TOTAL MERCAPTURIC ACID SYNTHESIS BY LIVER AND KIDNEY*

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It has been known since 1879 that certain aromatic hydrocarbons and their halogen derivatives may be converted by the animal body to mercapturic acids as well as to various hydroxylated derivatives (1, 2). The mercapturic acids generally have been isolated from the urine after high dosage of the hydrocarbon precursors. Synthesis of a mercapturic acid by isolated tissues has not been reported previously, although metabolism of hydrocarbons by oxidation to hydroxylated derivatives has been demonstrated. Benzene was converted to phenol by isolated livers (3) and naphthalene to dihydrodihydroxy derivatives by rat liver slices (4). We considered the possibility that with small dosage levels no mercapturic acid would be formed by metabolic reactions, owing to competition by the more biologically economical reactions of oxidation. In a test of this hypothesis in the intact animal, however, we found that the relative amounts of mercapturic acid and hydroxy derivatives which were produced were essentially independent of the dosage level (5). The present report describes the conversion of iodobenzene-I\(^{131}\) to p-iodophenylmercapturic acid by slices of rat liver and kidney. An ethereal sulfate and glucuronide yielding p-iodophenol on hydrolysis have also been detected.

EXPERIMENTAL

Iodobenzene was labeled with I\(^{131}\) as described previously (5). An additional distillation of the compound \textit{in vacuo} yielded a product with less than 0.2 per cent of non-volatile impurities. Transfer of iodobenzene to methyl Cellosolve was effected by adding 3.5 ml. of a 6:1 carbon tetrachloride-methyl Cellosolve solution to 15 ml. of a solution of iodobenzene in petroleum ether. The petroleum ether and carbon tetrachloride were then removed \textit{in vacuo} at 0\(^\circ\). If carbon tetrachloride was omitted, the solution separated into two phases during evaporation with resultant loss of much of the radioactive iodobenzene.

For incubation experiments, difficulty was encountered in bringing the iodobenzene into proper contact with the tissue slices. Methyl Cellosolve has been used as a solvent for naphthalene in similar experiments, but it

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would not keep iodobenzene in solution when added to a Krebs-Ringer buffer. The iodobenzene was satisfactorily dispersed when added in methyl Cellosolve solution to rat plasma. When 0.26 mg. of radioactive iodobenzene in 0.25 ml. of methyl Cellosolve was added to 7 ml. of rat plasma, only 8.5 per cent of the iodobenzene was lost by volatilization during a 90 minute incubation period at 37°. However, appreciable dilution of the plasma with 0.9 per cent saline greatly reduced its capacity to hold the iodobenzene.

A typical incubation experiment is described in Table I. The samples were shaken at 37° with 95 per cent oxygen and 5 per cent carbon dioxide as the gaseous phase. In the control experiment, the tissue slices in saline were heated to 100° for 10 minutes prior to the addition of iodobenzene dispersed in the unboiled plasma. At the end of the incubation period, the slices were homogenized and the proteins were precipitated with 50 ml. of ethanol. The protein precipitates were washed twice with 10 ml. portions of ethanol, and the washes were combined with the original supernatant fluid. This fraction was concentrated at 50° to about 5 ml. It was then extracted four times with 3 ml. portions of diethyl ether to remove lipides and unchanged iodobenzene. An aliquot of the aqueous phase was used for paper chromatograms. The p-iodophenylmercapturic acid spot on these chromatograms was obscured by the large amount of decomposition products of iodobenzene which are mentioned below. Consequently it was

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>Viable slices</th>
<th>Boiled slices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodobenzene originally present</td>
<td>1490</td>
<td>1490</td>
</tr>
<tr>
<td>&quot; lost during incubation</td>
<td>980</td>
<td>030</td>
</tr>
<tr>
<td>Water-soluble products</td>
<td>9.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Glucuronide metabolite</td>
<td>1.8*</td>
<td>0</td>
</tr>
<tr>
<td>Ethereal sulfate metabolite</td>
<td>0.58*</td>
<td>0.0</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>0.31</td>
<td>0.15</td>
</tr>
<tr>
<td>p-Iodophenylmercapturic acid</td>
<td>0.061</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Viable slice system, 2.2 gm. of rat liver slices, 4 ml. of 0.9 per cent NaCl, 6 ml. of iodobenzene-plasma solution. 3 hour incubation.

