The Uptake of Radiophosphorus in the Phospholipid Fraction of Mouse Epidermis in Methylcholanthrene Carcinogenesis*

C. J. Costello, M. D., C. Carruthers, Ph. D., M. D. Kamen, Ph. D. and R. L. Simoes, M. D.

(From the Departments of Surgery and Anatomy, Barnard Free Skin and Cancer Hospital, St. Louis 3, and the Mallinckrodt Institute of Radiology, Washington University, St. Louis 10, Mo.)

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The integration of the chemical, physical, and histological changes in mouse epidermis during carcinogenesis induced by methylcholanthrene has been recently reviewed in a second summarizing report by Cowdry (4). Investigations on the lipids (13, 14), minerals (1), B vitamins (11), and succinic dehydrogenase and cytochrome oxidase (2) have been reported. Experiments are being planned which will enable us to study the uptake of radiophosphorus in the nucleoprotein, acid soluble and phospholipid fractions of mouse epidermis undergoing carcinogenesis by methylcholanthrene. In this paper we wish to describe our investigations on the uptake of P32 in the epidermal phospholipid fraction.

EXPERIMENTAL PROCEDURE

The procedures for shaving the mice, applying the carcinogen, and removing the epidermis from dermis have been described (2). In this study methylcholanthrene in benzene, and benzene alone were applied on alternate days for 3, 6, and 12 treatments, and the mice were sacrificed 5 days after the last application of the carcinogen or solvent. For the final stage in our series, the transplantable squamous cell carcinoma of Cooper, Firminger, and Reller was employed (3).

The phospholipid fractions of normal, benzene-treated, and methylcholanthrene-treated epidermis and of the carcinoma were extracted twice with a reflux condenser using about 25 cc. portions of a mixture of 3 volumes of alcohol and 1 volume of reagent grade chloroform. The latter solutions containing the phospholipid were evaporated to dryness on a steam bath, and the lipid was reextracted with petroleum ether (b.p. 30 to 60°). The ether was evaporated on a steam bath, the residues digested in a Pyrex digestion tube with a mixture of 2 cc. of reagent grade nitric acid and 2 cc. of reagent grade perchloric acid. The digests were made up to a volume of 50 cc., and the orthophosphate content was determined on an aliquot by the method of Truog and Meyer (12) in a Coleman spectrophotometer.

To determine the rapidity of P32 uptake in the epidermis, solutions of P32 as sodium phosphate were injected subcutaneously into mice. The phospholipid fraction from mice so treated with P32 was extracted and digested in the same manner as described above. The perchloric acid solution containing the phosphate was diluted with a few cubic centimeters of water, neutralized with sodium carbonate, and quantitatively transferred to a 25 cc. volumetric flask. One cubic centimeter of the neutralized solution was delivered onto a small watch crystal, and evaporated to dryness in a vacuum dessicator. The resultant samples were mounted in a standard position under a "bell-jar" type of Geiger Mueller counter equipped with a thin aluminum window. Corrections for self-absorptions were not necessitated because the sample thickness in no case exceeded a few mgm./cm. Decay corrections were made when required by response to a control sample of radioactive phosphate prepared from the same solution used for injection. The total weight of sample was determined in each case by drying the alcohol ether extracted tissue at 105°C. to constant weight. The P32 content was then corrected to unit dry weight of lipid free tissue so that all samples were directly comparable as to specific P32 content.

RESULTS

The lipid-phosphorus content of normal-un-treated, benzene-treated, and of methylcholanthrene-treated epidermis and of the carcinoma is expressed as milligrams of lipid-phosphorus per 100 mgm. of dry fat-free tissue (Table I). This value for untreated epidermis was 0.163 which agreed rather well with that of the epidermis of mice which had received 3 applications of benzene (0.173), and 6 treatments with benzene (0.153). On the other hand, the phosphorus fat-free dry weight value of the epidermis which had been

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painted 3 times with the carcinogen was 0.123, a decrease of about 25 per cent from the normal. Those animals receiving 6 and 12 applications of the carcinogen had quantities of 0.120 and 0.112, respectively, which were 26 and 31 per cent less than that of the normal. In the carcinoma, the value increased to 0.311 which was nearly twice that of the normal-untreated epidermis.

