METHIONINE LABELED WITH RADIOACTIVE SULFUR
AS AN INDICATOR OF PROTEIN FORMATION
IN THE HEPATECTOMIZED DOG

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The evidence indicating the site of origin of plasma proteins has been
reviewed by several authors (1-3). It is now generally believed that the
major part of the plasma protein originates in the liver, but that part, par-
ticularly of the globulin fraction, may be formed in other tissues. Fibrino-
gen probably arises in the liver because this fraction falls when the liver
function is inhibited with agents such as chloroform, or when the liver is
removed. With regard to the origin of albumin and globulin, the evidence
is more ambiguous. In Eck fistula dogs the rate of regeneration of plasma
proteins is estimated to be only one-tenth of that in normal dogs (4, 5) and
in the hepatectomized dog protein may enter the circulation from some
organ other than the liver (6, 7). Globulin probably arises to some extent
in organs other than the liver, as is indicated by the high plasma globulin
values in multiple myeloma, kala azar, and lymphogranuloma inguinale, all
of which implicate the bone marrow or white blood cells as a source. The
weight of recent immunological evidence shows that the reticulo-
endothelial system and, in particular, the lymph nodes and lymphocytes
are concerned in the formation of γ-globulin (3, 8-11).

In previous experiments we have shown that labeled methionine fed in
small amounts is incorporated into body proteins (12). The assumption is
made that this indicates protein synthesis; that is, the incorporation of
methionine into protein by the formation of new peptide bonds. Methio-
nine sulfur is normally converted to cystine sulfur, which is likewise in-
corporated in the protein molecule, presumably as the result of peptide
bond formation (13). The present experiments were designed to determine
whether in the liverless dog methionine can be incorporated into tissue
proteins. We have also investigated the cystine sulfur of the tissue pro-
teins of the liverless dog to see whether this animal is able to convert
methionine sulfur into cystine sulfur, as is the case in the normal animal.

To check the efficiency of washing of proteins to remove free labeled
methionine, and to rule out the possibility of any simple exchange between
free methionine and protein-bound methionine, whole blood has been in-
cubated with labeled methionine for different periods of time. The proteins
were precipitated and washed repeatedly. Analyses were then made to determine the extent of binding of the labeled methionine.

**Methods**

**Experimental Animals and Technique of Hepatectomy**—Total removal of the liver was carried out in a one-stage operation according to the technique of Firor and Stinson (14). Two control animals underwent sham operations designed to simulate the operative conditions of hepatectomy. In these controls, the portal vein and the inferior vena cava were obstructed for the same length of time as in those animals in which the liver was actually removed. Sodium pentobarbital by intraperitoneal injection was employed as the anesthetic agent throughout the course of the experiments. In addition, 100 ml. samples of blood were removed from the control animals to provide blood for *in vitro* experiments. Shortly after the completion of the operation, an appropriate dose of methionine was injected intravenously into the animals. The animals were maintained throughout the duration of the experiments with injections of 25 per cent glucose. After varying intervals of time, the animals were sacrificed and samples of blood and tissues were taken. The proteins of these samples were then analyzed, as described under "Preparation and treatment of protein samples" below.

**In Vitro Incubation**—100 ml. of whole heparinized blood from each of the animals having the sham operation were incubated with labeled methionine for either 2 or 5 hours at 37°. After incubation, the plasma was separated, precipitated with 10 per cent trichloroacetic acid, and washed six or ten times with 4 per cent trichloroacetic acid. (The first three of these washes contained 15 mg. per cent of ordinary methionine to act as the carrier.) The washed proteins were made into samples for radioactivity determination.

**Methionine**—Labeled methionine was synthesized from barium sulfate, as previously described (12, 13). The dose of methionine given to the experimental animals varied between 0.62 and 1.05 mg. per kilo of body weight.¹

**Preparation and Treatment of Protein Samples**—The tissues were minced and washed four to six times with 4 per cent trichloroacetic acid, after precipitation with a 10 per cent solution of the same acid. The first two wash solutions contained 12 mg. per cent of cystine and 15 mg. per cent of methionine. For convenience the residue will be referred to as protein. The protein sulfur was converted to sulfate and determined as previously described (12, 13).