Boiled slice system, 2.2 gm. of boiled rat liver slices, 4 ml. of 0.9 per cent NaCl, 6 ml. of iodobenzene-plasma solution. 3 hour incubation.

* Calculated from the distribution of the radioactivity on the paper chromatograms.
necessary to use a different technique for the mercapturic acid determination. The remainder of the water solution was acidified with 0.1 volume of concentrated HCl and extracted six times with 3 ml. portions of chloroform. Approximately 20 mg. of carrier p-iodophenylmercapturic acid were added to each of the chloroform extracts, the chloroform was distilled, and the mercapturic acid was crystallized from the residues. For purification, recrystallization was carried out successively from water, from a neutralized sodium hydroxide solution, and from an acetone-water mixture. This purification procedure removed all of the radioactivity from the carrier mercapturic acid obtained from the control system, while the mercapturic acid from the viable incubation mixture had a specific activity of 48 c.p.m. per µM. Repetition of the above crystallization sequence reduced the specific activity of this sample to 45 c.p.m. The radioactivity of the purified mercapturic acid carrier accounted for 20 per cent of the I\textsuperscript{131} in the chloroform extract. The product was subjected to a constant solubility test (6). Table II shows that with an allowance of 5 per cent counting error no impurity was detected by this test.

In each of two other incubation experiments the p-iodophenylmercapturic acid of constant specific radioactivity was converted to p-iodophenylcysteine by heating the acetyl compound 2 hours with 6 N HCl under reflux. In both cases the specific activity of the recrystallized p-iodophenylcysteine was about 20 per cent below that of the mercapturic acid. The retention of radioactivity through the chemical reaction confirmed the original synthesis of mercapturic acid. The decrease in radioactivity might have been due to a loss of iodine during the acid hydrolysis. Azouz, Parke, and Williams (7) found that the iodine of p-iodocatechol is readily removed by acid.

Paper chromatograms of the aqueous concentrate of the incubation mix-

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Radioactivity of solution* (c.p.m. per 0.5 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5.5</td>
<td>19</td>
</tr>
<tr>
<td>6.5†</td>
<td>20</td>
</tr>
</tbody>
</table>

* The solvent was 2 ml. of water at 37°. p-Iodophenylmercapturic acid (2.90 mg.) was present initially.
† Additional mercapturic acid (2.50 mg.) was added at this time.
ture before chloroform extraction had two radioactive spots which were absent from the chromatogram of the control sample. The spots migrated to the positions occupied by the first and fifth metabolites found in chromatograms of urine of animals fed iodobenzene (5). Both spots likewise yielded \( p \)-iodophenol on hydrolysis and hence were, respectively, the glucuronic acid and ethereal sulfate of \( p \)-iodophenol or possibly of a precursor, dihydrodihydroxyiodobenzene.

The paper chromatograms of the aqueous concentrates showed a large amount of radioactivity in a position which did not correspond to any urinary metabolites observed previously. This was found also on the chromatograms of the control incubation mixture and to a lesser extent on chromatograms of an iodobenzene-plasma mixture which was not incubated. The latter finding eliminated the compound from consideration as a product of tissue metabolism. However, it was necessary to test whether

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Tissue</th>
<th>Weight of slices</th>
<th>Iodobenzene present*</th>
<th>Mercapturic acid synthesized</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver</td>
<td>2.2</td>
<td>141</td>
<td>0.031</td>
</tr>
<tr>
<td>2</td>
<td>Kidney</td>
<td>1.2</td>
<td>95</td>
<td>0.018</td>
</tr>
<tr>
<td>3†</td>
<td>Liver</td>
<td>2.5</td>
<td>160</td>
<td>0.057</td>
</tr>
</tbody>
</table>

