The Distribution of $^{35}S$ from Labeled DL-Methionine in Mice Bearing Carcinoma of the Breast, Neoplasms of the Hematopoietic System, or Liver Abscesses

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The experiments on the distribution of radio-sulfur reported here were intended to give empirical information on the relative uptake of the isotope by neoplastic tissue, as compared to normal tissue, in order to evaluate chances for a localized radiation therapy of tumors with labeled methionine. The possibility of an accumulation of radioactive sulfur in neoplasms, after administration of the isotope in an amino acid, was suggested by reports that neoplasms take up amino acids more rapidly than do normal adult tissues (24) and by the evidence for an increased metabolic turnover rate in tumors (4, 17). It was realized, however, that other factors, such as increased permeability or increased retention of metabolites, might account for any concentration of radiosulfur in the tissues (11). Some preliminary data concerning the distribution of $^{35}S$ from labeled methionine in mice bearing mammary carcinomas or transplanted ependymoblastomas have been published from this laboratory (9). We have since found that varying amounts of $^{35}S$ are lost by the method of tissue preparation which was used in this preliminary work. Some of our earlier experiments have been therefore repeated with more dependable technical procedures. In addition, we have extended our studies to neoplasms of the hematopoietic organs and liver abscesses. The results reported now are similar to those obtained in the preliminary investigations: in both studies the concentration of radiosulfur, at the times chosen for analysis, was higher in tumor tissue than in many normal tissues, but lower than in liver, kidney, spleen, or the intestines.

METHODS

DL-Methionine-$^{35}S$ was prepared from thiourea-$^{35}S$ (2). The purity and identity of the compound were checked by paper chromatography with n-butanol and phenol. The amino acid was located on the paperogram by the ninhydrin reaction, and the isotope by scanning with a monitor and by radioautography on x-ray film. Spraying ninhydrin on the papers produced but one spot, whose Rf value agreed with that reported in the literature for DL-methionine (16) and with that of a concurrently run authentic sample of the compound. All the radioactivity was found to be localized within the ninhydrin spot.

All radioactivity measurements were carried out on barium sulfate. The organic materials were digested by the Carius method (7, 19, 23), and enough sodium sulfate-$^{35}S$ was added to the digest to yield a total precipitate of 170–180 mg. barium sulfate. The barium sulfate was filtered onto stainless steel planchets having a surface area of 1.77 cm.$^2$ and clad with Whatman No. 50 filter paper (1). Care was taken to obtain a smooth mat of barium sulfate with even thickness. The precipitate was washed with water and ethanol, air-dried, and counted with a thin window bell-type Geiger tube. The counting rates were corrected for background, coincidence, and decay in the customary manner (15). For urinary sulfate-$^{35}S$ determinations, digestion by the Carius procedure was omitted.

Radioactivity assays on individual tissues and tissue fractions were run in duplicate or triplicate wherever sufficient material was available. The values for the $^{35}S$ concentrations were reproducible to within ±5 per cent in 46 tissues, to within ±10 per cent in 16 tissues, to within ±20 per cent in 9 tissues, and to within ±33 per cent in 5 tissues. The reproducibility was generally better for

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tissues with high isotope concentrations, such as liver, than for tissues with low isotope concentrations, such as muscle. Some of the discrepancies may have been due to inhomogeneity of the tissues, so that what was thought to be a duplicate sample from a tissue actually had a different radiosulfur concentration.

Proteins were obtained by dispersing the tissues in a glass homogenizer with 10 per cent aqueous trichloroacetic acid; the homogenate was centrifuged, and the residue washed 4 times with 10 per cent trichloroacetic acid, 3 times with ethanol, and then thrice with ethyl ether. Finally, the residue was dried in vacuo over phosphorus pentoxide at room temperature; it was considered the "protein" fraction for our purposes (3). The wet tissue minus this "protein" fraction was designated as the "nonprotein" fraction; the latter comprised water and the solutes which were removed from the wet tissues by treatment with aqueous trichloroacetic acid, ethanol, and ethyl ether.

The distribution of $^{35}$S was studied in the tissues of eleven mice. Two animals served as normal controls, two had mammary carcinomas, two carried transplanted lymphosarcomas, two had liver abscesses, two had lymphatic leukemias, and one had myelogenous leukemia. Further details concerning these animals are given in Table 1.

The animals received intraperitoneal injections of an aqueous solution of labeled DL-methionine containing 20–40 µc. of $^{35}$S, and were decapitated 24 hours later. It was assumed that administration of the labeled compound in a single dose, rather than continuous dosage, would favor selective uptake. We studied the distribution of the isotope at 24 hours, rather than at some other interval, because our preliminary experiments led us to expect a maximum concentration of the isotope in the tumors at this time. Also, the data of Maas (10), Friedberg (3), and Tarver (21) seemed to suggest that the distribution of radio-sulfur 24 hours after its administration would approximate the relative distribution of the isotope retained in the tissues over most of its biological life. Up to the time of the experiment, the mice were on an ad libitum fox chow diet, but they were put on a fast after the administration of the amino acid. No untoward effects were observed from the injection.

