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University of Wyoming Agricultural Experiment Station

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Metabolic Effects and Metabolism of Se in Animals

Agricultural Experiment Station
University of Wyoming
April 1964 Bulletin 414
Metabolic Effects and Metabolism of Selenium in Animals

Agricultural Experiment Station
Division of Biochemistry
University of Wyoming
PREFACE

The studies presented in these reports are part of a long-term program dealing with various phases of selenium metabolism and the metabolic interferences produced by selenium in animals.

While the work was in progress, portions of these studies were presented at various meetings and seminars, and summaries in abstract form appeared in a number of publications.

The continuation of the program was greatly assisted by the partial support provided by the Atomic Energy Commission. This assistance is gratefully acknowledged.

Dr. Irene Rosenfeld
Research Professor
Division of Biochemistry
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Part I
Effect of Chronic Selenosis on \( P^{32} \) Metabolism in the Liver

By Irene Rosenfeld

The storehouse of available phosphate in bone and the importance of dietary phosphate on the skeletal system tend to obscure the function of phosphate in metabolically more active tissues in the body. A vast amount of research has been devoted to the nutritional requirements for phosphate and to the relation of phosphorus to defective mineralization of the skeletal system. Soft tissues contain comparatively small amounts of phosphorus, but they are of vital importance in the regulation of metabolism. In these tissues, the generation of high-energy phosphate compounds is controlled by a myriad of enzyme systems.

In recent years a number of investigators have studied the mechanism of phosphorylation in normal animals by radioactive phosphorus. Distribution and metabolism of phosphates in tissues under pathologic conditions were investigated mainly in relation to the synthesis of nucleoproteins (1). Reinhard et al. (2) reviewed the literature and summarized the results with radioactive phosphorus in treatment of various blood dyscrasias and malignant neoplastic diseases.

Metabolism of phosphorus in tissues of intact animals during selenium poisoning has not been investigated up to the present time. Anderson and Moxon (3) observed a decrease in the inorganic phosphate of blood in acute selenium poisoning. Wright (4) reported that toxic doses of sodium selenite increased blood-sugar levels, while lethal doses decreased liver glycogen.

The continued injection or intake of selenium compounds produces emaciation, tubular kidney damage, necrosis and cirrhosis in the liver and, frequently, ascites in the abdominal cavity (5). Until now, there have been no studies to indicate what effect chronic tissue damage by selenium has on phosphorylation \textit{in vivo}.

The present report on phosphorus metabolism, divided into two sections, deals with the effect of chronic selenosis on phosphorus metabolism by use of \( P^{32} \). Section I discusses phosphorylation in the liver and Section II, phosphorylation in other tissues.

Primary report (AEC U-2315), 1953.
Chronic selenosis was produced in male and female Sprague-Dawley rats by daily administration (stomach tube) of a solution of 1.5 mg of selenium as sodium selenate per kilo of body weight. Average weight of the male rats at the beginning of the experiment was 350 ± 25 g and of females, 225 ± 25 g. The animals were raised and maintained on Purina Laboratory Chow. Chronic selenosis developed in the rats during a 5- to 8-week period of selenium administration. The symptoms indicating chronic selenosis were decreased food intake and weight loss (50-75 g) or ascites; at autopsy the liver showed varying degrees of necrosis and cirrhosis (6). The experimental animals (selenium-injected) and the control animals (receiving no selenium) of the same age and approximate weight were injected intraperitoneally with radioactive phosphorus (3.7 x 10^8 counts/min.) as H_3P^{32}O_4 at a pH of 7.0. The animals were then sacrificed at various time intervals as indicated in the results. Tissues were excised rapidly under nembutal anesthesia and immediately dropped into dry ice. The brittle, frozen tis-

*Obtained from Oak Ridge National Laboratory.

**TABLE 1 — Scheme of Fractionation of Homogenized Tissue**

(All procedures were carried out from 0 ± 5°C.)

- Aliquot (about ½ of the total volume) was centrifuged in refrigerated centrifuge.
- Precipitate (protein-bound phosphorus) washed twice and the washings added to the acid-soluble fraction.
- Made up to volume
- Aliquot digested as indicated on the left.
- Residue was made up to definite volume.
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for total acid-soluble P^m and P^p.
- Determined for inorganic P^m and P^p.
- Supernatant (total acid-soluble fraction)
- V
- Aliquot precipitated with magnesia mixture.
- Aliquots used for separation of organic acid-soluble compounds.
- Determined for inorganic P^m and P^p.
- V
- Aliquot precipitated with magnesium mixture.
- Aliquots used for separation of organic acid-soluble compounds.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
- V
- Aliquot digested as indicated on left and determined for total protein-bound P^m and P^p.
- Determined for inorganic P^m and P^p.
sues were pulverized between cold iron bricks and weighed; then they were placed in a modified Waring microblendor (surrounded by ice), which contained 5 volumes of ice-cold 10% trichloroacetic acid, and blended for two minutes. The fractionation scheme of the homogenized tissues is indicated in Table 1.

A calcium precipitation method by Fiske and Subbarow (7) for inorganic phosphate determination was tried and proved to be unsatisfactory since considerable amounts of labile phosphates were hydrolyzed. A magnesia-mixture-precipitation method gave more consistent results; therefore this method was used for determination of inorganic \( P_{31} \) and \( P_{32} \). Acid-soluble fraction was used for the separation of phosphorylated esters. The separation of organic phosphates was carried out by the barium-and-alcohol-precipitation method of Umbreit et al. (8) at 0 ± 5° C. to give: (1) barium-insoluble phosphates (ATP, ADP), (2) barium-soluble alcohol-insoluble phosphates (phosphorylated glucose intermediates), and (3) residue, containing barium and alcohol-soluble phosphates (propanedial and aminoethylphosphates). ATP and other phosphorylated intermediates were determined according to the method of Umbreit et al. (8).

The various fractions of liver extracts were digested with 10 N \( H_2SO_4 \) and concentrated \( HNO_3 \) and cleared with the addition of a few drops of 30% \( H_2O_2 \); the phosphate was then determined by the Fiske and Subbarow method (9). One or 2 ml aliquots of tissue fractions in duplicate or triplicate (depending on the \( P_{32} \) activity of the fractions) were transferred to stainless-steel planchets and dried at 70-80° C. Activity was measured with a Geiger-Mueller counter. All samples were corrected for decay; values are given in terms of activity at the time of injection. Results are the averages of five or more animals and are based on wet weight of tissues. \( P_{31} \) is expressed as mg P/100 g hepatic tissue, and \( P_{32} \) as percent of the injected dose recovered. Specific activity is expressed as counts per minute/mg P.

**RESULTS**

Distribution of \( P_{31} \) and \( P_{32} \) in the liver of animals with chronic selenosis showed deviation from normal. \( P_{31} \) and \( P_{32} \) total tissue and “bound phosphates” in the chronic-selenosis group were lower than in the control group. There was an increase in total acid-soluble \( P_{31} \) and \( P_{32} \) in selenized liver (Table 2).
<table>
<thead>
<tr>
<th>Sacrificed after $^{75}P$ inj.</th>
<th>Wt. of Liver</th>
<th>Total tissue phosphates</th>
<th>Total acid-soluble phosphates</th>
<th>&quot;Bound phosphates&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$^{75}P$</td>
<td>$^{75}P$</td>
<td>$^{75}P$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g</td>
<td>%</td>
<td>g</td>
</tr>
<tr>
<td>0.5</td>
<td>11.90</td>
<td>12.50</td>
<td>11.7</td>
<td>13.0</td>
</tr>
<tr>
<td>1.0</td>
<td>9.17</td>
<td>8.63</td>
<td>13.8</td>
<td>15.5</td>
</tr>
<tr>
<td>2.0</td>
<td>8.44</td>
<td>7.98</td>
<td>17.7</td>
<td>16.6</td>
</tr>
<tr>
<td>3.0</td>
<td>9.52</td>
<td>10.01</td>
<td>11.9</td>
<td>17.9</td>
</tr>
<tr>
<td>4.0</td>
<td>8.02</td>
<td>9.20</td>
<td>11.0</td>
<td>15.8</td>
</tr>
<tr>
<td>6.0</td>
<td>7.93</td>
<td>8.19</td>
<td>10.5</td>
<td>12.7</td>
</tr>
<tr>
<td>12.0</td>
<td>7.80</td>
<td>8.46</td>
<td>7.7</td>
<td>9.0</td>
</tr>
<tr>
<td>24.0</td>
<td>6.70</td>
<td>8.21</td>
<td>6.5</td>
<td>8.5</td>
</tr>
<tr>
<td>48.0</td>
<td>9.56</td>
<td>3.2</td>
<td>3.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{75}P$</td>
<td>mg%</td>
<td>$^{75}P$</td>
</tr>
<tr>
<td>$^{31}P$</td>
<td>320.2</td>
<td>280.3</td>
<td>100.6</td>
<td>128.8</td>
</tr>
<tr>
<td>S. E.</td>
<td>± 1.3</td>
<td>± 6.4</td>
<td>± 1.6</td>
<td>± 3.2</td>
</tr>
</tbody>
</table>

* $^{75}P$ values are percent of the administered dose (3.7 x 10^6 c/m) recovered. Mean values for $^{31}P$ are in mg P/100 g fresh tissue. Measure of variability is standard error of the mean.

** Control received no selenium injection.
the liver of the control group, indicating some malfunction in phosphorus metabolism or an increase in cellular permeability to phosphate ions. The “bound phosphates”, which contained phospho- and nucleo-proteins, showed a decreased rate of synthesis at all time intervals studied in the experimental group.

