Effect of Selenium Deficiency and Vitamin E Deficiency on Glutathione Metabolism in Isolated Rat Hepatocytes

Kristina E. Hill and Raymond F. Burk
From the Gastroenterology Division, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284

Selenium deficiency and vitamin E deficiency both affect xenobiotic metabolism and toxicity. In addition, selenium deficiency causes changes in the activity of some glutathione-requiring enzymes. We have studied glutathione metabolism in isolated hepatocytes from selenium-deficient, vitamin E-deficient, and control rats. Cell viability, as measured by trypan blue exclusion, was comparable for all groups during the 5-h incubation. Freshly isolated hepatocytes had the same glutathione concentration regardless of diet group. During the incubation, however, the glutathione concentration in selenium-deficient hepatocytes rose to 1.4 times that in control hepatocytes. The selenium-deficient cells also released twice as much glutathione into the incubation medium as did the control cells. Total glutathione (intracellular plus extracellular) in the incubation flask increased from 47.7 ± 8.9 to 152 ± 16.5 nmol/10^6 selenium-deficient cells over 5 h compared with an increase from 46.7 ± 7.1 to 92.6 ± 17.4 nmol/10^6 control cells and from 47.7 ± 11.7 to 79.5 ± 24.9 nmol/10^6 vitamin E-deficient cells. This overall increase in glutathione concentration suggested that glutathione synthesis was accelerated by selenium deficiency. The activity of γ-glutamylcysteine synthetase was twice as great in selenium-deficient liver supernatant (105,000 × g) as in vitamin E-deficient or control liver supernatant (105,000 × g). Hemoglobin-free perfused livers were used to determine the form of glutathione released and its route. Selenium-deficient livers released 4 times as much GSH into the caval perfusate as did control livers. Plasma glutathione concentration in selenium-deficient rats was found to be 2-fold that in control rats, suggesting that increased GSH synthesis and release is an in vivo phenomenon associated with selenium deficiency.

Glutathione plays a very prominent role in hepatic xenobiotic metabolism and detoxification. While the effects of selenium deficiency and of vitamin E deficiency on glutathione metabolism have been reported (4, 5), little is known about how these deficiencies influence glutathione synthesis and turnover. The present studies were undertaken with two major aims. One was to determine whether hepatocytes isolated from selenium-deficient rats and from vitamin E-deficient rats would be suitable for studies of drug metabolism and toxicity. The other was to examine glutathione metabolism in these isolated hepatocytes to learn whether any of the effects of selenium or vitamin E might be mediated through alterations in it.

EXPERIMENTAL PROCEDURES

Materials—Collagenase, class II (145 units/mg) was obtained from Worthington. All amino acids, GSH, GSSG, glutathione reductase, NADPH, and ATP were purchased from Sigma. L-[3H]Glutamic acid was purchased from New England Nuclear. All other chemicals were reagent grade or better.

Animals—Livers from male Sprague-Dawley rats (250-450 g) which had been fed the experimental diets from weaning for at least 14 weeks were used in all experiments. The diet was prepared as described previously (6). In the control diet, 0.5 mg/kg of selenium as Na₂SeO₃ and 100 IU/kg of vitamin E as dl-a-tocopheryl acetate were added. The selenium-deficient diet omitted the selenium, and the vitamin E-deficient diet omitted the dl-a-tocopheryl acetate and contained tocopherol-stripped corn oil. The rats had free access to food and water up to the time of the experiment. Selenium deficiency was verified by measurement of GSH and vitamin E concentrations in the livers of some of the rats fed the selenium-deficient diet (7). Vitamin E deficiency was verified by measurement of a-tocopherol in the plasma of some of the rats fed the vitamin E-deficient diet (8).

Solutions—All buffer solutions were bubbled with O₂/CO₂ (95:5) and warmed to 37 °C prior to use.