### TABLE 1

**MICE USED IN THE STUDY OF $^{35}$S DISTRIBUTION**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Pathology</th>
<th>Wt. of Mouse (gm.)</th>
<th>Age (mo.)</th>
<th>Methionine Dose</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Lung</th>
<th>Heart</th>
<th>Brain</th>
<th>Tumor</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>26.5</td>
<td>F</td>
<td>0.6</td>
<td>4.2</td>
<td>1.5</td>
<td>0.26</td>
<td>0.47</td>
<td>0.40</td>
<td>0.96</td>
<td>A.K.; strain Strong A</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Normal control</td>
<td>54.0</td>
<td>12</td>
<td>M</td>
<td>1.6</td>
<td>3.7</td>
<td>1.1</td>
<td>0.28</td>
<td>0.78</td>
<td>0.54</td>
<td>0.95</td>
<td>A.K.; strain Strong A</td>
</tr>
<tr>
<td>3</td>
<td>Mammary carcinoma</td>
<td>40.0</td>
<td>8</td>
<td>F</td>
<td>1.3</td>
<td>3.1</td>
<td>1.4</td>
<td>0.21</td>
<td>0.51</td>
<td>0.35</td>
<td>0.95</td>
<td>8.2 J.J.B.; strain Z Ce F; lactating</td>
</tr>
<tr>
<td>4</td>
<td>Mammary carcinoma</td>
<td>58.0</td>
<td>11</td>
<td>F</td>
<td>1.6</td>
<td>4.0</td>
<td>1.4</td>
<td>0.45</td>
<td>0.51</td>
<td>0.31</td>
<td>0.84</td>
<td>2.0 J.J.B.; strain Strong A</td>
</tr>
<tr>
<td>5</td>
<td>Transplanted lymphosarcoma</td>
<td>20.5</td>
<td>2</td>
<td>M</td>
<td>1.3</td>
<td>5.9</td>
<td>1.0</td>
<td>0.45</td>
<td>0.55</td>
<td>0.56</td>
<td>1.6</td>
<td>5.8 A.K.; line 1016; 1st transfer of spontaneous lymphosarcoma which had originated in this line</td>
</tr>
<tr>
<td>6</td>
<td>Transplanted lymphosarcoma</td>
<td>19.5</td>
<td>2</td>
<td>M</td>
<td>2.0</td>
<td>2.7</td>
<td>0.96</td>
<td>0.25</td>
<td>0.39</td>
<td>0.29</td>
<td>1.6</td>
<td>8.4 A.K.; line 1016; 6th transfer of spontaneous lymphosarcoma which had originated in this line</td>
</tr>
<tr>
<td>7</td>
<td>Liver abscess</td>
<td>28.0</td>
<td>?</td>
<td>F</td>
<td>0.6</td>
<td>9.1</td>
<td>1.4</td>
<td>1.1</td>
<td>0.88</td>
<td>0.96</td>
<td>1.4</td>
<td>G.M.; strain Strong A; fed N-acetylaminofluorene</td>
</tr>
<tr>
<td>8</td>
<td>Liver abscess</td>
<td>28.0</td>
<td>?</td>
<td>M</td>
<td>0.6</td>
<td>6.9</td>
<td>1.3</td>
<td>1.8</td>
<td>1.2</td>
<td>0.58</td>
<td>1.1</td>
<td>10.0 G.M.; strain Strong A; fed N-acetylaminofluorene</td>
</tr>
<tr>
<td>9</td>
<td>Myelogenous leukemia</td>
<td>28.0</td>
<td>?</td>
<td>M</td>
<td>0.6</td>
<td>6.8</td>
<td>1.7</td>
<td>4.3</td>
<td>1.3</td>
<td>0.99</td>
<td>1.2</td>
<td>A.K.; line 15; 40th transfer</td>
</tr>
<tr>
<td>10</td>
<td>Lymphatic leukemia</td>
<td>17.5</td>
<td>2</td>
<td>M</td>
<td>1.5</td>
<td>5.1</td>
<td>1.1</td>
<td>2.8</td>
<td>1.3</td>
<td>0.43</td>
<td>1.6</td>
<td>1.4 A.K.; line 676; 30th transfer</td>
</tr>
<tr>
<td>11</td>
<td>Lymphatic leukemia</td>
<td>27.0</td>
<td>23</td>
<td>M</td>
<td>0.2</td>
<td>5.9</td>
<td>0.87</td>
<td>0.55</td>
<td>0.55</td>
<td>6.0 A.K.; strain CBA; had received 200 r x-ray at age 1 mo.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mg. of methionine injected per gram body weight.
† The notations A.K., J.J.B., and G.M. indicate mice from the colonies of Drs. A. Kirschbaum, J. J. Bittner, and George Moore, respectively.
RESULTS AND DISCUSSION