The separation of acid-soluble phosphates in the liver indicated that inorganic \(^{31}\)P and \(^{32}\)P were lower in the control group than in the experimental group. Organic phosphates in the experimental group were decreased or showed considerable difference in their metabolic rate (Table 3). The mean value of adenosine triphosphate (ATP\(^{31}\)) of liver in the chronic-sele
nosis group was 6.7 mg percent, while in normal liver, it was 16.1 mg percent. There was a 60 percent decrease in this fraction.

Increase of inorganic phosphate in selenized liver extract suggests either that the rate of phosphorylation is reduced, which would result in the accumulation of inorganic phosphate, or that the phosphorylated compounds in presence of selenium are less stable than in non-seleniferous tissues. Wilson and Bandurski (10) reported an enzymatic reaction between ATP and selenate in a liver preparation. A nucleotide-bound selenate, APS\(e\) (adenosine-5'-phosphoselenate), was demonstrated by the use of proper enzyme systems by Wilson and Bandurski (11). Nucleotide-bound selenate was very unstable when compared with its sulfur analogue. Inorganic-phosphate liberation from APS\(e\) was greater than accumulation of nucleotide-bound selenate. Present data suggest that in the liver in chronic selenosis a similar reaction may take place if an increase in inorganic phosphate is a measure of the metabolic breakdown of nucleotide-bound selenate.

There was some decrease in the phosphorylated carbohydrates as indicated in Table 3 (column 4). Whether this decrease was due to inhibition of phosphorylating enzymes by selenate or to the decrease of liver glycogen cannot be determined from the present data. The glycogen level in the liver of selenized rats varied from 0.38 to 1.25 percent. The liver of control animals on the same diet varied from 3.5 to 5.6 percent.

Specific activities of total, acid-soluble, and protein-bound phosphates are indicated in Figure 1. In the chronic-selenosis group, the specific activities of total tissue as well as of acid-soluble phosphates are higher than those of the control animals. This difference is due to greater retention of \(^{32}\)P in fractions and to decrease of total tissue \(^{31}\)P. The rapid rise of \(^{32}\)P and the slower rate of decrease in the selenized liver, when compared with the control liver, may be related to the pathological changes that were present in chronic selenosis.

A considerable number of animals had ascites in the abdominal cavity, and the large amount of fluid would facili-
TABLE 3 — Distribution of $^{32}P$ and $^{31}P$ in Acid-Soluble Phosphates in Liver of Control and Chronic Selenosis Groups*.

<table>
<thead>
<tr>
<th>Sacrificed after $^{32}P$ inj.</th>
<th>Inorganic $%$</th>
<th>Adenosine triphosphate $%$</th>
<th>Barium-soluble alcohol-insoluble $%$</th>
<th>Barium and alcohol-soluble $%$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control**</td>
<td>Chronic selenosis</td>
<td>Control**</td>
<td>Chronic selenosis</td>
</tr>
<tr>
<td>hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3.4</td>
<td>5.4</td>
<td>2.0</td>
<td>.6</td>
</tr>
<tr>
<td>1.0</td>
<td>4.4</td>
<td>6.7</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>2.0</td>
<td>5.1</td>
<td>6.9</td>
<td>2.9</td>
<td>1.5</td>
</tr>
<tr>
<td>3.0</td>
<td>4.2</td>
<td>6.6</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>4.0</td>
<td>2.2</td>
<td>4.9</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>6.0</td>
<td>2.1</td>
<td>2.3</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>12.0</td>
<td>1.3</td>
<td>1.6</td>
<td>.8</td>
<td>0.5</td>
</tr>
<tr>
<td>24.0</td>
<td>.3</td>
<td>.8</td>
<td>.8</td>
<td>.5</td>
</tr>
<tr>
<td>48.0</td>
<td>.2</td>
<td>.3</td>
<td>.3</td>
<td>.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean value $^{31}P$</th>
<th>$^{31}P$ $\mu g$</th>
<th>$^{31}P$ $\mu g$</th>
<th>$^{31}P$ $\mu g$</th>
<th>$^{31}P$ $\mu g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{31}P$</td>
<td>21.2 mg%</td>
<td>29.1 mg%</td>
<td>16.1 mg%</td>
<td>6.7 mg%</td>
</tr>
<tr>
<td>S. E.</td>
<td>± .1</td>
<td>± .9</td>
<td>± .5</td>
<td>± .6</td>
</tr>
</tbody>
</table>

* $^{32}P$ values are percent of the administered dose ($3.7 \times 10^9$ c/m) recovered. Mean values for $^{31}P$ are in mg P/100 g fresh tissue. Measure of variability is standard error of the mean.

** Control received no selenium injection.
state the distribution of phosphate ions.
In all animals there were various degrees of kidney damage and decreased urinary flow, which may account for the slower rate of phosphate elimination. The specific activities of the "bound phosphates" free of phospholipids were low in both groups, a fact that reaffirms the less active participation of the component in this fraction in phosphate transfer.

The specific activity of serum, liver inorganic phosphate, and ATP of the selenized group at various time intervals is indicated in Figure 2. The serum and liver inorganic phosphates appear to come into isotopic equilibrium within two and one-half hours. There is an isotopic equilibrium between the serum inorganic phosphates and ATP at the end of three hours, at which time the specific activity of ATP is decreasing.

![Graph showing the specific activity of phosphates in liver fractions](image)

**FIG. 1—Comparison of time-course of specific activity of phosphates in liver fractions in animals with chronic selenosis and control groups.**
In the control livers, isotopic equilibrium was reached between serum, liver inorganic phosphate, and ATP in less than two hours, at which time the maximum synthesis of ATP was attained. Similar results were obtained in earlier studies by Kaplan and Greenberg (12) and by Sacks (13) in normal liver, suggesting that the turnover rate of labile P in selenized liver differs from that in normal liver. Zilversmit et al. (14) postulated that the specific activity of precursor and product intersect at the time of maximum specific activity of the product.

In the control group, the specific activity of serum and liver inorganic phosphates met this requirement, while in the experimental group the ATP turnover rate did not attain the specific activity of serum inorganic phosphate until the end of three hours, and liver inorganic phosphate at the end of five hours. The data indicate only that there is a rapid breakdown of ATP in selenized liver. The rate of decomposition or decreased formation of ATP in the selenized liver obscures the origin of the precursor of labile P in this nucleotide.

These results suggest that the increase of P\(^{31}\) and P\(^{32}\) in acid-soluble and inorganic phosphate fractions is due to metabolic disturbances in phosphorus metabolism caused by chronic selenosis.

**DISCUSSION**

The injected labeled phosphate was rapidly distributed into various liver fractions. P\(^{32}\) appeared in organic phosphates 30 minutes after administration of the isotope, indicating that liver cells have a high degree of permeability to phosphate ions. Probably the primary entrance of radio-phosphorus in various organic compounds is achieved by exchange of phosphorus-containing radicals, and later by synthesis. This was indicated by the continued rate of increase and by gradual decline in specific activity of the various organic components of the fractions at various time intervals.
The importance of the liver in normal metabolism is reflected in the many aberrations that are related to chronic hepatic dysfunction. The liver is the first organ to be affected by emaciation or protein restriction. Changes in phosphate components of liver can be induced by nutritional, hormonal, or toxic substances (15).

Distribution of $P^{31}$ and $P^{32}$ in the liver of the chronic-selenosis group showed considerable deviation from normal. Flock et al. (16) in studying the effect of diets on the phosphorus compounds in liver reported that high fat diets reduced acid-soluble phosphates in the liver about 50 percent with a corresponding decrease in the phosphorylated ester. Ennor and Stocken (17) reported an increase in ADP, ATP, and phosphocreatine in fatty livers induced by CCl$_4$ treatment. Evidently accumulation of fat in the liver under pathological conditions is not equivalent to nutritional imbalance produced by the fat diet; this difference was indicated by phosphate metabolism.

In animals with chronic selenosis there is decreased food consumption (18). In latter stages of the toxicosis, aphagia develops with subsequent emaciation. The majority of animals used in these experiments had not only consumed less food, but some developed a considerable degree of ascites and severe liver damage. Liver damage during selenium intoxication interferes with protein synthesis (18).

Aberrations of phosphate metabolism in selenized liver may be related to the decreased protein synthesis as well as to the direct effect of selenate on the enzyme systems in the intact animals. At present there is considerable evidence to indicate that selenium compounds inhibit the activity of a number of enzyme systems in vitro (19). The changes in phosphate metabolism in the liver were indicated by decreased total tissue $P^{31}$ and increased acid-soluble and inorganic $P^{31}$ and $P^{32}$. Rapid influx of $P^{32}$ into the liver suggests that in selenosis, permeability of liver cells was altered.

Sacks (20) studied $P^{32}$ turnover rate in alloxan diabetes and found a marked increase in inorganic phosphate and decrease in the amounts of ATP, ADP and glucose-6, and glucose-1 phosphates in the liver. These results were contrary to the results obtained by Kaplan and Greenberg (12). They reported an increased rate of phosphorylation in the liver following glucose injection. This divergence in the above results may be due to the differences of phosphorus metabolism in normal animals with a temporary imbalance of glucose and in animals with a pathological condition, diabetes. The results of Sacks (20) appeared to be in agreement with the results obtained in the liver of the chronic-selenosis group, except that in the selenized liver there was a decrease in liver glycogen. Both experiments had one common factor, that is, an abnormal metabolism of
phosphorus with pathological lesions in organs directly involved in phosphorylation.

The specific activity of total tissue and total acid-soluble phosphates in the chronic-selenosis group was higher than in the normal animals, which may be explained by the rate of influx of ions into the liver. Increase in P\textsuperscript{31} and P\textsuperscript{32}, and the high specific activity of inorganic phosphate in selenized liver, may be related to the reduced utilization of phosphate in the intact animals. The retention of P\textsuperscript{32} in serum is prolonged, but isotopic equilibrium is established with inorganic phosphate of the liver without any metabolic interference (Fig. 2.) The synthesis of organic phosphorylated esters is reduced, and high-energy phosphate compounds either are unstable or their formation is depressed in the selenized liver. The reduction in synthesis is evident by the decrease of phospho-proteins.