**Table I: Lipid Phosphorus/Fat-Free Dry Weight Ratio of Mouse Epidermis**

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Number of applications</th>
<th>Time after first treatment to killing of mice, (days)</th>
<th>Phosphorus/fat-free dry weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3</td>
<td>10</td>
<td>0.174</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>10</td>
<td>0.161</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>10</td>
<td>0.155</td>
</tr>
<tr>
<td>29 (Total)</td>
<td></td>
<td></td>
<td>Average 0.163</td>
</tr>
</tbody>
</table>

**Table II: Uptake of P$_{32}$ in the Phospholipid of Mouse Epidermis**

<table>
<thead>
<tr>
<th>Material</th>
<th>Time after injection of P$_{32}$, counts per minute per 100 mgm. dry fat-free tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, untreated epidermis</td>
<td>6, 12, 100, 18, 15, 750, 24, 22, 640, 36, 11, 620</td>
</tr>
<tr>
<td>Methylcholanthrene-treated epidermis, 3 paintings</td>
<td>12, 13, 560, 24, 13, 640, 36, 12, 520, 48, 10, 200</td>
</tr>
<tr>
<td>Benzene-treated epidermis, 3 paintings</td>
<td>6, 11, 210, 12, 18, 000, 18, 15, 500, 24, 11, 210</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>12, 33, 620, 24, 33, 810, 36, 27, 380, 48, 24, 330, 60, 18, 030</td>
</tr>
</tbody>
</table>

In Fig. 1 the specific activity (counts per minute per mgm. lipid-phosphorus) is plotted against the time. The uptake of P$_{32}$ in the benzene-treated epidermis was rapid and maximal at 12 hours and fell quickly, while that of the normal rose slowly reaching its peak at 24 hours, but dropped at about the same rate. On the other hand, the uptake in hyperplastic epidermis and in the carcinoma was rapid and was maintained at near maximal levels for nearly 36 hours. Moreover, the rate of fall of the P$_{32}$ was almost the same for these tissues. The specific activity of the normal epidermis at the time of maximum uptake was somewhat higher than the other tissues, but not significantly so, in view of other data to be shown later in this report.
For the study of the influence of repeated applications of methylcholanthrene in benzene or of benzene alone on the uptake of P32 in the phospholipid fraction of the epidermis and of the carcinoma, the time of maximum uptake was that shown in Fig. 1.

In conjunction with the study of the time of maximum uptake of P32, a series of untreated controls and of benzene-treated controls was run at the same time with the samples of P32 of nearly the same activity. The results (Table III) expressed as counts per minute per 100 mgm. dry fat-free tissue showed 22,118 counts for normal epidermis, whereas those for mice receiving 3, 3, 6, and 12 applications of benzene were, respectively, 22,075, 24,372, 20,641, and 20,758. Therefore, benzene alone has no appreciable effect upon the uptake of P32.

In another larger experiment with another sample of P32, the uptake of normal, benzene-treated, and methylcholanthrene-treated epidermis was determined (Table IV). The data are expressed as in the preceding table. The count for untreated controls was 9,500, and for mice treated 3 times with benzene, the value was 9,320. The values for the epidermis which had been painted 3, 6, and 12 times with the carcinogen were, respectively, 8,073, 7,600, and 7,706. In the carcinoma, the uptake rose to 20,450 counts per minute. These
counts are considerably lower than those given in Table III, which is due to the fact that a solution of radiophosphorus of different specific activity was administered. No attempt was made to inject the same amount of P³² per gram body weight since the activity of our solutions varied too much for this purpose. We chose to compare the specific activity values from Fig. 1 at maximal uptake time in the normal and treated epidermis and in the carcinoma are not significantly different, and calculated ratios for the normal and benzene-treated epidermis of mice shown in Table III would be quite constant. Although the uptake varies somewhat in the different experiments, the specific activities are quite constant, which demonstrates no appreciable significance in the phospholipid turnover of the differently treated epidermises, and of the carcinoma studied here.