Solution A: 112 mM NaCl, 5.6 mM KCl, 1.0 mM NH₄Cl, 1.4 mM KH₂PO₄, 1.5 mM MgSO₄, 29.5 mM NaHCO₃, pH 7.4.

Solution B: 119 mM NaCl, 4.7 mM KCl, 0.8 mM NH₄Cl, 1.2 mM KH₂PO₄, 1.3 mM MgSO₄, 24.8 mM NaHCO₃, 48.8 mM lactate, 5.3 mM fumarate, 48.8 mM L-glutamate, 11.4 mM α-δ-(+)glucose and amino acid mixture, pH 7.4.

Solution C: 118 mM NaCl, 4.7 mM KCl, 1.0 mM NH₄Cl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24.7 mM NaHCO₃, 2.5 mM CaCl₂, 48.7 mM lactate, 5.3 mM fumarate, 48.7 mM L-glutamate, 11.4 mM α-δ-(+)glucose, and amino acid mixture, pH 7.4.

Amino acid mixture (final concentrations of l-amino acids in solutions B and C): 0.43 mM alanine, 0.014 mM a-amino butyric acid, 0.033 mM aspartic acid, 0.19 mM arginine, 0.067 mM aspartagine, 0.076 mM citrulline, 0.060 mM cysteine, 0.38 mM glutamine, 0.076 mM histidine, 0.065 mM isoleucine, 0.16 mM leucine, 0.38 mM lysine, 0.6 mM methionine, 0.086 mM ornithine, 0.076 mM phenylalanine, 0.17 mM proline, 0.27 mM serine, 0.19 mM threonine, 0.29 mM threonine, 0.067 mM tryptophan, 0.070 mM tyrosine, 0.19 mM valine.
Hepatocyte Preparation—The rat was anesthetized with pentobarbital (100 g of body weight); the peritoneal cavity was opened; the portal vein was cannulated; and solution A was perfused through the liver. The liver was removed and transferred to the perfusion apparatus (9) and perfusion with solution B was begun. The first 50 ml of solution B perfused through the liver was discarded in order to remove the remaining blood. The liver system was then temperature regulated at 37 °C, and the liver was perfused for 30 min with 100 ml of solution B containing 50 mg of collagenase. After the collagenase treatment, the liver connective tissue was well digested and the liver was soft. The liver was removed from the perfusion apparatus and was minced in solution C. With minimal agitation, the hepatocyte suspension was filtered through nylon mesh to remove connective tissue and clumps of cells. The suspension was centrifuged for 1 min at 50 × g, the supernatant was discarded, and the cells were resuspended in solution C. This wash procedure was repeated three times. After the final wash, the hepatocytes were suspended in solution C containing 1.5% gelatin. The cells were then placed in a shaking incubator at 37 °C and maintained in a O₂/CO₂ (95:5) atmosphere for the duration of the experiment. The final cell concentration was adjusted to 3 × 10⁶ cells/ml.

Cell viability was measured by mixing an aliquot of the hepatocyte suspension with an equal volume of solution C containing 1% trypan blue. Cells which excluded trypan blue after 5 min were considered to have intact cell membranes and to be viable (10).

Liver Perfusion—The technique of hemoglobin-free, nonrecirculating liver perfusion employed previously (11) was used with some modifications. The bile duct was cannulated and bile was collected separately from the caval perfusate. The perfusion buffer contained 118 mm NaCl, 5.9 mm KCl, 4.3 mm MgSO₄·7H₂O, 1.2 mm Na₂SO₄, 1.3 mm NaH₂PO₄·2H₂O, 3.3 mm CaCl₂, 35 mm NaHCO₃, 2.1 mm lactate, 0.3 mm glucose. It was equilibrated with O₂/CO₂ (95:5) to give a final pH of 7.5. The flow rate used for all livers was 2.5 ml/g/min. The perfusion buffer was warmed so that the effluent temperature was 37 °C. Lactate dehydrogenase activity (12) was measured in all perfusate samples as an index of cell membrane damage.

