The Metabolism of 1,2-Benzanthracene in Mice and Rats*

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With an appendix on absorption spectra by E. R. Holiday, M.A., B.M.**

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From previous metabolic studies it is known that carcinogenic hydrocarbons can be converted by the animal body into phenolic derivatives. In mice and rats, 1,2,5,6-dibenzanthracene is converted into a dihydroxy derivative (3, 8), possessing properties similar to those of synthetic 4',8'-dihydroxy-1,2,5,6-dibenzanthracene, formula I (4), while 3,4-benzpyrene is converted into a monohydroxy derivative (11, 5, 6, 7), which, from recent studies (2), appears to be the 8-hydroxy compound, formula II.

A similarity in the positions of the hydroxy groups in the two cases becomes apparent when the respective authors in the study of the metabolism of 3,4-benzpyrene (1). About 2 ml. of a saturated solution of 1,2-benzanthracene in arachis oil was injected intraperitoneally per rat, and 0.4 ml. of the solution per mouse.

For 10 to 14 days after injection, the feces were collected, dried, and ground to a fine powder. This powder was extracted with cold benzene by percolation, and the pooled benzene extracts were passed through columns of alumina. The columns were developed with excess of benzene, and the pooled benzene extracts were passed through columns of alumina. The columns were developed with excess of benzene, and in each case the zone showing a strong bluish white fluorescence in ultraviolet light was cut and eluted with methanol. After evaporation, the residue was methylated with dimethyl sulfate in the presence of aqueous NaOH, and the product transferred into benzene. The benzene solution was dried with anhydrous Na$_2$SO$_4$, and then passed through another column of alumina. The fluorescent filtrate, containing the methylated metabolite, was evaporated to dryness, and sublimed in high vacuum. The sublimate consisted of an oily pale yellow material, showing a tendency to crystallize.

The phenolic nature of the metabolite (i.e., before methylation) was indicated by its chromatographic behavior from different solvents, and by its solubility in strong alkali, the latter being accompanied by the

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* Because of the difficulties of international communication, proof of this article was not read by the authors.

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characteristic change in fluorescence from bluish violet to yellow.

On oxidation with chromic acid it yielded a yellow product. This was not isolated, but it was found to differ from the 9,10-quinone (obtained by oxidation of benzanthracene itself with chromic acid) by its stronger adsorbability when a mixture of the two was passed through alumina. This result is compatible with the view that the oxidized yellow product of the metabolite is a hydroxyquinone and, therefore, that the OH group in the metabolite was not in the 9- or 10- position.

As in the study of the metabolism of 3,4-benzpyrene (1), conversion of the phenolic metabolite into its more stable methylated derivative proved advantageous as a practical expedient for its identification. For comparison, the following methoxy derivatives of 1,2-benzanthracene were synthesized.

4'-Methoxy-1,2-benzanthracene.—Prepared by methylation of the 4'-hydroxy compound, produced according to the method of Sempronj (12). The product was purified by chromatography and crystallization, yielding colorless needles; m.p. 160–161° C.

Analysis (Strauss and Weiler).—10.72 per cent OCH₃ (Theor. 12.02 per cent).

9,10-Dimethoxy-1,2-benzanthracene.—Prepared by reductive methylation of the 9,10-quinone, followed by purification by chromatography and crystallization. The final product consisted of colorless rhombic crystals; m.p. 137–138° C.

Analysis (Strauss and Weiler).—20.7 per cent OCH₃ (Theor. 21.5 per cent).

The 3-methoxy-1,2-benzanthracene, synthesized by Fieser and Dietz (9), was not prepared for direct comparison, as the description of its absorption spectrum by Jones (10) made it possible to establish whether or not it was identical with the methylated metabolite under investigation.

Chromatographic behavior from mixtures, fluorescence spectra, and ultraviolet absorption spectra were used as criteria for identification.

Chromatographic behavior.—Tests for identity or nonidentity of two substances can often be performed by passing the mixture of the two through chromatographic columns, and developing the columns with a suitable solvent. The resolution of the mixture into two separate zones is proof of nonidentity. When separation into two distinct zones does not occur, proof of nonidentity can still be obtained sometimes by testing successive samples of eluate from a fluid chromatogram for characteristic properties. While chromatographic resolution of a mixture is proof of nonidentity of its constituents, failure of resolution, though indicative, is not proof of identity.

Such tests were carried out with mixtures of the methylated metabolite and the synthetic compounds mentioned above. While mixtures of the methylated metabolite with the 9,10-dimethoxy compound could be resolved into the two components, no such resolution was found possible from mixtures of the methylated metabolite and synthetic 4'-methoxy-1,2-benzanthracene.

Fluorescence spectra.—This method proved to be very helpful in obtaining evidence of nonidentity between related derivatives of polycyclic hydrocarbons, and in indicating identity where the spectra were the same. In view of the relative simplicity of the technic, its high sensitivity, and its relative specificity in the presence of impurities (especially if the latter were themselves nonfluorescent), this method, together with chromatographic analysis, was used with great advan-

![Image](https://cancerres.aacjournals.org/content/5/5-6/687/F1.large.jpg)

**Fig. 1.—** Semiquantitative representation of fluorescence spectra of 1,2-benzanthracene and some of its metabolic (methylated) and synthetic derivatives, in benzene.
anthracene available or described in the literature. The close agreement between the absorption spectra of the methylated metabolite and of the synthetic 4'-methoxy-1,2-benzanthracene (see appendix) suggested that they were identical.