DISCUSSION

In an investigation of the rate of phospholipid turnover in 4 types of transplantable tumors in mice (a mammary carcinoma, a lymphoma, a lymphoblastoma, and sarcoma 180), Jones, Chai-koff, and Lawrence (6, 7) observed that the phospholipid turnover resembled that in tissues such as liver, kidney, and intestine, rather than in tissues like muscle or brain, which are less capable of regeneration and growth. They also observed that the rate of turnover for the types of tumors was not uniform, but that each displayed a characteristic activity. In another study, the same authors demonstrated that the total phosphorus turnover of three neoplastic tissues (a mammary carcinoma, a lymphoma, and a lymphosarcoma) showed a high and rapid uptake of P³² in the early intervals after its administration, and that the malignant tumors had a pronounced capacity for retaining P³² for a long time in contrast to normal tissues with equal levels of P³² (8). In our study on epidermal carcinogenesis, the specific activity time curves showed no appreciable difference at maximum uptake time between normal benzene-treated, and methylcholanthrene-treated epidermis and the carcinoma. However, the specific activity time curves of the carcinoma and hyperplastic epidermis, the latter being chemically pre-cancerous (2), was quite similar in that both re-

activities in the different experiments. The specific activity of the epidermal lipid phosphorus of the mice shown in Table IV did not differ significantly (Table V). The specific activity of normal, and benzene-treated epidermis was respectively 58,282, and 60,915, while the respective values for the hyperplastic epidermis and carcinoma were 65,635, and 65,755. The specific activity values from Fig. 1 at maximal uptake time in the normal and treated epidermis and in the carcinoma are not significantly different, and calculated ratios for the normal and benzene-treated epidermis of mice shown in Table III would be quite constant. Although the uptake varies somewhat in the different experiments, the specific activities are quite constant, which demonstrates no appreciable significance in the phospholipid turnover of the differently treated epidermises, and of the carcinoma studied here.

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tained their radiophosphorus at a higher level for a longer period of time, and decreased at nearly the same rate.

From experiments on the rate of turnover by lecithins and cephalins of rat carcinosarcoma 256, Haven (5) concluded that the rapid turnover of the lecithins indicated participation of lecithins in metabolic activities. The slower rate of turnover by cephalins suggested relationship to cell structure. Marshak (9) has made a detailed investigation of the uptake of P³2 by the nuclei of liver and tumor cells, and has shown, among other things, that the nuclei of tumor cells accumulate more P³2 than do normal liver nuclei. This may be due to greater mitotic activity of the tumor cells and not to the possession by them of a different and characteristic type of metabolism (9). Investigations on tumors employing labelled phosphorus have dealt chiefly with the leukemias (references cited by Scott [10]) which are obviously quite different from carcinomas in our experiments.

**SUMMARY**

The effect of methylcholanthrene on lipid phosphorus and on the uptake of phosphorus in the lipid fraction of mouse epidermis undergoing carcinogenesis induced by methylcholanthrene was investigated by the use of P³2. The carcinogen caused a drop of 30 per cent in the lipid phosphorus content of the epidermis, but in the carcinoma, the lipid phosphorus content was nearly twice that of the normal. The specific activity time curves of normal, benzene-treated, and methylcholanthrene-treated epidermis and of the carcinoma showed no appreciable difference in the specific activities at the time of maximum P³2 uptake. The rate of uptake in the hyperplastic epidermis and in the carcinoma was rapid, these tissues retained their labelled phosphorus for a considerable time, and both showed a similar fall in activity. The uptake in benzene-treated epidermis was at a maximum at 12 hours, while that of the normal was slower and at a maximum at 24 hours. The rate of fall in each was quite rapid and similar. Other experiments demonstrated no significant difference in the specific activities of normal epidermis of epidermis treated 3, 6, and 12 times with benzene alone or with the carcinogen in benzene on alternate days during 10, 20, and 30 days. Although the carcinoma had a high and rapid uptake of P³2, its specific activity was similar to that of the treated epidermis.

**REFERENCES**

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