Plasma proteins were fractionated by the sodium sulfate method (15).

¹ There is no reason to believe that this difference in dosage would cause any perceptible difference in the per cent incorporated into protein.
The fractions were each washed with trichloroacetic acid until free of sulfate and made into samples for radioactivity determination.

For the isolation of cystine, part of the total protein of liver (in the sham-operated controls), kidney, pancreas, and intestinal mucosa of each animal was completely hydrolyzed by refluxing for 24 hours with $8 \times HCl$ (5 ml. of HCl were used per gm. of dried protein). The HCl was removed at reduced pressure, the residue dissolved in water, filtered, and the filtrate adjusted to pH 4.0. The cystine was precipitated from the filtrate with cuprous chloride and washed with buffer, its sulfur being converted into sulfate for determination (13).

RESULTS AND DISCUSSION

Table I shows that in a period of 2 to 5 hours considerable sulfur is incorporated into the tissue proteins of both the control sham-operated animals and the animals with their livers completely removed. The figures show that in the animals without the liver as much, or more, of the labeled amino acid was incorporated into their proteins as in the animals with the liver. Thus the liver is not essential for synthesis of protein in other tissues. It is reasonable to expect that animals without the liver might show a greater incorporation of amino acid into other tissues because the amount of amino acid available for these tissues is greater in such animals. The liver in the control animal takes up a fairly large fraction of the total dose injected.

It is again shown that intestinal mucosa is exceptionally active in the incorporation of amino acid into protein.

The activity of the total plasma protein in the liverless animal is about one-twentieth that of the control animals, and the albumin is reduced to approximately the same extent. The globulin in the liverless animal, however, has about one-seventh the activity of this fraction in the normal animal. Consequently it is necessary to conclude that the liverless animal retains a significant fraction of the control animal's capacity to synthesize globulin.

Analyses of the plasma albumin fractions in the hepatectomized animals provide definite evidence for the incorporation of labeled methionine. In these experiments, however, it is not certain that plasma protein fractionation by the sodium sulfate method permitted a complete separation of albumin from globulin. The albumin fraction resulting from sodium sulfate fractionation of human plasma may contain 3 to 35 per cent of globulin (16). If all the activity in the albumin fraction in the present experiments is due to globulin contamination, it becomes necessary to assume up to 40 per cent admixture of albumin with globulin. It remains uncertain, consequently, whether or not there is any significant albumin synthesis in the hepatectomized dog.
The activity in the fibrin in the liverless animal is quite low and is most probably due to contamination with globulin.

Minor similarities and differences in the standard replacement values for the same proteins in different animals should not be emphasized. The short

<table>
<thead>
<tr>
<th>Tissue protein</th>
<th>Controls (Dog A)</th>
<th>Duration of experiment</th>
<th>Hepatectomized (Dog C)</th>
<th>Duration of experiment</th>
<th>Hepatectomized (Dog D)</th>
<th>Duration of experiment</th>
<th>Hepatectomized (Dog E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.21</td>
<td>2 hrs.†</td>
<td>2.26</td>
<td>3 hrs.†</td>
<td>2.98</td>
<td>5 hrs.†</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2.04</td>
<td>5 hrs.†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>1.00</td>
<td>3 hrs.†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intestinal mucosa</td>
<td>3.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Lymph nodes</td>
<td>1.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, plasma</td>
<td>1.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;albumin&quot;</td>
<td>1.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&quot;globulin&quot;</td>
<td>1.70</td>
<td></td>
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<tr>
<td>&quot;fibrin&quot;</td>
<td>3.0</td>
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<td></td>
</tr>
</tbody>
</table>

* Values are in standard replacement (cf. preceding paper of this issue).