The concentration of $S^{35}$ in the wet tissues (Table 2, roman type) and protein and nonprotein fractions (Table 3) is expressed in terms of fraction of total counts per minute administered per mg. sample $\times$ weight of mouse in milligrams. This expression normalizes values obtained in animals of various weights and injected with various amounts of the isotope to animals of unit size and injected with unit doses of labeled methionine. The percentage of the total dose in the various whole tissues is also given (Table 2, figures in italics).

**TABLE 2**

CONCENTRATION AND DISTRIBUTION OF $S^{35}$ IN TISSUES 24 HOURS AFTER INTRAPERITONEAL INJECTION OF DL-METHIONINE-$S^{35}$

| Exp. no. | Pathology                  | Liver | Both Kidneys | Spleen || Stomach* | Large intestine† | Small intestine† | Lang | Whole blood | Heart | Brain | Muscle | Tumor |
|---------|---------------------------|-------|--------------|--------|----------|----------------|----------------|-------|-------------|-------|-------|--------|-------|
| 1       | Normal control            | 0.67  | 0.50         | 0.44   | 0.43     | 0.30           | 0.29           | 0.27  | 0.11        | 0.07  |       |        |       |
| 2       | Normal control            | 3.8   | 0.67         | 0.11   |          | 0.14           | 0.11           | 0.10  |             |       |       |        |       |
| 3       | Mammary carcinoma         | 4.8   | 0.94         | 0.17   |          | 0.87           | 0.11           | 0.15  |             |       |       |        | 0.19  |
| 4       | Mammary carcinoma         | 3.8   | 0.88         | 0.11   |          | 0.14           | 0.07           | 0.12  |             |       |       |        | 0.16  |
| 5       | Transplanted lymphosarcoma| 4.7   | 0.51         | 0.07   | 0.07    | 0.55           | 0.45           | 0.65  |             |       |       |        | 0.69  |
| 6       | Transplanted lymphosarcoma| 5.8   | 0.88         | 0.17   |          | 0.88           | 0.80           | 0.16  |             |       |       |        | 0.69  |
| 7       | Liver abscess             | 1.2   | 0.79         | 0.21   |          | 0.69           | 0.22           | 0.15  | 0.07        | 0.07  |       |        | 0.07  |
| 8       | Liver abscess             | 1.1   | 0.31         | 0.11   |          | 0.14           | 0.07           | 0.07  |             |       |       |        | 0.07  |
| 9       | Myelogenous leukemia       | 7.6   | 1.0          | 0.71   |          | 0.65           | 0.16           | 0.21  |             |       |       |        | 1.0   |
| 10      | Lymphatic leukemia         | 4.7   | 0.51         | 0.11   |          | 0.71           | 0.31           | 0.19  |             |       |       |        | 0.07  |
| 11      | Lymphatic leukemia         | 5.8   | 0.88         | 0.21   |          | 0.88           | 0.34           | 0.24  | 0.16        | 0.16  |       |        | 0.07  |

* Glandular portion only.
† Wall only.
‡ Abscess, purulent material.

All figures except those in italics represent the concentration in fraction of total counts per minute administered $\times$ weight of mouse in mg. tissue.

Figures in italics represent distribution in per cent dose in organ.

**TABLE 3**

DISTRIBUTION OF $S^{35}$ BETWEEN PROTEIN AND NONPROTEIN FRACTIONS OF LIVERS, MUSCLES, AND TUMORS

<table>
<thead>
<tr>
<th>Experiment no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

Liver:

- Protein content, per cent
- Concentration of $S^{35}$ in protein fraction
- Concentration of $S^{35}$ in nonprotein fraction*

Muscle:

- Protein content, per cent
- Concentration of $S^{35}$ in protein fraction
- Concentration of $S^{35}$ in nonprotein fraction*

Tumor:

- Protein content, per cent
- Concentration of $S^{35}$ in protein fraction
- Concentration of $S^{35}$ in nonprotein fraction*

* Calculated from protein content and concentration of $S^{35}$ in wet tissue and protein.