Lipid peroxidation was quantitated by determining the amount of thiobarbituric acid-reactive substances present (13). A 250-μl aliquot of hepatocyte suspension was mixed with 2.0 ml of thiobarbituric acid-trichloroacetic acid solution containing 0.01% butylated hydroxytoluene. The mixture was heated in boiling water for 15 min and was centrifuged at 1500 × g for 10 min. The absorbance of the supernatant was measured at 535 nm. Malonaldehyde bis-(dimethyl acetal) (Aldrich) was used as a standard.

Total glutathione concentration was measured with the glutathione reductase-DTNB recirculating assay by the method of Tietze (14) as modified by Griffith (15). A 1.0-ml sample of hepatocyte suspension was centrifuged in a Beckman Microfuge B for 10 s. The extracellular glutathione concentration was determined in the supernatant. The cells were deproteinized with 3% metaphosphoric acid. The acid supernatant was neutralized with 0.3 m NaHPO₄, and the intracellular glutathione concentration was determined. Each assay contained 0.2 mM NAPDH, 0.6 mM DTNB, 0.5 units of glutathione reductase and 200 μl of sample containing 1-5 μmol of glutathione in a final volume of 1 ml. The reaction was started by the addition of glutathione reductase. The rate of formation of reduced DTNB was followed at 412 nm. Bile from perfused livers was assayed for glutathione as described by Eberle et al. (16). Plasma glutathione was measured in untreated and NaBH₄-treated plasma samples as described by Anderson and Meister (17). Untreated plasma samples were deproteinized with 3% metaphosphoric acid. A second plasma sample (0.2 ml) was treated with 0.1 ml of NaBH₄ in EDTA (5 mM, pH 7.1). HCl (0.15 N) was added after 1 h to decompose any remaining NaBH₄. The samples were then neutralized to pH 7.5 with 0.3 m NaHPO₄, and assayed for GSH. The extent of hemolysis in plasma samples was determined as described by Wendum and Cikryt (18).

Oxidized glutathione was measured in the perfused liver experiments by two methods. Direct determination of GSSG was carried out with a coupled assay in which NADPH was oxidized enzymatically by glutathione reductase (19). An Aminco DW-2a spectrophotometer (American Instrument Co., Silver Spring, MD) was used in the dual wavelength mode (340 minus 380 nm) to determine the change in absorbance due to oxidation of NADPH. The minimum amount of GSSG detectable in the perfusate was 1.2 nmol of GSSG/g of liver/min. Indirect determination of GSSG was made with the recirculating assay after reduced glutathione present in the sample had been reacted with 2-vinylpyridine (15). The minimum amount of GSSG detectable with this assay was 0.6 nmol of GSSG/g of liver/min.

γ-Glutamylcysteine synthetase activity was determined by the method of Minnich et al. (20) using liver supernatant (105,000 × g). The supernatant was dialyzed overnight against 0.01 m Tris-HCl, 1 mM EDTA (pH 7.1). Each assay contained 40 μmol of imidazole-HCl (pH 8.25), 4.0 μmol of cysteine-HCl, 1.2 μmol of ATP, 4.0 μmol of dithiothreitol, 6.0 μmol of MgCl₂, 3.6 μmol of L-[³H]glutamic acid (0.17 Ci/mmol) and cystein in a total volume of 0.35 ml. The protein concentration was determined from 0.05-0.30 mg/total assay volume. The reaction was stopped by the addition of 0.03 ml of 60% trichloroacetic acid. Each sample was assayed after 10-, 20- and 30-min intervals. The precipitated protein was removed by centrifugation. The γ-[³H] glutamy1cysteine produced was isolated as the cadmium salt and precipitated at pH 5.2 with 0.5 N NaOH. After being washed twice with water, the cadmium precipitate was dissolved in 0.6 ml of 7% trichloroacetic acid. A 0.5-ml aliquot was dissolved in 10.0 ml of Bray’s solution (21) and [³H] was determined in a Beckman LS 9800 liquid scintillation counter (Beckman Instruments, Inc. Palo Alto, CA).