Decrease of high-energy phosphates in selenized liver may be due to the direct effect of selenate on the enzyme systems or it may be related to the decrease in metabolic activity in the liver. Complete inhibition of enzyme systems in vitro by various selenium compounds has been demonstrated by a number of investigators. However, the unequivocal demonstration of enzyme inhibition by selenium in intact animals has not been presented (19).

It has been pointed out by Kamen and Spiegelman (21) that the formation and conversion of phosphate esters in living cells are mediated by a mosaic of enzymes. In normal mature cells, various regulating mechanisms produce a steady state in which the over-all chemical composition remains constant. This regulating mechanism in the latter stages of selenosis is disturbed and may explain the deviation in phosphorus metabolism from normal.

**SUMMARY**

There is a high rate of P\textsuperscript{32} uptake by the liver of normal animals and of animals with chronic selenosis following intraperitoneal administration of the isotope.

In the liver of animals with chronic selenosis there is decrease of total phosphorus and increase of acid-soluble phosphates. The increase in this fraction is mainly associated with increased inorganic phosphate.

ATP and other labile phosphate formations are decreased in the liver of animals with chronic selenosis.

Biochemical changes, in relation to the pathological condition produced by chronic selenosis, are discussed.
REFERENCES


—15—
Part II
Distribution of P$^{31}$ and P$^{32}$ in Tissues of Normal Animals and in Animals with Chronic Selenosis

By Irene Rosenfeld

Observations from a number of laboratories indicate that enzymes involved in phosphate transfer show quantitative and qualitative variations in tissues of different species or strains of animals (1, 2). Alkali-metal ions inhibit kidney mitochondrial adenosine triphosphatase activity in a non-specific manner (3). It has been postulated that the metal-ion-binding groups at the cell surface are polyphosphates that produce changes in spatial arrangements of polyphosphate chains and may result in an inhibition of enzymatic activity (4).

Numerous studies indicate that selenium compounds inhibit a number of enzyme systems in vitro. There is also evidence that most of the enzyme systems which require the -SH group for their function are effectively inhibited by selenium compounds. Demonstration of the direct effect of selenium compounds on enzyme systems in intact animals is not as well substantiated as in in vitro studies. These differences between in vitro and in vivo studies may be due to the numerous metabolic pathways by which the body attempts to remove the toxic agent (selenium) as well as the substitution of selenium for sulfur in these pathways (5).

The manner in which chronic selenium toxicity alters phosphorus metabolism in various tissues in vivo is not known at present. In the preceding report (Part I), phosphate metabolism in the liver of animals with chronic selenosis indicated that selenium interferes with phosphorylation of tissues in intact animals. Although studies presented in this report are not as detailed as those with the liver, they indicate that chronic selenosis may produce abnormal distribution of phosphate in some tissues.

The present discussion attempts to correlate changes in phosphate metabolism with pathological changes observed in the chronic-selenosis group. The correlation does not imply that these pathological changes are responsible for abnormal phosphate metabolism in animals with chronic selenosis. Selenium compounds in excess or by their absence produce a multitude of bio-
chemical changes in vivo as well as in vitro. These biochemical changes were reviewed by Rosenfeld and Beath (6).

The distribution of $P^{31}$ and $P^{32}$ in soft and osseous tissues in animals with chronic selenosis is presented and in some cases the results are compared with the distribution in control animals. Total tissue distribution of $P^{31}$ and $P^{32}$ in normal animals was presented in an earlier report (7).

**METHODS**

Experimental procedures were the same as those described in the preceding report (Part I).

After development of chronic selenosis in Sprague-Dawley rats (experimental group), $P^{32}$, as phosphate, (3.7 x $10^8$ c/m) was injected intraperitoneally and the animals were sacrificed at time intervals indicated in the results. Tissues were removed under nembutal anesthesia and dropped immediately into dry ice. Frozen pulverized tissues were homogenized in 9 volumes of 10 percent TCA.* Total tissue, acid soluble, inorganic, and TCA-precipitated $P^{31}$ and $P^{32}$ were separated and determined as indicated in Part I. Specific activity is expressed as a ratio $c/m P^{32}$ between = in fractions. Percent uptake was calculated from the amount of $P^{32}$ retained per gram or per organ.

Simultaneous studies on the control group (receiving no selenium) were carried out.

* trichloroacetic acid.

**RESULTS**

Distribution of $P^{31}$ and $P^{32}$ in the kidney of control and experimental groups is presented in Table 1. $P^{31}$ distribution in the selenized group showed considerable variation from normal. On the basis of $P^{31}$ content of selenized kidney, the results were divided into three groups: in one group the $P^{31}$ content of the kidney was about 35 percent higher than in the control group; in the second group the $P^{31}$ content was about 10 percent higher; and in the third group the $P^{31}$ content was similar to that of the control group.

These differences in $P^{31}$ retention were interpreted to be due to functional damage produced by selenium to the kidney. Although no histopathological studies were made on the kidney, gross pathology indicated chronic damage to this organ in all animals. In a few animals the $P^{31}$ content was about 10 percent lower than in the control animals, suggesting various degrees of functional damage to the kidney in chronic selenosis. First, the reabsorption mechanism is probably damaged, at which time the phosphate excretion is increased; this is followed by de-
**TABLE 1 — Distribution of P\textsuperscript{31} and P\textsuperscript{32} in Kidney**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Control</th>
<th>Chronic Selenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P\textsuperscript{31}</td>
<td>P\textsuperscript{32} *</td>
</tr>
<tr>
<td></td>
<td>mg%</td>
<td>%</td>
</tr>
<tr>
<td>Total tissue</td>
<td>260.8 ± 5.8</td>
<td>1.80</td>
</tr>
<tr>
<td>Acid-soluble</td>
<td>80.5 ± 2.4</td>
<td>1.41</td>
</tr>
<tr>
<td>Inorganic</td>
<td>26.6 ± 1.5</td>
<td>0.74</td>
</tr>
<tr>
<td>TCA ppt</td>
<td>183.8 ± 3.7</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* Percent of the administered dose recovered one hour after P\textsuperscript{32} (3.7 x 10\textsuperscript{8} c/m) injection intraperitoneally. (Results are based on five rats in each group.)

** Division was based on the results obtained from 100 rats used in the experiments.

Increased excretion of urine. Higher retention of P\textsuperscript{31} occurred in animals showing anuria. Reduction of urinary excretion and development of anuria varies from animal to animal during selenosis; this would be consistent with variations in the results of P\textsuperscript{31} content of the kidney in selenized animals.

P\textsuperscript{32} uptake by the various tissue fractions of kidney one hour after injection of the isotope is given in Table 1. P\textsuperscript{32} in total tissue of the experimental group showed significant variation from that of the control group. Acid-soluble and inorganic phosphates showed corresponding variations. Inorganic phosphate (P\textsuperscript{31}) in the experimental groups was 29.6, 37.5, and 43.4 mg percent, while in the control group it was 26.6 ± 1.5 mg percent. In this experiment, P\textsuperscript{32} showed similar differences between the two groups. TCA precipitate, which contains the phosphoproteins and phospholipids, also showed considerable variation in P\textsuperscript{31} content. In this fraction, P\textsuperscript{31} in the experimental groups varied from 169.4 to 233.6 mg percent, while in the control group it was 183.8 ± 3.7 mg percent. P\textsuperscript{32} uptake in this fraction was low in both groups. The difference in P\textsuperscript{32} uptake in the TCA precipitate between the experimental groups and control group was about 20 percent.

Specific activity of inorganic and acid-soluble phosphates in kidney up to six hours is indicated in Figure 1. In the control group, both fractions at the end of 0.5 hour showed maximum activity, while in the selenized group, the maximum specific activity was attained at the end of two hours. The rate of decrease in the selenized kidney was slower than that of the control. The rate of penetration and the rapid exchange of P\textsuperscript{32} between inorganic...
FIG. 1—Specific activity of kidney inorganic and acid-soluble fractions in chronic selenium and control groups. P$^{32}$ (phosphate) $3.7 \times 10^8$ c/m injected intraperitoneally. Results are based on 5 animals at each time interval.

Studies on the amount of P$^{32}$ absorbed into the circulation and on the amount passed from the circulatory system into organs indicate that in normal animals, the maximum uptake of P$^{32}$ in rat plasma was 14 minutes after intraperitoneal injection of labeled phosphate (9). The rapid decrease of P$^{32}$ in plasma is associated with absorption and exchange with P$^{31}$ in the organs and with excretion first by the urinary and subsequently by the gastrointestinal tracts. The present results indicate that in normal animals the maximum transfer from plasma to kidney occurs in less than 30 minutes. Rapid clearance of phosphate by the urinary tract in normal animals is indicated by the rapid decrease in specific activity of the acid-soluble fraction. Over 70 percent of P$^{32}$ was excreted at the end of six hours. In the experimental group, specific activity of the acid-soluble fraction increased up to two hours and declined very slowly. The apparent lower specific activity in acid-soluble and inorganic phosphates in selenized kidney in Figure 1 is due to higher P$^{31}$ content of these fractions as indicated in Table 1.

