site of application.

The discovery and study of the principles influencing the delay or acceleration of resorption of 3,4-BP from the skin may be carried out much more easily by the suggested method than by other methods described above, because it enables the animals to be preserved for repeated investigations and for studying the dynamics of the process. In addition, by means of this method one individual animal can be studied and, finally, the number of animals used may be much smaller without any reduction in the reliability of the results.

LITERATURE CITED

1. C. G. Ahlström and N. O. Borg, Acta Path. Microbiol. Scand., 24 (1947), p. 483.
2. S. Beelk and P. B. Peacock, Brit. J. Exp. Path., 21 (1940), p. 227.
3. F. G. Bock and M. Burnham, Cancer Res., 21 (1961), p. 510.
4. F. G. Bock, Nat. Cancer Inst. Monogr. 10 (1963), p. 361.
5. A. [illegible] Graff, Krebsforsch., Bd. 52 S. 165 (1942).
6. [illegible] J., 24 (1930), p. 505.
7. [illegible] Int. Cancr., 15 (1959), p. 178.
8. G. [illegible] Scand., Suppl. 96 (1953).
9. G. H. I. Sloane and C. A. Loeser, Cancer Res., 23, (1963), p. 1555.
10. J. M. Twort and C. C. Twort, Am. J. Cancer, 35 (1939), p. 80.
11. F. Weigert and J. C. Mottram, Cancer Res., 6 (1946), p. 97.