γ-Glutamyltranspeptidase was measured in liver homogenates with a commercial kit (Sigma, Kit No. 410). The enzymatic activity was determined by the rate of formation of p-nitroaniline from L-γ-glutamyl-p-nitroanilide. The minimum activity detectable was 0.2 μmol of p-nitroaniline produced/mg of protein/min.

Cysteine concentration was determined in liver homogenate by the method of Gaitonde (22).

RESULTS

Isolated hepatocytes from selenium-deficient, vitamin E-deficient and control rats were incubated for 5 h. The hepatocytes from all groups maintained similar levels of viability (Fig. 1) with the exception of those isolated from severely vitamin E-deficient rats. Lipid peroxidation in the cells, as measured by thiobarbituric acid-reactive substances, was low and not significantly different in the three major groups, but it was markedly elevated in the hepatocytes isolated from the severely vitamin E-deficient rats (Fig. 2).

The initial glutathione concentration in the hepatocytes was the same for all the diet groups (Fig. 3, top). As the incubation proceeded, however, the amount of glutathione in the selenium-deficient hepatocytes rose to about 1.4 times the control level, while the glutathione content of the vitamin E-deficient hepatocytes remained about the same as that in control hepatocytes. More strikingly, the selenium-deficient hepatocytes released twice as much glutathione into the medium as the control or the vitamin E-deficient hepatocytes released (Fig. 3, bottom). The intracellular glutathione concentration of the vitamin E-deficient hepatocytes whose via-

FIG. 1. Viability of isolated hepatocytes measured by the percentage of cells which excluded trypan blue. O, control hepatocytes; △, selenium-deficient hepatocytes; □, vitamin E-deficient hepatocytes; ⋄, vitamin E-deficient hepatocytes isolated from rats fed the diet at least 21 weeks. Each point represents the mean of at least four experiments and one standard deviation is indicated by the bracket.

1 The abbreviation used is: DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).
In order to extend the observations in isolated hepatocytes to the intact liver, glutathione release by isolated perfused livers was measured. Because glutathione is released by the liver into the caval perfusate and into the bile, the bile duct was cannulated and the bile was collected separately from the caval perfusate. The glutathione released into the caval perfusate and that released into the bile was measured. Table III shows that the selenium-deficient livers released approximately 4 times as much glutathione into the caval perfusate as did the control livers. The amount of glutathione released into the bile was the same in both diet groups. Lactate dehydrogenase activity was measured in each sample of the caval perfusate and was less than 1 milliunit/ml.

### Table I. y-Glutamylcysteine synthetase activity

<table>
<thead>
<tr>
<th>Diet group</th>
<th>n</th>
<th>y-Glutamylcysteine formed&lt;sup&gt;a&lt;/sup&gt; (nmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>4.49 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenium-deficient</td>
<td>7</td>
<td>8.35 ± 1.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E-deficient</td>
<td>4</td>
<td>3.46 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± S.D.
<sup>b</sup>Values significantly different from each other (p < 0.01) by Duncan's multiple range test have the same superscript letter (b or c).

### Table II. Cysteine content of liver

<table>
<thead>
<tr>
<th>Diet group</th>
<th>n</th>
<th>Cysteine&lt;sup&gt;c&lt;/sup&gt; (nmol/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0.100 ± 0.021&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenium-deficient</td>
<td>8</td>
<td>0.065 ± 0.004&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E-deficient</td>
<td>5</td>
<td>0.119 ± 0.019&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>c</sup>Values are means ± S.D.
<sup>d</sup>Values significantly different from each other (p < 0.05) by Duncan's multiple range test have the same superscript letter (b or c).