From these results it may be inferred that the metabolite excreted in the feces was 4'-hydroxy-1,2-benzanthracene.

DISCUSSION

The results so far obtained in the studies on the metabolism of carcinogens in mice and rats appear to conform to a certain pattern. While considerably more evidence is required before the chemical mechanism involved can be understood, some tentative conclusions seem justifiable at this stage.

1. There appears to be a similarity in the positions of metabolic oxidation in the molecule as far as 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 1,2-benzanthracene are concerned; compare formulas I, II, and III.

2. The positions in the molecule metabolically attacked are not those that are most reactive chemically (the latter being the 9,10- positions in the case of benzanthracene and dibenzanthracene, and the 5- position in the case of benzpyrene). This point has already been stressed by Cason and Fieser (4).

3. It is interesting to observe that with benzanthracene and dibenzanthracene, the positions in the molecule metabolically attacked (4'- and 4',8'- respectively) are also those where sulfonation occurs in vitro, provided the most reactive positions (9,10-) are blocked, as in the case of quinones.

4. These results are compatible with the view that in the process of metabolic oxidation some group, possibly an enzyme, blocks the most reactive position in the molecule, so that metabolic oxidation occurs in the next reactive positions.

SUMMARY

A fluorescent phenolic derivative has been isolated from the feces of mice and rats injected with 1,2-benzanthracene by the intraperitoneal route.

On methylation of the metabolite a product was obtained that possessed identical chromatographic behavior, fluorescence spectrum, and absorption spectrum (in the range longer than 300 mp) with those of synthetic 4'-methoxy-1,2-benzanthracene. It is suggested, therefore, that the metabolite is 4'-hydroxy-1,2-benzanthracene.

The mechanism of metabolic oxidation of carcinogenic hydrocarbons is discussed briefly.

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Six cases of malignancy of the rectum were diagnosed by means of the proctoscope. In one case x-ray failed to reveal the lesion. Use of the proctoscope is an essential part of the examination of patients with rectal or colonic complaints.—M. E. H.


The author concludes from studies and observations during a period of 10 years on more than 200 patients with large gastric ulcers that surgical intervention is the treatment of choice. In most cases it cannot be definitely ascertained preoperatively whether the lesion is benign or malignant. Large benign ulcers tend to heal poorly, bleed dangerously, and rarely to undergo malignant change.—G. H. H.

LEUKEMIA, LYMPHOSARCOMA, HODGKIN'S DISEASE


The mode of onset, presenting symptoms, and features of the physical examination in cases of acute and chronic leukemia are briefly described. Details of the blood smear are given. Bone marrow biopsy is often of great assistance in making the diagnosis. The differential diagnosis includes agranulocytosis, pertussis, infectious mononucleosis, pyogenic infections, tuberculosis, and the leukemic reaction to tumors metastasizing to bone marrow.—E. E. S.


The author discusses the previously proposed classification of skin lymphoblastomas and points out the uncertainty in placing many lesions in one of these groups. Two cases are described in detail and illustrated. One was a 74 year old male with multiple recurring lymphosarcoma cutis. Autopsy revealed extensive internal metastases. The other was a 9 months old male infant with a single subcutaneous lymphoma. Postmortem examination revealed no internal metastases but the right cervical region was extensively involved. The lesions in both instances were very radiosensitive, but new crops of nodules would appear rapidly. The author cites the following criteria as characteristic of lymphosarcoma cutis: (1) local lymph node invasion; (2) skin invasion without ulceration, papules, exfoliation, or hemorrhagic dermatitis; (3) no pronounced changes in blood picture except myelophthisic anemia; (4) mature and malignant lymphocytes in the biopsy; (5) radiosensitivity; (6) generalized nodular spread into internal organs. He considers lymphosarcoma cutis actually to be secondary to initial involvement of internal or contiguous lymph nodes.—V. F. M.

PITUITARY


In the records of the Johns Hopkins Hospital there are 113 cases of tumor of the craniopharyngeal duct, arising from the cells that later form the pars intermedia and the pars tuberalis of the hypophysis. In this group the author found 12 cases of adamantinoma. He reports one in detail and summarizes 11 briefly. Photomicrographs demonstrate the characteristic structure of adamantinoma.—S. A. G.

THYROID


A case report.—G. H. H.

STATISTICS


There was a further reduction in the number of notified cases of epidermolatous ulceration—113 (8 fatal) as compared with 128 (11 fatal) in 1941. Eighty-five (2 fatal) of these were due to pitch and tar, and 28 (6 fatal) to mineral oil. Since the beginning of the war the Registrar General has been unable to continue his usual practice of notifying fatal industrial cases that had not been notified during life; if this were possible the figures would be somewhat higher.—E. L. K.


Deaths from malignant disease in 1942 numbered 8,556 being 120 more than in 1941 and 431 above the average for the 5 years from 1937 to 1941. This is the largest number hitherto registered in Scotland but it is probable that the greater part of this increase may be attributed to the aging of the population. The death rate is 171 per 100,000 of the population estimated for 1939 (168 in 1941).—E. L. K.

Correction

The authors of "The Metabolism of 1,2-Benzanthracene in Mice and Rats" (3:686, 1943) point out that the last compound in Fig. 3, page 690, should be 9,10-Dimethoxy-1,2-benzanthracene, instead of 9,10-Dimethyl-1,2-benzanthracene. As this figure is a reproduction by photography of the authors' chart the fault lies in the original.
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