* Iodobenzene present at the close of the incubation period.
† Coenzyme A (Pabst, equivalent to 0.13 mg. of pure coenzyme A), adenosinetriphosphate (4.3 mg.), and 17 mg. of glucose were added to this incubation sample.

the substance could serve as a precursor of mercapturic acid and iodophenol. This was done by collecting the water-soluble radioactive products from the boiled slice digest and from the plasma-iodobenzene mixture and using this material in a new incubation experiment with viable slices. After 3 hours of incubation, the products were fractionated as described above. The proteins were precipitated and the aqueous solution was extracted with chloroform. Carrier \( p \)-iodophenylmercapturic acid was added to the chloroform extract and was recovered by crystallization. Two recrystallizations were sufficient to remove all radioactivity from the sample. A paper chromatogram of the original aqueous concentrate showed only the starting material.

The amounts of mercapturic acid formed in two experiments with liver slices and one with kidney slices are shown in Table III. The increased amount of mercapturic acid produced in the presence of adenosinetriphosphate and coenzyme A is not at present considered significant.

In another experiment, the amount of ethereal sulfate metabolite syn-
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thesized by 2 gm. of liver slices in 3 hours was found to be 1.2 per cent of the 0.3 mg. of iodobenzene used.

DISCUSSION

The isolation of mercapturic acid from incubation mixtures by isotopic dilution constitutes the evidence for synthesis in vitro. Two criteria were used to demonstrate that the radioactivity in the isolated carrier substance was due to p-iodophenylmercapturic acid-I\textsuperscript{31} synthesized by the tissue slices. After the carrier compound had been crystallized to constant specific activity, it was subjected to the constant solubility test, or to conversion to a derivative with retention of a substantial amount of radioactivity.

The predominance of iodophenol derivatives produced by the liver slices as compared to mercapturic acid production is contrary to results obtained with the intact animal. A relationship of mercapturic acid formation to the hydroxylation reactions has never been proved, and it is now generally accepted that the two types of derivatives arise from different pathways of metabolism. Hydroxylation is a more general reaction, since many hydrocarbons, particularly those of the carcinogenic type, are readily hydroxylated, but are not converted to mercapturic acids. In the present study it was observed that liver slices were able to carry out the hydroxylation reaction more readily than the mercapturic acid synthesis.

Mercapturic acid synthesis is obviously a two-step process involving combinations between the aromatic ring, cysteine, and the acetyl fragment. It has been shown previously that the animal body can acetylate an arylcysteine as the final step of synthesis (8). Gutmann and Wood have shown likewise that the acetylation step can be performed by liver or kidney slices (9). Acetylation is a common reaction in biological as well as organic chemistry. The conjugation of the aromatic ring with the sulfur moiety, however, is a biological reaction for which no counterpart exists in organic chemistry. The actual mechanisms and intermediate compounds involved in the attachment of the cysteine sulfur to the aromatic ring remain obscure. The synthesis of mercapturic acids by an in vitro system provides a means of further study of the reaction.

SUMMARY

Rat liver and kidney slices synthesized p-iodophenylmercapturic acid from iodobenzene-I\textsuperscript{31} dispersed in rat plasma. The mercapturic acid was recovered after addition of carrier and was crystallized to constant specific activity. The constant solubility test and conversion to radioactive p-iodophenylcysteine were used as criteria for the identification of the isolated radioactive compounds.
A larger portion of the iodobenzene-I\textsuperscript{131} was converted by the liver slices to glucuronide and ethereal sulfate conjugates yielding p-iodophenol on hydrolysis. Of these two conjugates, the glucuronide was found in larger amounts.

BIBLIOGRAPHY

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