FIG. 2—Specific activity of inorganic phosphate in heart, spleen, and brain of control and selenized groups. P$^{32}$ (phosphate) injected intraperitoneally, dose $3.7 \times 10^8$ c/m.
TABLE 2 — Distribution of P\textsuperscript{31} and P\textsuperscript{32} in Total Tissue at Various Time Intervals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group*</th>
<th>p\textsuperscript{31} mg%</th>
<th>1 hr.</th>
<th>2hrs.</th>
<th>6 hrs.</th>
<th>12 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p\textsuperscript{31} S.A.***</td>
<td></td>
<td>p\textsuperscript{31} S.A.***</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% X10\textsuperscript{1}</td>
<td>% X10\textsuperscript{4}</td>
<td>% X10\textsuperscript{4}</td>
<td>% X10\textsuperscript{4}</td>
</tr>
<tr>
<td>Spleen</td>
<td>Se</td>
<td>307.9 ± 12.8</td>
<td>0.27  30.1</td>
<td>0.66  39.9</td>
<td>0.28  30.5</td>
<td>0.28  30.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>290.8 ± 6.5</td>
<td>0.36  38.8</td>
<td>0.44  42.8</td>
<td>0.34  35.5</td>
<td>0.31  35.5</td>
</tr>
<tr>
<td>Heart</td>
<td>Se</td>
<td>198.0 ± 6.8</td>
<td>0.36  28.1</td>
<td>0.53  46.1</td>
<td>0.41  39.9</td>
<td>0.33  27.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>207.9 ± 5.1</td>
<td>0.37  32.8</td>
<td>0.62  57.1</td>
<td>0.43  47.3</td>
<td>0.34  30.1</td>
</tr>
<tr>
<td>Brain</td>
<td>Se</td>
<td>308.2 ± 4.4</td>
<td>0.04  2.3</td>
<td>0.06  3.4</td>
<td>0.03  4.1</td>
<td>0.08  6.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>298.8 ± 4.9</td>
<td>0.05  2.6</td>
<td>0.05  3.1</td>
<td>0.09  4.8</td>
<td>0.06  4.4</td>
</tr>
</tbody>
</table>

* Se: Chronic-selenosis group; C: Control group.
** Percent of injected P\textsuperscript{32} (3.7 x 10\textsuperscript{4} c/m) recovered at various time intervals.
*** Specific activity = \(\frac{\text{c/m}}{\text{mg P}}\).

Distribution of P\textsuperscript{31} and P\textsuperscript{32} and specific activity of the spleen, heart, and brain at 1, 2, 6, and 12 hours in total tissue and acid-soluble phosphates are given in Tables 2 and 3.

Although the differences in the control and experimental groups of P\textsuperscript{31} and P\textsuperscript{32} uptake in total tissue and acid-soluble phosphates are not significant, there are considerable differences as to the inorganic phosphate in these organs (Fig. 2). Specific activity of inorganic phosphate in the spleen of the control group showed rapid rise and decline within six hours. In the spleen of the experimental group, the rise and decrease was slow; the maximum never reached the magnitude of the control group. These significant differences between the control and experimental groups may be related to functional changes that occur in selenosis. In rats

TABLE 3 — P\textsuperscript{31} and P\textsuperscript{32} in Acid-Soluble Fractions of Tissues at Various Time Intervals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group*</th>
<th>p\textsuperscript{31} mg%</th>
<th>1 hr.</th>
<th>2hrs.</th>
<th>6 hrs.</th>
<th>12 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p\textsuperscript{31} S.A.***</td>
<td></td>
<td>p\textsuperscript{31} S.A.***</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% X10\textsuperscript{1}</td>
<td>% X10\textsuperscript{4}</td>
<td>% X10\textsuperscript{4}</td>
<td>% X10\textsuperscript{4}</td>
</tr>
<tr>
<td>Spleen</td>
<td>Se</td>
<td>84.0</td>
<td>0.20  57.0</td>
<td>0.32  101.5</td>
<td>0.36  129.6</td>
<td>0.26  80.9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>88.0</td>
<td>0.28  65.5</td>
<td>0.29  83.0</td>
<td>0.21  60.3</td>
<td>0.18  53.4</td>
</tr>
<tr>
<td>Heart</td>
<td>Se</td>
<td>97.1</td>
<td>0.50  91.0</td>
<td>0.50  90.0</td>
<td>0.69  229.9</td>
<td>0.29  115.6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>91.4</td>
<td>0.51  99.0</td>
<td>0.58  135.6</td>
<td>0.61  147.8</td>
<td>0.29  107.2</td>
</tr>
<tr>
<td>Brain</td>
<td>Se</td>
<td>87.7</td>
<td>0.04  7.8</td>
<td>0.06  8.8</td>
<td>0.07  13.9</td>
<td>0.08  14.6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>84.6</td>
<td>0.05  6.1</td>
<td>0.05  6.3</td>
<td>0.06  10.0</td>
<td>0.06  11.0</td>
</tr>
</tbody>
</table>

* Se: Chronic-selenosis group; C: Control group.
** Percent of injected P\textsuperscript{32} (3.7 x 10\textsuperscript{4} c/m) recovered at various time intervals.
*** Specific activity = \(\frac{\text{c/m}}{\text{mg P}}\).
with chronic selenosis, anemia is one of the frequent manifestations of toxicosis (10). Spleen pulp is decreased and replaced by fibrous tissue (11). These changes may be responsible for the decreased phosphate content in the spleen of the experimental group.

There was considerable difference in the specific activity of inorganic phosphate of the heart in both groups (Fig. 2). In the control group there was a rapid turnover of $P^{32}$, while in the selenized group the inorganic phosphate showed increase up to six hours. The accumulation of inorganic phosphate in the heart suggests either the formation of unstable seleno-phospho-compounds or decrease in the formation of labile phosphate esters with subsequent accumulation of the precursor (inorganic phosphate) in this organ.

Studies on the rate of renewal of creatine phosphate and ATP molecules in cardiac-muscle slices indicate that these labile phosphates are renewed within 30 minutes at 37.5° C. (12). It has also been demonstrated that in resting muscle of intact animals (rat), a three-fold increase in inorganic phosphate takes place within one minute with concomitant decrease of creatine phosphate and ATP (13). In animals with chronic selenosis, the heart muscle is soft and flabby and there is considerable loss of muscle tone (11). These physiological changes may be responsible for the changes in phosphorylation in the heart.

In the brain, the rate of penetration of labeled phosphates is lower than in any other organ. This difference is due to the blood-brain barrier which prevents rapid change in ionic composition of the extracellular fluid of this organ. In the brain, $P^{32}$ was present mainly in the acid-soluble fraction as indicated by results in Tables 2 and 3. Inorganic phosphate was slightly increased in the brain of the experimental group (Fig. 2). Limited studies on the rate of formation of labile phospho-compounds in the brain indicated a rapid rate of $P^{32}$ incorporation to ATP. At the end of one hour, specific activity of acid-soluble, inorganic, and ATP fractions in the control group was 5.88, 8.46, and 9.73 x 10$^4$, respectively. Specific activity of ATP in the brain of the experimental group was 5.65 x 10$^4$, almost 40 percent less than in the control group. Labeling of ATP in the brain was detected at about the same time as it occurred in other organs. At the end of 12 hours, specific activity of ATP increased (18.8 x 10$^4$) in the control group. In the experimental group there was an increase of specific activity with increase in time; however, it was consistently lower than that of the control group.

Distribution of $P^{31}$ and $P^{32}$ in various soft and bone tissues is indicated in Table 4. There are some differences in total phosphate content of the control and experimental groups as indicated by the data. Of special interest may be the continued increase of $P^{32}$.
in the skeletal muscle of the experimental group up to 12 hours. Although no separation of inorganic phosphate was carried out in this tissue, the increase possibly is due to accumulation of inorganic phosphate in a manner similar to that observed with cardiac muscle (Fig. 2). In the bone of both groups, $P^{32}$ showed slow increase during the experiment. Higher specific activity of the selenized group was due to lower $P^{31}$ content of the bone in this group.

Lacroix et al. (14) demonstrated the incorporation of $P^{32}$ in the epiphyseal bone of mature animals up to at least 76 days. The uptake of $P^{32}$ by the skeleton of rats, and continued and prolonged migration of $P^{32}$ from soft tissues to bone, occur up to 98 days (8). Although the present experiments were of short duration, the slow migra-