### Table III. Glutathione release by perfused liver

<table>
<thead>
<tr>
<th>Diet group</th>
<th>n</th>
<th>Bile&lt;sup&gt;e&lt;/sup&gt; (nmol GSH/g liver/min)</th>
<th>Per fusate&lt;sup&gt;e&lt;/sup&gt; (nmol GSH/g liver/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>1.41 ± 0.13 0.64 ± 0.08 5.62 ± 0.85&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>Selenium-deficient</td>
<td>3</td>
<td>1.42 ± 0.17 0.54 ± 0.18 23.8 ± 6.47&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;1.2</td>
</tr>
</tbody>
</table>

<sup>e</sup>Values are mean ± S.D. where the value for each animal is the average of three time points of the perfusion (10, 20, and 30 min). All values are expressed in units of nanomoles of GSH/g of liver/min.

<sup>f</sup>Values significantly different from each other (p < 0.01) by Duncan's multiple range test.

<sup>g</sup>Oxidized glutathione was not detectable in the perfusate by the methods used. The minimum concentration detectable is 1.2 nmol/g of liver/min (expressed as GSH equivalents).
increased GSSG formation. GSH release into bile or into caval
occur as a result of redox changes in the liver leading to
Biliary GSSG release is increased by infusing hydroperoxides
have reported finding GSH as well as GSSG in the bile (16).
added xenobiotics in these experiments.
xenobiotic or ascribed to release from the cell. We have not
rat hepatocytes. Therefore, turnover of glutathione in the
synthesizing glutathione. Glutathione breakdown is catalyzed
by y-glutamyltranspeptidase, which is virtually absent from
hepatocyte would have to be ascribed to conjugation with a
The forms of glutathione released by the perfused livers
were determined (Table III). Approximately 40% of the biliary glutathione was in the oxidized form in both diet groups.
Oxidized glutathione was not detectable in caval perfusate
from either selenium-deficient or control livers, indicating that
at least 95% of the glutathione released into the caval perfusate
by selenium-deficient livers was in the reduced form. Thus,
selenium deficiency accelerated hepatic glutathione synthesis
and increased the release of reduced glutathione into the blood.
The increased release of glutathione by the selenium-defi-
cient liver was confirmed by determining plasma glutathione concentrations. As shown in Table IV, plasma from selenium-
deficient rats contained 3 times as much glutathione as control plasma. Upon treatment with NaBH₄, to recover any GSH
involved in mixed disulfides, the selenium-deficient plasma
contained 2 times as much glutathione as control plasma. These data support our conclusion that the increased gluta-
thonie release in selenium deficiency is an in vitro as well as an in vivo phenomenon.

DISCUSSION

In this study, we have isolated selenium-deficient and vi-
tamin E-deficient hepatocytes and have maintained them in
a complete medium for 5 h. With the exception of cells isolated
from severely vitamin E-deficient rats, the viability of deficient
hepatocytes were similar to the viability of control hepatocytes. The cells isolated from rats fed the vitamin E-
deficient diet for at least 21 weeks lost viability rapidly and
had an increased production of thiobarbituric acid-reactive
substances. This implies that severe vitamin E deficiency
makes the cell more susceptible to oxidative stresses and that
other oxidant defenses are unable to prevent lipid peroxidation
under these conditions. These studies show that selenium-
deficient hepatocytes and vitamin E-deficient hepatocytes can
be isolated without special precautions. Thus, isolated hepa-
tocytes can be used to study the effects of these nutrient
deficiencies on xenobiotic metabolism and hepatotoxicity.
Selenium deficiency has a striking effect on glutathione
turnover in the hepatocyte while the effect of vitamin E-
deficiency appears to be minimal. All cells are capable of
synthesizing glutathione. Glutathione breakdown is catalyzed
by y-glutamyltranspeptidase, which is virtually absent from
rat hepatocytes. Therefore, turnover of glutathione in the
hepatocyte would have to be ascribed to conjugation with a
xenobiotic or ascribed to release from the cell. We have not
added xenobiotics in these experiments.
Sies et al. (23) have studied glutathione release from the
perfused liver and have reported that GSSG is released into
the bile and GSH is released into the caval perfusate. Others
have reported finding GSH as well as GSSG in the bile (16).
Biliary GSSG release is increased by infusing hydroperoxides
and certain xenobiotics into the liver (23). It is thought to
occur as a result of redox changes in the liver leading to
increased GSSG formation. GSH release into bile or into caval
perfusate is unaffected under these conditions and factors
which influence it have not been identified. Thus, previous
studies provide no explanation of our observation that GSH
release into the caval perfusate is increased by selenium
deficiency.

