The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites

III. Distribution of 1,2,5,6-Dibenzanthracene in Rats Following Subcutaneous Injection in Olive Oil

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The experiments described in this paper were undertaken with the object of investigating the distribution of 1,2,5,6-dibenzanthracene in the rat following subcutaneous injection of the hydrocarbon in olive oil. Analyses of the excreta of rats which had received similar injections were discussed in the previous paper (4), where it was observed that only a very small percentage of the total hydrocarbon injected could be detected, either as unchanged hydrocarbon or as a phenolic metabolite, 4',8'-dihydroxy-1,2,5,6-dibenzanthracene. The results described here show that an appreciable amount of the injected hydrocarbon may remain encapsulated near the site of injection for a considerable time but there is also qualitative evidence that such encapsulation may be greatly reduced by modification of the injection technic.

The analyses were carried out on two rats each of which had received 8 injections of 20 mgm. of dibenzanthracene in olive oil at weekly intervals during the 8 weeks immediately preceding the autopsy.

MATERIALS AND METHODS

POST-MORTEM EXAMINATION OF THE RATS

Rat No. 1 (female) had multilocular abscesses on both flanks which showed on fluorescence in ultraviolet light. On opening the body cavity a perirenal cystic structure (2 cm. diam.) was present which contained a clear fluid which fluoresced in ultraviolet light as also did the wall of the sac. This cyst, which presumably was caused by the accidental penetration of the perirenal tissue during injection, was excised, and on opening it an oily fluid exuded. The contents were collected in ether and stored together with the sac itself.

The visera were grossly normal as were the following organs: heart, lung, thymus, salivary gland, thyroid, liver, spleen, pancreas, kidney, colon, bladder, ovary, adrenal, stomach, brain, cerebellum, pituitary, bone, bone marrow, all of which were examined grossly by ultraviolet light without observing any pronounced fluorescence in any of the organs such as might indicate a local concentration of carcinogenic hydrocarbon. The intestines and urinary bladder, however, showed a distinct blue fluorescence. The subcutaneous tissue of the back and sides was riddled with fluorescent vesicles containing oily material.

Rat No. 2 (male) resembled the first rat closely except for the absence of a perirenal sac. The testicles of rat No. 2 showed no gross fluorescence. The hair of both rats fluoresced brilliantly but this was not due to the effect of the hydrocarbon since the hair of control rats had a similar fluorescence.

The skins were removed from both rats, the carcasses being carefully washed with ether so as to collect quantitatively the oily fluid which exuded from the vesicles. The dissecting trays were also washed with ether, the washings being added to those obtained above. The greater part of the hair was removed from the skin and collected separately, after which the skins were added to the ether washings from the body cavity and stored.

After removal of the livers which were stored separately, the carcasses were preserved under ether together with the various organs which had been examined for fluorescence. The tissues were divided as follows into five separate samples for subsequent extraction and spectrographic analysis: A. hair; B. livers; C. skins together with ether washings of the body cavity; D. carcasses and remaining organs; E. perirenal sac and part of the kidney of rat No. 1. These samples were stored under ether at 5° C. until required for spectrographic analysis.

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1 Kindly supplied by Dr. K. Dobrincher of the Memorial Hospital, New York.
EXTRACTION AND SPECTROGRAPHIC ANALYSIS OF ORGANS

With the exception of the hair, the various fractions were prepared for spectrographic analysis in the following manner. The ether washings were decanted and preserved, the residual tissue washed twice with ether, the ether being added to that collected above, and the tissue minced with an equal weight of anhydrous sodium sulfate and extracted with chloroform until the fresh chloroform extracts were no longer fluorescent. The extraction of samples B and E was carried out in a Soxhlet extractor while samples C and D were extracted in a modified Wolfe-Hershberg extractor (2) adapted for the extraction of solid material. Fraction A was extracted with chloroform in a Soxhlet extractor without the addition of sodium sulfate.

The chloroform extracts so obtained from the several fractions were added to the respective ethereal washings and the solvent removed in a stream of nitrogen under reduced pressure. The residues were taken up in ether and extracted three times with N sodium hydroxide solution. The alkaline solution was back extracted 4 times with ether, the ether extracts bulked, washed with water, dried with anhydrous sodium sulfate, and the solvent removed to leave the neutral fraction. The aqueous alkaline extracts were saturated for 2 to 3 hours with a rapid stream of carbon dioxide and reextracted with ether. The ether extract so obtained was reextracted with alkali and the second alkaline extract reacidified with carbon dioxide and reextracted with ether. After washing with water, drying with anhydrous sodium sulfate, and removing the solvent in a stream of nitrogen under reduced pressure there remained the phenolic fraction. The double precipitation with carbon dioxide described here was necessary since the first treatment with N sodium hydroxide caused some emulsification and two treatments were found necessary to free the phenolic fraction from unchanged dibenzanthracene.

The results of the spectrographic analyses are summarized in Table I from which it will be seen that a considerable quantity of unchanged hydrocarbon (36 per cent) is recoverable from the bodies and organs of the rats. Comparison of the curves for the various neutral fractions with the master curve for the pure hydrocarbon by the method described in an earlier publication (3) (Figs. 1 to 5) indicates that the distortion of the curve due to background absorption is very small in the cases of the extracts from the perirenal sac (E), the skin (C), and the neutral extract of the bodies and other organs (D). The liver extract (B) shows very pronounced distortion, and the concentration of dibenzanthracene in the livers as calculated from this curve (Fig. 2) is probably much too high.