### Table 4 — Distribution of $P^{31}$ and $P^{32}$ in Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group*</th>
<th>$P^{31}$ mg%</th>
<th>$P^{32}$** S.A.*** g% $X 10^4$</th>
<th>$P^{32}$** S.A.*** g% $X 10^4$</th>
<th>$P^{32}$** S.A.*** g% $X 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum******</td>
<td>Se</td>
<td>9.3 ± 0.3</td>
<td>0.13 568.2</td>
<td>0.11 426.0</td>
<td>0.07 208.9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7.7 ± 1.7</td>
<td>0.08 249.1</td>
<td>0.70 194.6</td>
<td>0.03 88.3</td>
</tr>
<tr>
<td>Lung</td>
<td>Se</td>
<td>244.8 ± 8.2</td>
<td>0.34 36.2</td>
<td>0.46 53.9</td>
<td>0.36 39.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>243.9 ± 9.7</td>
<td>0.48 40.7</td>
<td>0.52 64.0</td>
<td>0.22 41.3</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Se</td>
<td>216.6 ± 1.5</td>
<td>0.09 14.5</td>
<td>0.18 36.2</td>
<td>0.11 18.3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>215.3 ± 3.1</td>
<td>0.08 22.9</td>
<td>0.14 26.1</td>
<td>0.50 38.2</td>
</tr>
<tr>
<td>Bone*****</td>
<td>Se</td>
<td>70.2 ± 0.3</td>
<td>0.65 2.6</td>
<td>0.65 2.5</td>
<td>0.81 11.9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>75.0 ± 0.3</td>
<td>0.85 3.0</td>
<td>0.85 3.1</td>
<td>0.93 5.7</td>
</tr>
<tr>
<td>Stomach</td>
<td>Se</td>
<td>176.9 ± 5.1</td>
<td>0.37 52.8</td>
<td>0.46 81.7</td>
<td>0.38 52.3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>185.0 ± 3.1</td>
<td>0.24 44.4</td>
<td>0.61 72.8</td>
<td>0.25 42.7</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Se</td>
<td>228.7 ± 2.7</td>
<td>0.71 76.9</td>
<td>0.78 81.1</td>
<td>0.45 60.9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>225.5 ± 2.2</td>
<td>0.49 51.4</td>
<td>0.82 116.7</td>
<td>0.46 54.4</td>
</tr>
<tr>
<td>Large intestine</td>
<td>Se</td>
<td>175.2 ± 2.3</td>
<td>0.38 65.7</td>
<td>0.30 59.4</td>
<td>0.23 25.3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>192.1 ± 2.2</td>
<td>0.40 66.6</td>
<td>0.70 86.3</td>
<td>0.40 50.6</td>
</tr>
<tr>
<td>Uterus</td>
<td>Se</td>
<td>187.4 ± 1.8</td>
<td>0.51 73.8</td>
<td>0.72 92.0</td>
<td>0.03 17.6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>172.5 ± 1.0</td>
<td>0.24 54.7</td>
<td>0.60 83.2</td>
<td></td>
</tr>
<tr>
<td>Testicle</td>
<td>Se</td>
<td>191.3 ± 5.5</td>
<td>0.06 3.4</td>
<td>0.07 5.5</td>
<td>0.08 5.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>188.8 ± 1.5</td>
<td>0.07 5.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Se: Chronic-selenosis group; C: Control group.
** Percent of injected $P^{31}$ (3.7 x $10^4$ c/m) recovered at various time intervals.
*** Specific activity = \( \frac{c/m}{mg\ P} \).
**** Protein-free filtrate.
***** g%.

---
tion of phosphate ions from the soft tissue is evident from the data.

Differences in P\textsuperscript{32} content of male and female gonads merit some comments. Total P\textsuperscript{31} content of these organs showed no significant variations; however, there is a significant difference in specific activity and percent of P\textsuperscript{32} retained by the uterus when compared with the male organ. Whether this difference is related to the reproductive cycle of females cannot be determined from present data. Bulliard et al. (15) studied the P\textsuperscript{32} uptake in resting ovary and corpus luteum of rabbits following administration of the isotope. The corpus luteum contained 10 times more P\textsuperscript{32} than an equal weight of ovary. Hevesy and v. Euler (16) studied the rate of phosphate penetration into tissues following subcutaneous injection of labeled phosphate. Their studies also indicated that the P\textsuperscript{32} uptake by testes was very low.

Different anatomical parts of the gastrointestinal tract showed variations as to P\textsuperscript{32} uptake. The small intestine with its highly selective absorption contained the highest percent of P\textsuperscript{32}, followed by the large intestine and stomach. It appears that the uptake of phosphate by the gastrointestinal tract is directly related to the physiological functions of each segment of this organ.

P\textsuperscript{32} uptake by the lung increased up to three hours and at the end of 12 hours showed considerable decrease, indicating a high turnover rate of phosphates in this organ.

P\textsuperscript{32} in protein-free filtrate of serum in the control group studied at various time intervals was lower than that in the experimental group (Table 4). The higher phosphate level in the serum of animals with chronic selenosis presents further evidence of a decreased rate of phosphate excretion by the kidney.

Fractionation of tissues in Table 4 may give further information on the effect of selenium on phosphorylation in these tissues.

**DISCUSSION**

The metabolism of phosphorus, studied by use of radioactive phosphorus in chronic-selenosis and control groups, indicates that, although total phosphate in tissues shows less variation, there is considerable difference in distribution of phosphates in the fractions and in the formation of phosphorylated compounds in the organs. The factors responsible for interference of phosphate metabolism in animals with chronic selenosis are manifold. In the kidney it may involve structural damage produced by repeated administration of selenate. In animals with chronic selenosis, tubular damage is the primary lesion (13). It is generally accepted that the tubular epithelium function is two-fold — excretory and reabsorptive. Phosphate is reabsorbed by an active mechanism which exhibits a limitation of transfer capacity. When tubu-
lar load exceeds reabsorptive capacity, the excess is excreted quantitatively \((17)\). The most probable energy sources for these mechanisms are energy-rich phosphates. Tubular secretion is inhibited in the presence of quinone and dinitrophenol inhibitors of dehydrogenases \((18)\). The sensitivity of dehydrogenases to selenium compounds has been reported by a number of investigators \((6)\). Whether selenium reduces cellular permeability or damages the tubular epithelium, thereby damaging the excretion mechanism directly or indirectly by inhibiting the energy source for transport mechanism, cannot be assessed from the present data. Studies on chronic selenosis indicate that at terminal stages of toxicity there is decrease in urine excretion, followed by anuria \((19)\).

Chronic selenosis produced by selenate indicates that the formation of ATP is depressed in the liver \((\text{Part I})\). Limited studies on ATP content of the kidney indicated that selenium administration depressed ATP formation in this organ in a manner similar to that of the liver. Specific activity of ATP in the kidney of the control group one hour after \(P^{32}\) administration was \(37.2 \times 10^5\); in the experimental group it was \(24.2 \times 10^5\). Although these studies were limited, the results indicated that the ATP content of the kidney in animals with chronic selenosis was consistently lower than that of the control group.

Accumulation of inorganic phosphate in the kidney and heart suggests that selenium may interfere with enzymatic reactions that involve phosphorylation in these tissues \((\text{Figs. 1 and 2})\). Tissues in which labile phosphate plays an important role in energy transfer show the highest concentration of inorganic phosphate.

Total \(P^{32}\) uptake in cardiac and skeletal muscle and the brain is low when compared with the kidney and liver, indicating low permeability of these tissues to phosphate ions. However, almost a steady rate of increase of \(P^{32}\) was maintained up to 12 hours. Kalckar et al. \((20)\), studying the rate of uptake and rejuvenation of phosphates in the skeletal muscle by use of radiophosphorus, concluded that in order to study the rate of uptake in the muscle, it is necessary to remove the highly radioactive extracellular phosphate by perfusion. Perhaps, if present studies were carried out on perfused tissues and the extracellular phosphates were removed, the differences in activity of the various fractions of cardiac muscle might be more evident.

These results indicate that phosphate uptake depends on the permeability of tissues to the ions as well as the absence of barriers between the organ and the body fluids. A negligible amount of \(P^{32}\) uptake by the brain after subcutaneous or intravenous injection of \(P^{32}\) was interpreted to indicate slow phosphorus turnover in the central nervous system \((21)\). However, it is
erroneous to assume that the turnover rate of phosphate in the brain is at a low level as was reported by Borell and Orstrom (22). The “blood-brain” barrier acts as an effective obstacle to exchange between the central nervous system and the organism; therefore, the uptake of ions from the total body reserve by the brain is not an index of the metabolism of the element within the brain. Although the percentage of $P^{32}$ uptake in the brain was low, the rate of formation of organic esters in the brain was higher than that of other organs with high phosphorylating activity.

In the control group, specific activity of inorganic and acid-soluble phosphates in the brain was lower than specific activity of ATP in all time intervals studied, indicating rapid synthesis of phosphorylated esters. The formation of ATP in the selenized group was somewhat depressed in the brain, but the depression was considerably less than those observed in the liver and kidney. Studies by a number of investigators indicate that only small amounts of selenium accumulate in the brain after single or repeated administrations of selenium (6).

Bakay and Lindberg (23) demonstrated that intracisternally injected $P^{32}$ spread very rapidly into the different parts of the brain and within 10 minutes the administered phosphate was found in organic linkage. Lindberg and Ernster (24) studied the turnover rate of $P^{32}$ in the brain by injection of the isotope into the subarachnoid space. They reported that within two minutes, 40 percent of the injected isotope was in organic linkage. At the end of eight hours, 70 percent was in organic form, after which it became approximately constant. The labile phosphate groups were renewed at an approximate rate of $40\gamma$ P/min/g of brain. These results are in agreement with the results obtained in these studies.

The spleen, although in direct contact with the circulatory system, contains a small amount of radioactive phosphorus, indicating that little if any phosphorylation takes place in this organ. In the experimental group, there was a decrease in inorganic phosphate in the spleen, which may be related to the reduction of cellular components in the tissues.

**SUMMARY**

Distribution of $P^{31}$ and $P^{32}$ in total tissue, total acid-soluble, and inorganic phosphates in the kidney, heart, spleen, and brain of normal animals and animals (rats) showing chronic selenosis was studied after administration of radioactive phosphate at a pH of 7.

Percentage uptake and specific activity of tissue fractions at various time intervals were presented. The permeability of cells to the phosphate ion determined the uptake of $P^{32}$ by the
tissues but was not an index of the turnover rate of the phosphate within the cell.

Distribution of $P^{31}$ and $P^{32}$ in the kidney of normal animals and animals with chronic selenosis showed considerable variation. These differences were assumed to be due to impaired kidney function induced by selenium poisoning. Total $P^{31}$ and $P^{32}$ content of soft and bone tissues in the experimental and control groups was presented.

The significance of alteration of phosphate metabolism to functional changes in chronic selenosis was discussed.

REFERENCES

19. Rosenfeld, I. see Part IV, infra.
Part III

Effect of Copper, Zinc, and Uranium on Chronic Selenosis

By Irene Rosenfeld

The effect of trace elements can be considered on the basis of deficiency or excess in the diet. The nutritional effect of copper and zinc, in excess or deficiency, was discussed by Underwood (1). The effect of other elements on selenium toxicity was summarized by Rosenfeld and Beath (2). The biological effect of uranium was discussed by Voegtlin and Hodge (3).

