THE METABOLISM OF AMINOETHYLPHOSPHORIC ACID, FOLLOWED BY MEANS OF THE RADIOACTIVE PHOSPHORUS ISOTOPE*

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(Received for publication, April 2, 1940)

Aminoethylphosphoric acid, \( \text{NH}_2\text{CH}_2\text{CH}_3\text{OPO(OH)}_2 \), was first isolated from bovine malignant tumors by Outhouse (1). Since benign tumors and a number of normal tissues did not yield this compound, it was thought to be specific for malignant tumor tissue. Recently, however, Colowick and Cori (2) reported the isolation of the acid from the small intestine of rabbits and pigs.

The possible relationship of this compound to the metabolism of phosphatides, particularly to that of cephalin, is obvious. It may be significant that it appears to be present in largest amounts in tissues in which the phosphatide metabolism is extremely vigorous.

The biological function of aminoethylphosphoric acid could be that of an intermediate in the synthesis of phosphatides by the organism, or that of a breakdown product of the phosphatides. Very little is known about the mechanism by which phospholipids are formed in the body. A study of the utilization of aminoethylphosphoric acid appeared, therefore, of interest. For this purpose a preparation was employed which contained the radioactive phosphorus isotope \( ^{32}\text{P} \). The present paper gives a report on the conversion of this compound into phosphatides in different organs. Some data on tumor-bearing animals are included.

EXPERIMENTAL

Synthesis of Radioactive Aminoethylphosphoric Acid

A method for the preparation of radioactive phosphorus oxychloride, involving the conversion of \(^{31}\text{P} \) into \(^{31}\text{PCl}_3 \) and the oxida-

* This work has been supported by a grant from the John and Mary R. Markle Foundation.

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tion of the latter to *POCl₃, has been briefly reported elsewhere (3). In the present work it was prepared from radioactive phosphate by means of the following reaction.

$$\text{Ag}_3^+\text{PO}_4 + 3\text{PCl}_5 = 4^+\text{POCl}_3 + 3\text{AgCl}$$

Radioactive sodium phosphate was converted into the silver salt. To 6.89 gm. of dry $\text{Ag}_3^+\text{PO}_4$ (0.016 mole) in a bomb tube 11.2 gm. of powdered $\text{PCl}_5$ (0.054 mole) were added. The sealed tube was heated to 130° for 10 minutes. The $^+\text{POCl}_3$ which had formed at that time was refluxed for a few minutes by careful heating with a microburner in order to break up the $\text{AgCl}$ lumps. The radioactive phosphorus oxychloride was finally purified by distillation in vacuo into a trap cooled with solid CO₂. It weighed 8.15 gm. (yield, 83 per cent).

The barium salt of radioactive aminoethylphosphoric acid was synthesized according to Outhouse (1).

Analysis—$\text{C}_2\text{H}_5\text{O}_4\text{N}^+\text{PBa}$. Calculated. P 11.2, N 5.0

276.4 Found. “ 11.4, “ 4.8

For the metabolism experiments this preparation was converted into the sodium salt.

Methods

The radioactivity of the various phosphatide fractions discussed later in this paper was determined in the dry state by means of a Geiger-Müller counter according to the method generally used in this laboratory (4, 5). The urine samples were concentrated, and the phosphorus, after acid digestion, precipitated as the ammonium molybdate complex. The weighed precipitates were dissolved in NaOH solution and their radioactivity was determined in solution by means of the technique previously described (4). The amount of radioactive phosphorus accumulated in the livers and tumors of the tumor-bearing animals was likewise determined in solution after digestion of weighed tissue portions with a mixture of nitric and sulfuric acids. All activities are expressed in KF units per mg. of $^+\text{P}$ (4).

As usual, all radioactivity measurements were accompanied by

1 The asterisk before the symbol for an element indicates an unstable isotope.
standard measurements in order to correct for the decay of the unstable phosphorus administered to the animals and to express the activity counts in terms of the radioactive preparation employed. The standards consisted (a) for the measurements in the dry state, of a suspension of the radioactive aminoethylphosphate preparation in vegetable lecithin, (b) for the measurements on the phosphomolybdate solutions, of a similar solution of the ammonium phosphomolybdate complex prepared from the aminoethylphosphate, (c) for the measurements on the acid digests of organs, of an aqueous solution of the aminoethylphosphate.

The methods for the extraction and isolation of the phosphatides were essentially the same as in previous publications (5, 6).