Our studies provide evidence for accelerated glutathione
synthesis in selenium-deficient hepatocytes when compared
with controls. The activity of y-glutamylcysteine synthetase
is twice as high in selenium-deficient liver as in control. This
enzyme catalyzes the initial step of glutathione synthesis and
is considered, along with cysteine availability and other fac-
tors, to be involved in the regulation of glutathione synthesis
(24–26). The glutathione content of the flask containing sele-
nium-deficient hepatocytes increased by 105 nmol/10⁶ cells
during the 5-h incubation while that of controls increased by
only 45 nmol/10⁶ cells. Since little glutathione conjugation or
degradation is likely to have occurred, this is strong evidence
for increased synthesis in the selenium-deficient cells.

The evidence for increased glutathione synthesis in sele-
nium deficiency is accompanied by evidence for an increased
grate of GSH release into the blood. The perfused liver exper-
iments (Table III) were necessary to determine that the
augmented release of glutathione in selenium deficiency was
into the blood instead of into the bile and that it consisted of
GSH and not GSSG. An additional conclusion to be drawn
from the perfusion experiments is that the increased GSH
release is not related to artificially raising the glutathione
synthesis rate by providing an excess of cysteine as could be
argued was the case in the isolated hepatocyte experiments.
The finding of 2 times as much glutathione in plasma from
selenium-deficient rats as in controls (Table IV) supports the
in vitro evidence of glutathione release.
Earlier studies have indicated that selenium deficiency has
little or no effect on liver glutathione concentration (4). Therefore,
the present data allow us to hypothesize that in vivo the
increased release of GSH into the blood caused by selenium
deficiency is balanced by an increased rate of glutathione
synthesis in the hepatocyte so that intracellular glutathione
concentration is maintained. The net result of this is an
increase in plasma glutathione concentration (presumably
GSH) and a decrease in cysteine concentration in the hepa-
tocyte.
A major question raised by these findings is why the sele-
nium-deficient liver releases so much GSH into the blood.
One possibility is that it leaks out due to membrane abnor-
mality. We found no evidence of impaired viability of sele-
nium-deficient cells in comparison to controls which might be
expected if their membranes were abnormal, and they did not
release lactate dehydrogenase into the perfusate. Thus, studies
will be required to determine whether cell membranes are
abnormal in selenium deficiency.

Another possibility is that GSH has an extracellular func-
tion and that the increased plasma concentration seen in
selenium deficiency is a compensatory mechanism. Glutathione
peroxidase and another selenoprotein are present in plasma
(27), and declining concentrations of them might be
expected if their membranes were abnormal, and they did not
release lactate dehydrogenase into the perfusate. Thus, studies
will have to determine whether extracellular GSH has an essential
biochemical function.
The results of this study may shed some light on other
phenomena observed in selenium deficiency. The accelerated
glutathione synthesis could explain the protection against
acetaminophen hepatotoxicity afforded by selenium defi-
cency in vivo (4). This protective action is considered to be
mediated by selenium deficiency. However, our studies provide
evidence of glutathione release.