Interaction of trace elements in plant and animal nutrition suggests that the absence or presence of one element profoundly influences utilization of another element or elements. Studies on the interrelation of iron and copper demonstrated the role of copper in hematopoiesis (4). The function of copper (5) and zinc (6) in a number of enzyme systems has been well substantiated.

The toxic manifestation caused by excess of one element may be reduced or prevented by addition of another element to the diet. This was demonstrated in molybdenosis, a disease in livestock produced by an excess of molybdenum in forage that can be prevented by administration of copper (7).

The presence of toxic amounts of selenium in plants in certain areas of the United States and other countries has been substantiated by a number of investigators (2). These plants, in addition to selenium, contain a number of trace elements (8, 9).

The aim of the present investigation was to study the effect of copper, zinc, and uranium on chronic or subacute selenosis and to determine whether these elements can influence the toxicity of selenium to any degree. Zinc, copper, and uranium were selected since they are known to be present in many of the plants that grow in seleniferous soils.

All of these elements, in excess, produce pathological changes in the same organs that are affected by selenium. The concentrations of copper and zinc used in the experiments were comparable with the amounts present in selenium indicator plants and forage in Wyoming (8). The results reported here are based on the effects of these elements on growth and weight changes of rats.
METHODS

The experiments were divided into three sections. Sections 1 and 2 include the effect of copper and zinc on selenium toxicity in weanling and full-grown rats; section 3 deals with the effect of uranium on selenium toxicity.

Section 1

Weanling Wistar strain, white albino rats weighing 50 ± 5 grams were used as experimental animals. They were divided into six groups; each group contained five males and five females. The elements were added to distilled water as follow: 12 ppm copper (CuSO$_4$·5H$_2$O); 12 ppm copper + 7.5 ppm selenium (K$_2$SeO$_4$); 12 ppm zinc (ZnCl$_2$); 12 ppm zinc + 7.5 ppm selenium; and 7.5 ppm selenium. The control received distilled water without additive. The drinking water with the elements was made fresh and changed every day; the unused portion was discarded. The animals were weighed three times a week and the experiment was terminated at the end of five weeks. Water with the elements, and Purina laboratory chow, were given ad libitum.

The decision to add the elements to the drinking water was made to obtain a more nearly exact intake of the elements. Previous studies in our laboratory have shown that there is considerable scattering of food by rats when their diets contain selenium.

Section 2

Full-grown male and female rats were used as experimental animals. The weights of the males varied from 372 to 433 g and of females, from 210 to 255 g. The concentration of selenium (K$_2$SeO$_4$) was increased to 15 and 20 ppm; of copper (CuSO$_4$·5H$_2$O) to 18 ppm; and of zinc (ZnCl$_2$) to 27 ppm with or without selenium, or selenium without the other elements. The control received distilled water without additive. The animals were grouped and weighed as above. The daily water consumption was recorded and the residue discarded. Freshly made drinking water and food were given ad libitum. The experiment was terminated when all animals died. The experiments with copper, zinc, and control were terminated at the end of 100 days.

Section 3

Full-grown male rats were used. The weights of the males ranged from 414 to 484 g. Since the radioactive element, uranium, was used in combination with selenium, the elements were given by intraperitoneal injection. Uranium(UO$_2$ (NO$_3$)$_2$·6H$_2$O), 1.0 and 2 mg/kg of body weight, was injected intraperitoneally with or without 1.0 mg Se/kg (K$_2$SeO$_4$). Uranium, by intraperitoneal injection, was more toxic to rats than has been indicated by various investigators (3). The schedule of injection as originally planned was not continued. The number of injections, time intervals between injections, and duration of life are given in the results.
Studies following oral administration (stomach tube) of a decreased amount of uranium — 1.0 mg/kg as uranyl nitrate and 1.5 mg Se/kg (K₂SeO₄) — were carried out in male and female rats. The males weighed between 364 and 418 g, and the females between 190 and 240 g. At the terminal stage of toxicosis, the animals were sacrificed, and blood NPN (non-protein nitrogen) determination was made by micro-Kjeldahl method and by direct nesslerization (10).

RESULTS

The relative effect of the different treatments on the growth of weanling rats is indicated in Figure 1. The growth rate of the rats was not affected by addition of 12 ppm of copper; however, the same amount of zinc retarded growth to some degree. Selenium (7.5 ppm) retarded growth to a greater extent than any of the other elements used in this experiment.

![Graph showing growth rate of Wister albino male and female weanling rats with different elements in the diet.](image-url)

**EXPERIMENTAL PERIOD — WEEKS**

**FIG. 1**—Wister albino male and female weanling rats had access to water that contained the following amounts of elements: 12 ppm copper (CuSO₄·5H₂O), and 12 ppm zinc (ZnCO₃), with or without 7.5 ppm selenium (K₂SeO₄). Weights of rats at beginning of the experiments were 50 ± 5g.
Addition of copper (12 ppm) to selenium (7.5 ppm) neither enhanced nor retarded the growth rate of weanling rats when compared with the growth rate of this group with the selenium group. Growth retardation was greatly enhanced by the combined effect of zinc and selenium. Growth retardation of weanling rats by selenium (2) and zinc (1) has been reported. Smith and Larson (11) reported that addition of zinc (1.0 and 0.7%) to an otherwise adequate diet for rats induced anemia and subnormal growth. No growth retardation was present when zinc (0.5%) was added to milk powder diets (12).

Present data suggest that a lower level of zinc is required for growth repression when zinc is added to the water than when mixed with the diets. Dietary zinc is excreted unabsorbed by the gastrointestinal tract, a small proportion that has been absorbed being re-excreted into the intestine, chiefly by the pancreatic juice (13). Absorption of zinc is not only influenced by the diet, but also varies with chemical form or combination ingested by the animals (1).

The effect of zinc (27 ppm), copper (18 ppm), and selenium (15 and 20 ppm) on weight of full-grown rats and the combined effect of zinc and copper with different levels of selenium are indicated in Figure 2. Full-grown rats are slightly less sensitive to zinc (27 ppm) than weanling rats. The animals (zinc group) lost about 10 to 15 percent of their weight during the experiment. During the same period, the group receiving copper (18 ppm) showed a slight increase in weight. Again, copper had no significant effect on the weight loss of animals that received 20 and 30 ppm selenium, while zinc greatly enhanced the toxicity of selenium with subsequent loss of weight.

Although there was no evident effect of copper (18 ppm) on selenium (15 and 20 ppm) on weight loss, there were some differences in the duration of life of male and female rats in the copper + selenium groups when compared with the selenium groups (Table 1).

Addition of zinc to selenium increased the weight loss (Fig. 2) and reduced the survival time of the animals (Table 1), indicating that zinc (27 ppm) greatly enhanced the toxicity of selenium.

In the studies on effect of uranium on selenium, the original plan was to inject simultaneously one-half of the chronic toxic dose of both elements. The dose of selenium for males is 2 mg Se/kg, therefore 1.0 mg was used. The dose for uranyl nitrate was reported to be between 8 and 10 mg/kg of body weight (3). The acute toxic dose for females is approximately one-half that of the males. Table 2 indicates the dose of uranyl nitrate and the effect of uranium on selenium toxicity. It is evident that intraperitoneal administration of uranium and selenium (1 mg/kg) enhanced the toxicity of selenium.
Weig hts
Weight a t
be gi nn ing o f
ex pe ri m en t
Weight a t
en d o f
ex pe ri m en t

27 ppm
Zn
15 ppm
Se
20 ppm
Se
15 ppm
Se
20 ppm
Se
15 ppm
Se
20 ppm
Se
15 ppm
Se
20 ppm
Se

FIG. 2—Effect of zinc and copper with or without selenium on full-grown rats.

TABLE 1—Duration of Life and Total Intake of the Elements*

<table>
<thead>
<tr>
<th>Elements**</th>
<th>Duration of life Days</th>
<th>Selenium mg.</th>
<th>Zinc mg.</th>
<th>Copper mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Se (15)</td>
<td></td>
<td></td>
<td>79</td>
<td>49</td>
</tr>
<tr>
<td>Se (20)</td>
<td></td>
<td></td>
<td>56</td>
<td>38</td>
</tr>
<tr>
<td>Zn (27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se (15) + Cu (18)</td>
<td>93</td>
<td>63</td>
<td>20.4</td>
<td>10.3</td>
</tr>
<tr>
<td>Se (15) + Zn (27)</td>
<td>55</td>
<td>39</td>
<td>12.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Se (20) + Cu (18)</td>
<td>69</td>
<td>46</td>
<td>19.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Se (20) + Zn (27)</td>
<td>43</td>
<td>29</td>
<td>12.9</td>
<td>5.8</td>
</tr>
</tbody>
</table>

* Intake in water during the experiment.
** Se as K₂SeO₃; Zn as ZnCl₂; Cu as CuSO₄·5H₂O.
( ) = parts per million.
*** All animals lived at the termination of the experiment (100 days).
Since intraperitoneal administration of uranium and selenium produced severe toxicosis and death of the animals, limited studies were carried out by oral administration (stomach tube) of the elements. These studies indicated an increase in the nephropathic effects of uranium and selenium. In the early stages of toxicosis there was an increase in urine excretion. Subsequently the volume of urine decreased and the urine appeared highly pigmented. At this stage of toxicosis there was considerable loss of weight, anorexia, paralysis of hind quarters, and coma. Animals sacrificed at this stage had increased NPN in the blood — up to 180 mg%. Irritability of the central nervous system was more prominent in the females than in the males.

Duration of life of the females was between 8 to 12 days. Males survived from 14 to 18 days. Both groups showed severe kidney damage, but the blood NPN was higher in the females than in the males.