Metabolism of Aminoethylphosphoric Acid

Two adult rats (body weight 270 and 295 gm. respectively) each received 80 mg. of disodium aminoethylphosphate, dissolved in 2 cc. of water, by subcutaneous injection; i.e., in each case a total of 13.4 mg. of *P with an activity of 300,000 KF units (calculated on the same basis as the figures given in Table I). The animals, which received no food for 24 hours prior to and during the experiments, were killed 24 hours after the administration of the radioactive material and examined in the usual manner. The results of the experiment are summarized in Table I. The lecithin and cephalin fractions of the brain are not included, since they showed no measurable radioactivity. As stated before (6) the amount of cephalin obtainable from the kidneys of one rat does not suffice for activity measurement and analysis. Data on the amount of *P excreted in the urine will be found later in this paper.

Utilization of Aminoethylphosphoric Acid by Tumor-Bearing Animals

A few orienting experiments were carried out concerning the metabolism of aminoethylphosphoric acid in tumor-bearing rats. Two adult rats with well developed carcinomas2 (body weight 282

2 Carcinoma of the rat breast (No. 2426) which had been transplanted into animals of the inbred strain in which the tumor arose. We wish to thank Dr. W. H. Woglom of the Institute of Cancer Research of this University for the animals used.
TABLE I

Formation of Phosphatides from Aminoethylphosphoric Acid

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Organ</th>
<th>Phosphatide</th>
<th>Weight</th>
<th>P</th>
<th>Radioactivity* in 1 mg. *P</th>
<th>Minimum amount of newly formed phosphatide, in per cent of total phosphatide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver</td>
<td>Lecithin</td>
<td>120.3</td>
<td>3.9</td>
<td>1630</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalin</td>
<td>14.9</td>
<td>3.7</td>
<td>780</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Intestinal tract</td>
<td>Lecithin</td>
<td>13.7</td>
<td>3.2</td>
<td>800</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalin</td>
<td>21.2</td>
<td>2.9</td>
<td>690</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>Kidney</td>
<td>Lecithin</td>
<td>19.2</td>
<td>3.8</td>
<td>590</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>&quot;</td>
<td>124.3</td>
<td>3.5</td>
<td>1470</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Intestinal tract</td>
<td>Lecithin</td>
<td>8.0</td>
<td>3.6</td>
<td>870</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalin</td>
<td>30.8</td>
<td>3.3</td>
<td>940</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Lecithin</td>
<td>12.6</td>
<td>3.0</td>
<td>420</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalin</td>
<td>21.7</td>
<td>3.6</td>
<td>620</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* The spread of the individual counts was within about 3 per cent of the mean values given.

TABLE II

Accumulation of *P Originating from Aminoethylphosphoric Acid in Tumor Rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>*P in 1 gm. fresh tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat 3</td>
</tr>
<tr>
<td>Liver</td>
<td>0.0601</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.0365</td>
</tr>
</tbody>
</table>

TABLE III

Phosphatides from Tumor Rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Organ</th>
<th>Weight</th>
<th>P</th>
<th>Radioactivity* in 1 mg. *P</th>
<th>Minimum amount of newly formed phosphatide, in per cent of total phosphatide</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Liver</td>
<td>24.5</td>
<td>3.4</td>
<td>1490</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>4.2</td>
<td>3.2</td>
<td>1190</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>Liver</td>
<td>4.9</td>
<td>3.4</td>
<td>1350</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>26.8</td>
<td>3.4</td>
<td>960</td>
<td>4.3</td>
</tr>
</tbody>
</table>

* The spread of the individual counts was within about 3 per cent of the mean values given.
and 223 gm. respectively) received the same amount of radioactive aminoethylphosphoric acid as the normal animals discussed in the preceding paragraph; i.e., each animal was given a subcutaneous injection of 13.4 mg. of \(^*\)P with an activity of 300,000 KF units, contained in 2 cc. of water. The animals, which were fasted for 24 hours prior to and during the experiment, were killed 24 hours after the administration of the radioactive material. The livers and tumor tissues were removed. A portion of each organ was destroyed with acid and the total accumulation of \(^*\)P determined by measurement of the radioactivity, as described above; the phosphatides were prepared from the remaining tissue in the usual manner. The amounts of \(^*\)P originating from the radioactive aminoethylphosphate which were contained in the liver and tumor tissue 24 hours after the administration of the material are compared in Table II.

The relative activities of the phosphatide preparations from the tumor and liver of the animals are given in Table III. These values are strictly comparable to the figures given in Table I. No attempt was made in this case to separate the ether-soluble phosphatides into their components, since only portions of the tissues were available for examination.