S. R. = \( \frac{\text{S. A. of sample} \times 10^8 \times W}{\text{S. A. of methionine} \times C} \)

Example of calculations from original data for liver of control Dog A.

\[ \text{S. A. of methionine} = \frac{2.05 \times 10^8 \times 149}{6.86 \times 2} = 2.23 \times 10^4 \]

\[ \text{S. A. of sample} = \frac{478}{0.483} = 990 \]

\[ \text{S. R.} = \frac{990 \times 10^8 \times 10.2}{2.23 \times 10^6 \times 2.05 \times 10^6} = 2.21 \]

† Dog A, male, 10.2 kilos; methionine, 6.86 mg. with 205,000 counts per minute, intravenously. Dog B, female, 11.1 kilos; methionine, 6.86 mg. with 205,000 counts per minute, intravenously. Dog C, male, 14.0 kilos; methionine, 13.71 mg. with 410,000 counts per minute, intravenously. Dog D, female, 13.1 kilos; methionine, 13.71 mg. with 410,000 counts per minute, intravenously. Dog E, male, 14.5 kilos; methionine, 13.71 mg. with 410,000 counts per minute, intravenously.

duration of the experiments and the differences in age, sex, and nutritive conditions among the animals employed are complicating factors not readily susceptible to exact control. The actual values given in Tables I to III, however, are the averages of duplicate or quadruplicate determinations differing by not more than 5 per cent, or, in a few cases in which the
amount of protein employed was small (fibrin), by 10 per cent. None of the existing differences is sufficiently large to cast doubt on the conclusions reached.

**Table II**

*Incorporation of Cystine into Proteins of Normal and Hepatectomized Dogs Given Methionine*

<table>
<thead>
<tr>
<th>Tissue protein</th>
<th>Duration of experiment</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 hrs. (Dog A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.37</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td>1.22</td>
</tr>
<tr>
<td>Intestinal mucosa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See the notes below Table I.

**Table III**

*Blood Incubation Experiment*

<table>
<thead>
<tr>
<th>No. of washes</th>
<th>Actual radioactivity in plasma protein and calculated standard replacement</th>
<th>Incubation, 2 hrs.</th>
<th>Incubation, 5 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Counts per min.† per m.eq. sulfur</td>
<td>S. R.‡</td>
<td>Counts per min.† per m.eq. sulfur</td>
</tr>
<tr>
<td>6</td>
<td>15.5</td>
<td>0.004</td>
<td>22.3</td>
</tr>
<tr>
<td>10</td>
<td>9.7</td>
<td>0.003</td>
<td>22.8</td>
</tr>
</tbody>
</table>

* 100 ml. of whole blood from Dog A or Dog B were incubated *in vitro* at 37° with 0.57 mg. of methionine with 17,100 counts per minute.
† The actual radioactivity found was always less than 16 counts per minute above the background and so the counting error is large.
‡ Calculated on the basis of 10⁶ counts per minute added to 1 liter of whole blood (*cf. in vivo* experiments).

Table II shows that the liver is not essential for the conversion of methionine sulfur into cystine sulfur. From the results of these experiments it appears that all the tissues investigated bring about this conversion.

In Table III are shown the results of incubating methionine with whole blood *in vitro*. There is no appreciable incorporation of methionine into the proteins under these conditions. Part of the apparent incorporation may be due to failure to wash out all of the free methionine. There may be a small incorporation as the result of the activities of white blood cells. It is quite certain from these results, however, that the incorporation found in the *in vivo* experiments is the direct result of metabolic activity of the tissues.
We gratefully acknowledge our indebtedness to Dr. Maurice C. Fishler for his assistance at the operations, and to Dr. Joseph G. Hamilton and the staff of the Radiation Laboratory for the supply of radioactive sulfur used in these studies.

**SUMMARY**

1. The hepatectomized dog incorporates methionine into its tissue proteins at the same rate as does the normal dog.
2. The rate of globulin synthesis in the normal dog is 7 times that found in the hepatectomized animal.
3. The rate of albumin synthesis in the normal dog is 20 or more times that found in the hepatectomized animal. The rate of albumin synthesis as determined in these experiments is overestimated rather than underestimated.
4. The hepatectomized dog can convert methionine sulfur into cystine sulfur in tissues such as the kidney, pancreas, and intestinal mucosa.

**BIBLIOGRAPHY**

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