The phenolic fractions after removal of the solvent were distilled in a current of steam until 500 ml. of aqueous distillate had collected, in order to remove volatile phenolic substances. The spectra of the residual nonvolatile fractions showed no trace of any structure which could be attributed to 4',8'-dihydroxy-1,2,5,6-dibenzanthracene. The curves from all fractions, however, had a similar shape with a broad indeterminate maximum between 2,700 and 2,800 A.; an example of the spectrum of one of these extracts is given in Fig. 6. All the phenolic fractions showed a distinct greenish blue fluorescence but unlike the blue fluorescence characteristic of 4',8'-dihydroxy-1,2,5,6-dibenzanthracene this fluorescence did not change to a yellowish color on addition of aqueous alkali. Experience of other work of a similar nature has convinced the author that the presence of blue fluorescence in an extract, unless accompanied by a characteristic absorption spectrum, cannot be accepted as indicative of the presence of carcinogenic hydrocarbons or their metabolites in fat-soluble tissue extracts of this kind. It appears therefore that in none of these body extracts was any trace of 4',8'-dihydroxy-1,2,5,6-dibenzanthracene detected.

The observations reported here are in agreement with the findings of other workers who have studied the metabolism of dibenzanthracene. The results of this work indicate that dibenzanthracene is not quickly metabolized to carcinogenic compounds in the bodies of the rats used in these experiments. The large quantity of unchanged hydrocarbon found in the bodies of the rats is of interest in view of the fact that the chief route of administration in these experiments was the peritoneal cavity.

Table I: Spectrographic Analysis of 1,2,5,6-Dibenzanthracene in Tissue Extracts

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight of extract</th>
<th>Intensity of maxima</th>
<th>Per cent hydrocarbon in extract</th>
<th>Weight of hydrocarbon in extract, in mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (hair)</td>
<td>237 mgm.</td>
<td>E 1 cm. 2,070</td>
<td>38</td>
<td>0.61</td>
</tr>
<tr>
<td>B (livers)</td>
<td>673 mgm.</td>
<td>E 1 cm. 2,070</td>
<td>6.2</td>
<td>0.10</td>
</tr>
<tr>
<td>C (skins)</td>
<td>18.93 gm.</td>
<td>E 1 cm. 2,070</td>
<td>23.6</td>
<td>0.38</td>
</tr>
<tr>
<td>D (carcasses)</td>
<td>18.65 gm.</td>
<td>E 1 cm. 2,070</td>
<td>8.5</td>
<td>0.14</td>
</tr>
<tr>
<td>E (perirenal sac and kidney)</td>
<td>5.43 gm.</td>
<td>E 1 cm. 3,485</td>
<td>1.31</td>
<td>0.27</td>
</tr>
<tr>
<td>Total weight of hydrocarbon recovered</td>
<td>114.8 mgm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total weight of hydrocarbon injected</td>
<td>320.0 mgm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>36%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1.—A, continuous line — —, neutral fraction, extract A (hair). B, dash line ———, master curve of 1,2,5,6-dibenzanthracene.

FIG. 2.—A, continuous line ———, neutral fraction extract R (livers). B, dash line ———, master curve of 1,2,5,6-dibenzanthracene.

FIG. 3.—A, continuous line ———, neutral fraction, extract C (skins). B, dash line ———, master curve of 1,2,5,6-dibenzanthracene.

FIG. 4.—A, continuous line ———, neutral fraction, extract D (carcasses). B, dash line ———, master curve of 1,2,5,6-dibenzanthracene.

FIG. 5.—A, continuous line ———, neutral fraction, extract E (perirenal nonsaem-volatile phenolic fraction of sac). B, dash line ———, master curve of 1,2,5,6-dibenzanthracene.

FIG. 6.—A, continuous line ———, nonsaem-volatile phenolic fraction of extract C (skins).
with those reported by Dobriner, Rhoads, and Lavin (1). Using a qualitative spectroscopic method these authors detected absorption bands in extracts of the intestinal contents of rats injected with dibenzanthracene, which they attributed to the unchanged hydrocarbon. No positive identification of dibenzanthracene in the liver could be obtained but extracts of whole rats gave a neutral fraction with intense hydrocarbon bands. The same authors detected absorption bands in the phenolic extract of the intestinal tract which they attributed to 4',8'-dihydroxy-1,2,5,6-dibenzanthracene but no similar bands could be discerned in the phenolic liver extract, while in extracts of whole rats there were faint bands which may have been due to this phenolic metabolite but were obscured by much background absorption.

**DISCUSSION**

Although these results cannot be quantitatively related to those discussed in the previous paper, it would appear that a significant part of the discrepancy between the amount of dibenzanthracene injected and that recoverable in the excreta may be due to the encapsulation of the hydrocarbon in vesicles near the site of subcutaneous injection, from which it may be removed only extremely slowly. Qualitative experiments suggest that intraperitoneal injection of the hydrocarbon in solution in tricaprylin gives rise to considerably less local accumulation than subcutaneous injections in this or other solvents. Quantitative experiments to test this are at present in progress.

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8 I wish to thank Miss M. G. Lewisohn for assistance with this part of the work.—Author.
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