### Table IV

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Collection of specimen; time after administration of radioactive material</th>
<th>Total P excreted</th>
<th>(^*)P excreted</th>
<th>(^*)P in total P excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 hrs.</td>
<td>12.5 mg.</td>
<td>3.91 mg.</td>
<td>31.2 per cent</td>
</tr>
<tr>
<td>2</td>
<td>18 hrs.</td>
<td>9.1 mg.</td>
<td>0.67 mg.</td>
<td>7.4 per cent</td>
</tr>
<tr>
<td>3</td>
<td>8 hrs.</td>
<td>11.7 mg.</td>
<td>3.93 mg.</td>
<td>33.7 per cent</td>
</tr>
<tr>
<td>4</td>
<td>8 hrs.</td>
<td>9.1 mg.</td>
<td>3.27 mg.</td>
<td>35.9 per cent</td>
</tr>
</tbody>
</table>

* Partly lost by accident.

**Excretion of Radioactive Phosphorus in Urine**

The excretion of \(^*\)P in the urine specimens collected within 8 hours after the administration of radioactive aminoethylphos-
Aminoethylphosphoric Acid

phoric acid was followed in all animals. The preparation of the material for the activity measurements has been described above. In the case of Rat 1 a subsequent 10 hour specimen was likewise examined. The results of this experiment are summarized in Table IV.

DISCUSSION

The most striking result of the experiments reported here is that the body apparently is unable to utilize aminoethylphosphoric acid as such for the synthesis of cephalin. The relative amounts of lecithin and cephalin newly formed in the liver and the intestinal tract, given in Table I, indicate that the new lecithin exceeded the new cephalin to an even higher degree than when inorganic phosphate was administered (5, 6).

The utilization of aminoethylphosphoric acid for the synthesis of phosphatides in the body could proceed along one of the following lines: (1) Aminoethylphosphoric acid combines with a diglyceride to form cephalin. (2) Aminoethylphosphoric acid is first methylated to cholinephosphoric acid which in turn combines with a diglyceride to form lecithin. (3) Aminoethylphosphoric acid is first split into ethanolamine and phosphoric acid, and the latter used for the synthesis of lecithin. Reaction 1 is made extremely unlikely by the experiments here described in which much larger amounts of newly formed lecithin than cephalin were found. Reaction 2 cannot be excluded on the basis of the present experiments, although the failure of ethanolamine to replace choline as lipotropic (7) or dietary (8) agent would seem to speak against it. The most likely assumption, for the time being, is that expressed in Reaction 3. Enzymes which hydrolyze aminoethylphosphoric acid have been found in kidney and feces (9), and the occurrence in liver of an enzyme which splits cholinephosphoric acid has been made probable (10). The simplest series of reactions would, therefore, appear to be enzymatic hydrolysis of aminoethylphosphoric acid in the tissues, utilization of the inorganic phosphate for the synthesis of lecithin, demethylation of

3 Under the conditions of the experiments the amount of methionine available to the animals must have been very small. It would be of interest to determine whether in the presence of a methyl group donor like methionine more lecithin is formed from aminoethylphosphoric acid.
lecithin to form cephalin. The aminoethylphosphoric acid normally occurring in the body tissues probably is the product of the catabolism of cephalin.

One other point appears noteworthy; viz., the large amount of radioactive phosphorus excreted in the urine during the first 8 hours of the experiment (Table IV). In this time more than a quarter of the radioactive phosphorus administered had passed through the kidneys. There was no difference in this respect between the normal and tumor-bearing animals. As can be seen, a high percentage, about one-third, of the total phosphorus excreted was radioactive phosphorus which came from the aminoethylphosphoric acid. During the next 10 hours the concentration of radioactive phosphorus dropped to 7 per cent of the total phosphorus eliminated.

The experiments on the metabolism of aminoethylphosphoric acid in tumor-bearing animals provide no basis for the assumption of a specific function of this compound in malignant growth. The liver showed a faster rate of phosphatide turnover and of total phosphorus uptake than the tumor (Tables II and III). A comparison of Tables I and III will, however, show that tumors belong to the most active tissues with regard to the formation of phosphatides from aminoethylphosphoric acid. This has been demonstrated before, as far as the utilization of inorganic phosphate is concerned (11, 12).

The authors would like to express their gratitude to Dr. J. R. Dunning of the Department of Physics of this University and to Dr. E. O. Lawrence of the Radiation Laboratory of the University of California for the radioactive phosphorus used in the experiments here described. They are indebted to Mr. B. Kress for general assistance.

SUMMARY

Experiments on the metabolism of radioactive aminoethylphosphoric acid indicate that this compound is not directly utilized for the synthesis of cephalin. After its administration to rats more newly formed lecithin than cephalin is found both in the liver and the intestinal tract. The bearing of the findings on the theory of phosphatide metabolism in the body is discussed.
Aminoethylphosphoric Acid

Experiments with tumor-bearing animals fail to demonstrate a specific function of aminoethylphosphoric acid in malignant growth. It is considered as a normal breakdown product of cephalin.

BIBLIOGRAPHY

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