A POSSIBLE MODE OF ACTION OF BENZPYRENE AS A TYPICAL CHEMICAL CARCINOGEN.

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Received 23rd August, 1943.

An outstanding feature of the chemical carcinogens is their long delayed action. When the skin of mice is painted with a great excess of the dissolved carcinogen the first tumours do not appear until many weeks, and even months have elapsed. The latent period can be interpreted by biological conceptions, which include the various precancerous states of the tissue until a normal cell is transformed into a malignant cell. We are interested here in a physico-chemical study of the problem whether the carcinogenic drug or one of its metabolites can persist within a living animal over periods which are commensurable with the latent period, and whether it is liable to a spontaneous transformation which may stimulate the change of the cell race. Experiments at the Mount Vernon Hospital have been in progress during the last four years.[1] Benzpyrene was chiefly studied, although it is not the most potent carcinogen, because it is distinguished from other carcinogenic hydrocarbons by a number of different typical fluorescence and absorption spectra.

From the experiments of Peacock, Chalmers and Berenblum and their collaborators [2] it follows that by far the greater part of the benzpyrene which has been introduced into a mouse or rat is changed to non-fluorescent derivatives which have not yet been accounted for. About 1 % is excreted unchanged and the rest appears as blue-fluorescent 'BPX' in the bile and is excreted in the fæces as 5-8-benzpyrene-quinone and as green-fluorescent 'BPF' from which 8-hydroxy-benzpyrene could be isolated.

This metabolism has now been followed in greater detail by fluorescence- and absorption-spectrography and by fluorescence-chromatography which led to the following tentative scheme :

$$\begin{array}{ccccccc} \text{Benzpyrene} & \longrightarrow & \text{'BPX'} & \longrightarrow & \text{'BPF'} & \xrightarrow{(?)} & \text{5-8-Benzpyrene-quinone} \\ & & \downarrow & & \downarrow & & \\ & & [\text{BPX}] & \longrightarrow & [\text{BPF}] & \longrightarrow & \text{8-Hydroxy-benzpyrene} \end{array}$$

[BPX] and [BPF] are prepared by fluorescence-chromatographic purification of extracts from 'BPX' and 'BPF' respectively, from which they are distinguished by their fluorescence spectra.

The blue-fluorescence spectrum of 'BPX' can be seen in the skin, liver, lung and kidney-cortex after application of benzpyrene to a mouse or rabbit. Furthermore, it appears in the bile and small intestine which are the chief route of excretion, and in the milk in the stomachs of suckling mice after intravenous injection of benzpyrene into the mother. Green-fluorescent 'BPF' appears in the alimentary canal beyond the ileo-cæcal valve, in the bladder-urine, and together with 'BPX' in discrete patches of the lung.

[1] Weigert, *Trans. Faraday Soc.*, 1940, **36**, 1033 ; *Nature*, 1942, **150**, 56 ; Weigert and Mottram, *Nature*, 1940, **145**, 895 ; 1942, **150**, 635 ; *Chem. Ind.*, 1941, **60**, 617 ; *Biochem. J.*, 1943, **37**, 497 ; Doniach, Mottram, and Weigert, *Brit. J. Exp. Path.*, 1943, **24**, 1, 9.

[2] Peacock, *Brit. J. Exp. Path.*, 1936, **17**, 164 ; *Amer. J. Cancer*, 1940, **40**, 251 ; Chalmers, *Biochem. J.*, 1938, **32**, 271 ; Chalmers and Crowfoot, *ibid.*, 1941, **35**, 1270 ; Berenblum, Crowfoot, Holiday and Schoental, *Cancer Res.*, 1943, **3**, 145, 151.

[BPX] and [BPF] are different with respect to their spectrographic and chromatographic behaviour:

(1) The blue-fluorescence spectra of [BPX] show the same banded structure as adsorbate on alumina and as eluate in alcohol. The fluorescent zone remains fixed at the top of the column even after long development.

(2) The green-fluorescence spectrum of the adsorbate of [BPF] on alumina shows no bands, but the blue-fluorescence spectrum of its eluate in alcohol has a banded structure. The fluorescent zone of [BPF] on alumina moves slowly down during development.

(3) The absorption spectrum of [BPF] in alcohol is very similar to that of 8-hydroxy-benzpyrene, while that of [BPX] is unrelated to any known benzpyrene derivative.

(4) Apart from these chief differences, minor distinctions can be seen according to the origin of the preparations. For instance, the blue-fluorescent zone of [BPX] at the top of the alumina column is narrow and sharp with extracts from bile and small intestine, but extended and diffuse with extracts from liver, while those of kidney-cortex and lung are diffuse but less extended. The diffuse green-fluorescent zones of the adsorbates of [BPF] which move down on development are not so typical, but the fluorescence spectra of their eluates in alcohol show slight displacements of the bands according to the origin of the extracts.

These results show that 'BPX' and [BPX], on the one hand, and 'BPF' and [BPF] on the other hand, comprise two groups, the X- and F-groups respectively, the members of which contain two different benzpyrene derivatives as prosthetic groups combined with various cell constituents. The fluorescence spectra of the adsorbates of the X-group give evidence that the carrier molecule is adsorbed by the alumina, and that the benzpyrene-group is not affected optically by the adsorption. In the F-group the disappearance of the fluorescence bands in the adsorbate indicates that the adsorbed portion of the molecule is the benzpyrene group itself, which is probably identical with 8-hydroxy-benzpyrene.

All members of the X-group are metastable and are readily transformed into members of the F-group. This happens in vivo when bile-'BPX' passes through the ileo-cæcal valve into the cæcum and when kidney-'BPX' enters into the urine. In the lung it occurs apparently in the 'BPX'-holding cells themselves. Post-mortem it can be seen with all 'BPX'-containing tissues, if they are kept for some time at 37°, and even after three days in the ice-chest. This transformation is obviously due to autolysis and to a detachment of the carrier molecules. It can be completely prevented by formalin and other preservatives. [BPX] which is adsorbed on alumina is transformed into [BPF] at elevated temperature in vacuo.

There is no direct evidence whether the 5-8-benzpyrene-quinone is produced directly from 'BPX' or via 'BPF'.

'BPX' appears and is fixed just in those tissues, skin (where it persists in the Malpighian layer for over three weeks), lung and liver, where tumours can be produced by benzpyrene. Hence it is likely that its metastability and its spontaneous transformation into 'BPF' according to the laws of probability may be the reason for the stimulation of the change of a normal into a malignant cell.

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