**TABLE 2**—The Effect of Uranyl Nitrate on Selenium Intoxication*

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of injections</th>
<th>Time intervals between injections</th>
<th>Amount/Injection</th>
<th>Total injection</th>
<th>Percent died</th>
<th>Number of days after last injection when death occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>U + Se 2</td>
<td>2</td>
<td>2nd inj. 2 days after 1st inj.</td>
<td>1.0 mg/kg + 1.0 mg.</td>
<td>2.0 mg. + 2.0 mg.</td>
<td>2.0 mg. + 2.0 mg.</td>
<td>80%</td>
</tr>
<tr>
<td>Se</td>
<td>2</td>
<td>Same intervals as above</td>
<td>0 mg/kg + 1.0 mg.</td>
<td>1.0 mg.</td>
<td>1.0 mg.</td>
<td>0%</td>
</tr>
<tr>
<td>U</td>
<td>2</td>
<td></td>
<td>2.0 mg/kg + 0 mg.</td>
<td>4.0 mg.</td>
<td>4.0 mg.</td>
<td>0%</td>
</tr>
<tr>
<td>U</td>
<td>3</td>
<td>2nd and 3rd inj. at 12 day intervals</td>
<td>2.0 mg/kg + 0 mg.</td>
<td>6.0 mg.</td>
<td>6.0 mg.</td>
<td>0%</td>
</tr>
<tr>
<td>U</td>
<td>4</td>
<td></td>
<td>2.0 mg/kg + 0 mg.</td>
<td>8.0 mg.</td>
<td>8.0 mg.</td>
<td>0%</td>
</tr>
<tr>
<td>U</td>
<td>5</td>
<td>4th and 5th inj. at 6 day intervals</td>
<td>2.0 mg/kg + 0 mg.</td>
<td>10.0 mg.</td>
<td>10.0 mg.</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Male rats weight 450 ± 30 gms.

** Intraperitoneal.
DISCUSSION

Interaction of selenium with essential trace elements and uranium indicates that some elements may enhance selenium toxicity while others have no influence. Zinc and uranium enhanced selenium toxicity, while copper had no appreciable effect under present experimental conditions.

Studies on the distribution of Zn$^{65}$ and Cu$^{64}$ in nuclei and cytoplasmic proteins indicate that, while a considerable amount of Cu$^{64}$ was bound with cytoplasmic proteins, Zn$^{65}$ was present mainly in the acid-soluble fraction of all tissues (14). The inability of tissue proteins to bind zinc ions may be an important factor in the additive toxic effect of zinc in these experiments.

Previous studies by Moxon (15) and by Moxon and DuBois (16) indicated that a number of elements (salts of the elements) increased the toxicity of seediferous wheat or inorganic selenium with the exception of arsenic and tungsten. On the basis of a number of studies by Moxon and co-workers (15, 16, 17), arsenic assumed greater importance in preventing chronic seleniumosis in livestock than it merits. Recent studies indicate that arsenicals markedly reduced the elimination of inorganic selenium compounds by the respiratory tract when the dose approached the acute toxic level (18). Arsenic had a minor effect on elimination of selenium by rats when the diets contained seediferous wheat (19), suggesting that arsenic may interfere with the detoxification mechanism of selenium.

Salts of zinc and uranium included in this study produced additive toxic selenium effects, indicated by decreased growth rate, loss of weight, and increased pathological damage. The latter was evident in the uranium and selenium group.

Copper, zinc, and uranium were used in low concentrations in these studies; much higher concentrations of these elements are present in a number of forage plants. If selenium is absent, these elements (in amounts present in forage) do not produce harmful effects in animals. However, data indicate that in all cases of chronic poisoning, some trace elements may enhance the toxic effect of selenium.

SUMMARY

The biological effect of copper sulfate and zinc chloride with selenium (K$_2$SeO$_4$) was studied in weanling rats. Zinc enhanced the toxic effect of selenium, which was indicated by the reduced growth rate. Copper had no influence on the depressed growth rate produced by selenium.

Copper had no effect on weight loss produced by selenium in full-grown animals, but zinc enhanced the toxic effect of selenium, which was indicated
by loss of weight and by decrease in duration of life. Addition of copper increased the duration of life of full-grown rats which received 20 and 30 ppm selenium.

Intraperitoneal administration of uranyl nitrate and selenium was extremely toxic to rats. Oral administration of uranium and selenium produced severe kidney damage with increase in NPN of the blood.

REFERENCES

9. Love, J. D. Personal communication.
Part IV
Excretion and Retention of Se$^{75}$ in Relation to Modes of Administration, Toxicity, and Pregnancy in Rats$^1$

By Irene Rosenfeld

Recent emphasis on selenium metabolism is based on the recognition of the dual function of the element. Selenium as a toxic agent and its effect on deficiency diseases were summarized by Rosenfeld and Beath (1); its effect in trace amounts on deficiency diseases was reviewed by Schwarz (2).

Up to the present, no comparable data have been available on the metabolism of selenium when repeated subtoxic doses and trace amounts (atoxic doses) of selenium are administered to animals.

In the prophylactic use of selenium for prevention of myopathies (White Muscle Disease) in lambs and calves, selenium is usually administered before or soon after parturition. A number of investigators administered single or repeated subtoxic doses of selenium subcutaneously or orally before parturition or post-partum in the assumption that one subacute dose has the same metabolic pathway as have single or repeated smaller doses. Administration of a single intramuscular dose of 30 mg of selenium to sheep was fatal within 24 hours, while 50 mg selenium (selenite) given orally was tolerated by the sheep and prevented the incidence of muscular dystrophy in the progeny (3).

These studies were initiated to determine the excretion and retention of selenium after various modes of single- and repeated-dose administration of tracer doses and subacute toxic doses of selenium, as well as to investigate the placental transmission of selenium with subtoxic and tracer doses. Tracer doses contain microgram amounts of selenium and do not produce any detectable interference with the normal metabolism of the animals. Subacute toxic doses refer to the administration of selenium that produces toxic effects and interferes with the normal metabolic activity of animals.

In all these studies the radioactive isotope, Se$^{75}$, was used as the means to determine the distribution and metabolism of selenium.

$^1$Presented in a seminar lecture at the University of California Medical School, San Francisco, in February 1963.
MATERIALS AND METHODS

Sprague-Dawley rats (weight of females, 200 - 300 g; weight of males, 300 - 450 g) were used as experimental animals. Selenium$^{75}$ ($\text{H}_2\text{Se}^{75}\text{O}_3$) contained 0.175 mg of Se/10 mcs. The daily tracer dose of Se$^{75}$ was given intragastrically (i.g.) by stomach tube, intraperitoneally (i.p.), or subcutaneously (s.c.), as indicated in the results. The dose of Se$^{75}$/day administered was calculated to contain 7.5 $\mu$g of selenium. A subacute toxic dose of 2.0 mg or 2.5 mg of selenium ($\text{H}_2\text{SeO}_3$)/kg was mixed with Se$^{75}$ ($\text{H}_2\text{Se}^{75}\text{O}_3$).

The animals receiving 7.5 $\mu$g of selenium as Se$^{75}$ are indicated as the atoxic group or tracer group. The animals receiving the subacute toxic doses of selenium are indicated for brevity as the toxic group.

The animals were placed in metabolism cages; the urine and feces were collected at 24-hour intervals. Feces were dried at 60° to 70° C. for several days. Concentrated HNO$_3$ was added to the weighed and dried feces and placed on a steam bath for 12 hours between 60° to 80° C. This temperature control was essential in order to prevent the loss of selenium by volatilization. The urine and the acid-digested feces were diluted to volume with water, and the Se$^{75}$ was determined each day. The number of doses administered and the duration of the experiment are indicated in the results. The experiments were terminated 3, 4, 90, or 150 days after administration of the final dose of selenium.

**Placental transmission studies.** Selenium (2 mg/kg) with Se$^{75}$ was administered by stomach tube to non-pregnant and pregnant rats between 12 and 13 days of pregnancy. A group of pregnant females received the same number of tracer doses of Se$^{75}$ as the toxic group, and they served as controls. The experiment was terminated two days before parturition, or 24 hours after the final dose was given.

At termination of the experiments, the animals were anesthetized with ether and decapitated. The blood and internal organs were removed and weighed. The weighed tissues were homogenized with a glass tissue homogenizer and diluted to volume. Retention in the bones was determined in the femur. The gastrocnemius muscle was used for determination of selenium retention in the muscle. The eviscerated carcass (including the muscles, skin, fur, and bones) was digested with HNO$_3$ on a steam bath between 60° to 80° C. until the digest could be pipetted readily and diluted to volume. The gastrointestinal content was removed by repeated washing of the various segments of the intestinal tract with saline (0.9% NaCl). The washings and intestinal contents were pooled and digested with HNO$_3$ on a steam bath.
Radioactivity in tissues, urine, and feces of each sample was determined in triplicate aliquots. All measurements for radioactivity were carried out in a well-type Scintillation gamma detector, and the results were corrected for decay.

The results for excretion are expressed as percent of the administered dose of Se\textsuperscript{75} excreted during the experiment. Percent distribution in tissues was based on the total Se\textsuperscript{75} retained in the whole animal (carcass, including internal organs), which was considered as 100. The sum total of Se\textsuperscript{75} per gram of all tissues was considered as 100, and percent per gram was calculated on this basis. Each group contained three to five animals.

RESULTS

The data obtained in these experiments will be presented in the following sequence: (a) urinary, fecal, and respiratory excretions; (b) distribution of the Se\textsuperscript{75} retained in the tissues; (c) placental transmission of Se\textsuperscript{75}; and (d) distribution of Se\textsuperscript{75} retained in maternal tissues.