Concentrations in fraction of total counts per minute administered $\times$ weight of mouse in mg.
The concentration of S\textsuperscript{35} was highest in the liver and intestines; on the average, decreasing concentrations were found in the kidneys, spleen, intestinal wall, stomach, lung, whole blood, heart, brain, and muscle. The concentration of radiosulfur in the tumors was higher than its concentration in most normal tissues, but lower than that in the intestines, livers, kidneys, or spleens. In contrast to its concentration in the tumors, the concentration of the isotope in both abscesses was lower than in any of the tissues in the same animal. Grossly normal lactating mammary gland tissue showed a lower concentration (0.18) than homologous tumor tissue (0.33) in the same animal (Experiment No. 8). The spread of the values for the concentration of the isotope in a given organ among various animals was of the order of ±0.1 of the average value. While this may appear to be a large variability, it is no greater than that of the ratio of organ to body weight.

A comparatively large fraction of the dose was present in the liver, as one would expect from the high concentration in this organ and from its size. The percentage of the dose found in the spleen varied by a factor of 40 from animals with normalized spleens to animals with enlarged spleens. However, it appeared that the large amounts of S\textsuperscript{35} found in the spleens in Exp. Nos. 8, 9, and 10 were not due to the mass of the organs alone, since the concentration of the isotope in the spleens tended to increase with their relative size. A slightly higher percentage of the dose was found in the lungs of mice with leukemia or liver abscesses than in the lungs of the other animals. The percentage of the dose in the tumors and the tumor weights given in Table 1 represent minimum values only, since it is likely that small metastatic and infiltrating lesions were missed at autopsy.

The protein fractions of the livers, tumors, and muscles, although they comprised only one-tenth to one-fifth of the mass of the tissues, contained one- to two-thirds of the total amount of radiosulfur in the tissues, and the concentration of the isotope in the protein fractions was 2–12 times greater than in the nonprotein fractions. The ratio of the total amount of radiosulfur in the protein fraction to the total amount of radiosulfur in the nonprotein fraction was relatively constant, despite large differences in the concentration of the isotope between various tissues. The concentration of radiosulfur in the protein fraction from lymphatic tumors was equal to or greater than its concentration in the protein fraction from the livers of the same animals (Exp. Nos. 5, 6, and 11). The fact that the concentration of the isotope in the wet tumor tissue of these animals was lower than in the wet liver tissue may be due to the low protein content of the tumors. Our experiments do not permit one to draw conclusions regarding the chemical form in which the radiosulfur was present in the tissues and tissue fractions; however, one may surmise from what is known about sulfur metabolism (5) that appreciable amounts of radiosulfur retained in the animals were no longer incorporated in methionine.

Within the 24-hour period of our experiments, the animals excreted up to 10 per cent of the injected methionine sulfur in the stools. An average of 47 per cent appeared in the urine, the value in individual animals ranging from 15 to 90. Inorganic sulfate accounted for 62–90 per cent of the isotope in the urine.

Our values for the percentage of methionine sulfur excreted in the urine are comparable to corresponding values in the literature (12, 13, 20, 22), obtained from balance experiments with methionine doses of 0.1–1 mg/gm body weight. If one assumes the normal methionine level in serum (about 10\textsuperscript{-2} mg/ml) (6, 18) to be representative of the normal methionine level in the body water, and on the premise that the body water accounts for about 60 per cent of the body weight, it seems likely that the amount of methionine which we injected into our animals may have raised the methionine concentration in their body fluids by a factor of 100. Following the administration of much smaller amounts of labeled methionine (10\textsuperscript{-2}–10\textsuperscript{-3} mg/gm body weight), Kinsell (8), Reed (14), and Tarver (21) found about 10 per cent of the radiosulfur in the urine within the first 24 hours. Comparison of our data with those of Kinsell (8), Tarver (21), and Friedberg (3) suggests, as far as comparison of the data is possible, that even a hundredfold change in methionine dosage has only a relatively slight effect on the percentage of methionine sulfur excreted, or on the distribution of the isotope in the tissues.

SUMMARY
The distribution of S\textsuperscript{35} in mice bearing mammary carcinomas, lymphosarcomas, leukemias, or liver abscesses was studied 24 hours after an intraperitoneal injection of D.L-methionine-S\textsuperscript{35}. The concentration of the isotope was highest in the intestinal wall and liver, lowest in muscle; it was considerably higher in protein fractions than in nonprotein fractions of the tissues. The concentration of S\textsuperscript{35} in the wet tumor tissue was lower than that in the wet liver tissue of the same animals; but the concentration of the isotope in the proteins of lymphosarcomas was equal to or greater than...
that in the liver proteins from the same animal. The fact that the $S^4$ in wet lymphosarcoma tissue is lower than in wet liver tissue is probably due to the low protein content of lymphosarcomas. In contrast to that in tumors, the concentration of $S^4$ in liver abscesses was very low. On the premise that the concentration of a radioactive isotope in tumor tissue must be much higher than in other tissues of the body if the isotope is to be useful in the treatment of neoplastic diseases, the data reported here do not favor methionine-$S^4$ as a possible therapeutic agent for mammary carcinomas, lymphosarcomas, or leukemias.

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REFERENCES

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