TABLE IV

<table>
<thead>
<tr>
<th>Diet group</th>
<th>GSH + GSSG*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Control</td>
<td>11.2 ± 2.2²</td>
</tr>
<tr>
<td>Selenium-deficient</td>
<td>30.9 ± 7.1¹</td>
</tr>
</tbody>
</table>

* Extent of hemolysis in all plasma samples was less than 0.4%. Values are means ± S.D., n = 7.
¹ Values with the same superscript are significantly different from each other (p < 0.001) by Student’s t test.
² Extent of hemolysis in all plasma samples was less than 0.4%. Values are ± S.D., n = 7.

Selenium deficiency has a striking effect on glutathione
synthesis in selenium-deficient rat hepatocytes

...continued from previous page...

selenium deficiency is accompanied by evidence for an increased
grate of GSH release into the blood. The perfused liver exper-
iments (Table III) were necessary to determine that the
augmented release of glutathione in selenium deficiency was
into the blood instead of into the bile and that it consisted of
GSH and not GSSG. An additional conclusion to be drawn
from the perfusion experiments is that the increased GSH
release is not related to artificially raising the glutathione
synthesis rate by providing an excess of cysteine as could be
argued was the case in the isolated hepatocyte experiments.
The finding of 2 times as much glutathione in plasma from
selenium-deficient rats as in controls (Table IV) supports the
in vitro evidence of glutathione release.

Earlier studies have indicated that selenium deficiency has
little or no effect on liver glutathione concentration (4). Therefore,
the present data allow us to hypothesize that in vivo the
increased release of GSH into the blood caused by selenium
deficiency is balanced by an increased rate of glutathione
synthesis in the hepatocyte so that intracellular glutathione
concentration is maintained. The net result of this is an
increase in plasma glutathione concentration (presumably
GSH) and a decrease in cysteine concentration in the hepa-
tocyte.
A major question raised by these findings is why the sele-
nium-deficient liver releases so much GSH into the blood.
One possibility is that it leaks out due to membrane abnor-
mality. We found no evidence of impaired viability of sele-
nium-deficient cells in comparison to controls which might be
expected if their membranes were abnormal, and they did not
release lactate dehydrogenase into the perfusate. Thus, studies
will be required to determine whether cell membranes are
abnormal in selenium deficiency.

Another possibility is that GSH has an extracellular func-
tion and that the increased plasma concentration seen in
selenium deficiency is a compensatory mechanism. Glutathione
peroxidase and another selenoprotein are present in plasma
(27), and declining concentrations of them might be
expected if their membranes were abnormal, and they did not
release lactate dehydrogenase into the perfusate. Thus, studies
will have to determine whether extracellular GSH has an essential
biochemical function.
The results of this study may shed some light on other
phenomena observed in selenium deficiency. The accelerated
glutathione synthesis could explain the protection against
acetaminophen hepatotoxicity afforded by selenium defi-
cency in vivo (4). This protective action is considered to be
mediated by selenium deficiency. However, our studies provide
evidence of glutathione release.

...continued from previous page...

...continued from previous page...

Selenium deficiency has a striking effect on glutathione
synthesis in selenium-deficient rat hepatocytes

...continued from previous page...
Selenium-deficient rats are more sensitive to inorganic mercury poisoning than are control rats. Several years ago, this finding was linked with lower levels of the protective mercury-binding protein metallothionein in rat kidney (28). One-third of the amino acid residues in metallothionein are cysteine. Thus, the low cysteine concentration seen in selenium deficiency could impair the induction of metallothionein and explain the sensitizing effect of selenium deficiency in inorganic mercury toxicity.

Acknowledgments—We are grateful to Dr. Rainer N. Zahlten for helpful discussions and to J. M. Lane for technical assistance. J. L. Bloom and A. Kirkland provided secretarial assistance.

REFERENCES
Effect of selenium deficiency and vitamin E deficiency on glutathione metabolism in isolated rat hepatocytes.

K E Hill and R F Burk


Access the most updated version of this article at http://www.jbc.org/content/257/18/10668

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/257/18/10668.full.html#ref-list-1