**TABLE 1**—Se\textsuperscript{75} Excreted in 24 Hours After a Single-Dose Administration of the Isotope

<table>
<thead>
<tr>
<th>Mode of Administration</th>
<th>Tracer group*</th>
<th></th>
<th>Toxic group*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td>Subcutaneous (4)</td>
<td>12.9</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal (4)</td>
<td>26.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragastric (4 M)</td>
<td>34.7</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragastric (4 F)</td>
<td>33.3</td>
<td>15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragastric (6 M)</td>
<td>10.2**</td>
<td>4.0**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragastric (6 PF)</td>
<td>8.9***</td>
<td>6.3***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

( ) Number of animals: M—males, F—females, PF—pregnant females.

* 0.1 mc Se\textsuperscript{75}, Sprague-Dawley rats (5 μg Se).

** 2.5 mg Se (Na\textsubscript{2}SeO\textsubscript{4}) /kg.

*** 2 mg Se (Na\textsubscript{2}SeO\textsubscript{4}) /kg.
and fecal) of the atoxic groups in 24 hours was as follows: intragastric, 47 to 48 percent; intraperitoneal, 30 percent; and subcutaneous, 16 percent. Administration of a single intragastric toxic dose of selenium reduced the combined urinary and fecal excretion of Se\textsuperscript{75} in males and pregnant females to less than 15 percent (Table 1).

The total excretion of Se\textsuperscript{75} after repeated administration of selenium (by various modes) for 21 days of the atoxic and the intragastric-toxic group is indicated in Figure 1. The urinary excretion of Se\textsuperscript{75} in decreasing order was subcutaneous > intraperitoneal > intragastric > intragastric-toxic group. The fecal excretion of Se\textsuperscript{75} was highest in the toxic-intragastric group (Fig. 1, group IV). Intragastric administration of atoxic amounts of selenium increased the fecal excretion of Se\textsuperscript{75} above that of the intraperitoneal and subcutaneous groups.

![MODE OF ADMINISTRATION](image)

**FIG 1**—Urinary and fecal excretion of Se\textsuperscript{75} by rats after various modes of administration of the isotope. Groups I, II and III received repeated tracer doses of Se (7.5 μg) as H\textsubscript{2}Se\textsuperscript{75}O\textsubscript{2} per day. Group IV toxic dose was 2.5 mg Se (Na\textsubscript{2}SeO\textsubscript{3}) per kg + Se\textsuperscript{75} per day. Duration of the experiment was 21 days. Total Se\textsuperscript{75}, 54.077 x 10\textsuperscript{6} c/m, = 100.
The repeated intragastric administration of the isotope at toxic levels reversed the route of maximum elimination of selenium (Fig. 1, group IV). The maximum selenium was excreted in the feces, and the urinary excretion was less than one-half that of the atoxic group.

A comparison of $\text{Se}^{75}$ excretion in the atoxic (tracer dose) and the toxic groups after intragastric administration of selenium up to 96 hours is indicated in Figure 2. The maximum urinary and fecal excretion of $\text{Se}^{75}$ in the atoxic group was at the end of 24 hours with rapid decrease between 24 to 48 hours and with almost constant amounts of $\text{Se}^{75}$ excretion from 72 to 96 hours. After the toxic dose of selenium administration, the maximum urinary excretion of $\text{Se}^{75}$ was less than one-third of the atoxic group with slow decrease up to 96 hours. In the atoxic group, the main pathway of $\text{Se}^{75}$ elimination was by the urinary tract; in the toxic group the main pathway was by the gastrointestinal tract (Fig. 1, group IV; Fig. 2).
These studies suggest that at least one of the contributing factors for the toxic effects of selenium may be due to the slow rate of elimination and to the extended contact of tissues with the toxic agent.

Pregnancy as well as subacute selenium influenced the excretions of selenium. Pregnant rats receiving tracer doses of Se\(^{75}\) intragastrically had slightly higher urinary excretion and lower fecal excretion of Se\(^{75}\) than the non-pregnant animals. The total excretion of Se\(^{75}\) in both atoxic groups was over 50 percent in 24 hours (Fig. 3).

Pregnancy and selenium toxicity put considerable stress on the gastrointestinal tract as well as on the urinary tract. The total Se\(^{75}\) excretion (urinary and fecal) in the toxic group at the end of 24 hours was about 25 percent of the administered dose. The urinary excretion of Se\(^{75}\) increased at 48 hours, with slow decrease up to 72 hours. The fecal excretion of Se\(^{75}\) was delayed, as indicated by the high concentration of Se\(^{75}\) in the feces at 72 hours. These

![Graph](image-url)

**Fig. 3**—Urinary and fecal excretions of Se\(^{75}\) at various time intervals by non-pregnant and pregnant rats. Toxic and tracer (atoxic) dose of selenium administered i.g. for 6 days. Tracer dose: (non-pregnant and pregnant rats) 7.5 \(\mu\)g Se\(^{35}\) day as H\(_2\)Se\(^{35}\)O\(_2\). Toxic dose: 2 mg Se (H\(_2\)SeO\(_3\))/kg + Se\(^{75}\)/day. Total Se\(^{75}\) given in 6 days, \(10.8 \times 10^7\) c/m, = 100.
results again indicate reversal of the mode of elimination of Se\textsuperscript{75} in the toxic group.

No long duration studies on the effect of selenium toxicity on the urinary and gastrointestinal functions in pregnancy in rats could be carried out because of the limited time of the gestation period of these animals. Livestock would be ideal experimental animals for study of subacute effects of selenium on pregnancy since their gestation period is longer; thereby the delayed and immediate toxic effects of selenium on the mode of elimination could be studied. At the same time, chemical analysis for total selenium (Se + Se\textsuperscript{75}) could be carried out.

High concentrations of selenium in feces of toxic groups at the end of 72 and 96 hours (Figs. 2 and 3) suggest that selenium may have entered into the metabolic pathways, and the end products may have been excreted in the feces.

Selenium is known to be excreted in the bile in acute and subacute poisoning of hogs, sheep, calves (4), and rabbits (5). The presence of Se\textsuperscript{75} in the bile pigments of dogs after intraperitoneal injection of radioactive selenium (6) and in sheep bile was reported (7). A large amount of Se\textsuperscript{75} in the intestinal tract may be due to re-excretion of Se\textsuperscript{75} via the bile and pancreatic juice to the intestinal tract.

A summary of Se\textsuperscript{75} excretion and retention in the carcass (including internal organs and blood) after various modes of administration is presented in Table 2. The selenium was given for 27 days, and the animals were sacrificed 3, 90, and 150 days after the final dose. It is evident from the data that the main pathways of selenium elimination are the urinary, gastrointestinal, and respiratory tracts. Elimination varied according to the mode of administration and also depended on whether toxic or atoxic (tracer) doses were administered. The elimination of atoxic doses was more efficient when the selenium was administered intragastrically. Over 90 percent of the Se\textsuperscript{75} was eliminated during the experiment and three days after the final dose (that is, within 30 days). Regardless of the modes of administration when atoxic doses were given, in all cases over 80 percent of the selenium was eliminated by the animals during the experiment (27 days) and three days after the final dose was administered.

Administration of toxic doses greatly reduced the urinary and gastrointestinal elimination of selenium. The intestinal content of the toxic group contained over 17 percent of the Se\textsuperscript{75} administered three days after the final dose, while in the atoxic groups the intestinal content contained only a fraction of 1 percent of the administered Se\textsuperscript{75}. Retention of Se\textsuperscript{75} in the carcass (including internal organs and blood) was highest in the group in which selenium was given subcutaneously, which may ac-
TABLE 2—Excretion and Retention of Se$^{75}$* After Repeated Tracer and Toxic Doses of Selenium

<table>
<thead>
<tr>
<th>Mode of administration</th>
<th>Dose of Se/day</th>
<th>Day sacrificed after final dose</th>
<th>Percent of administered dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td>Atoxic Group**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine and feces (M)</td>
<td>S. C.</td>
<td>7.5 μg</td>
<td>60.4 ± 4.8</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td>18.4 ± 2.5</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Respiratory tract***</td>
<td></td>
<td>21.2</td>
<td>21.2</td>
</tr>
<tr>
<td>Urine and feces (M)</td>
<td>I. P.</td>
<td>7.5 μg</td>
<td>63.1 ± 4.7</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td>11.5 ± 1.5</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Respiratory tract***</td>
<td></td>
<td>25.4</td>
<td>26.4</td>
</tr>
<tr>
<td>Urine and feces (M)</td>
<td>I. G.</td>
<td>66.1 ± 4.9</td>
<td>71.2 ± 5.3</td>
</tr>
<tr>
<td>Carcass (M)</td>
<td></td>
<td>8.6 ± 1.9</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>Respiratory tract (M)***</td>
<td></td>
<td>25.3</td>
<td>28.3</td>
</tr>
<tr>
<td>Urine and feces (F)</td>
<td>I. G.</td>
<td>70.4 ± 4.0</td>
<td>72.1 ± 2.5</td>
</tr>
<tr>
<td>Carcass (F)</td>
<td></td>
<td>9.4 ± 2.2</td>
<td>0.8 ± 0.04</td>
</tr>
<tr>
<td>Respiratory tract (F)***</td>
<td></td>
<td>20.2</td>
<td>27.1</td>
</tr>
<tr>
<td>Toxic Group**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine and feces (M)</td>
<td>I. G.</td>
<td>2.5 mg Se/kg + Se$^{75}$</td>
<td>50.2 ± 2.9</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td>5.0 ± 2.5</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Intestinal content</td>
<td></td>
<td>17.5 ± 7.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Respiratory tract***</td>
<td></td>
<td>27.3</td>
<td>36.2</td>
</tr>
</tbody>
</table>

* 2.0 x 10$^7$ c/m/day. (M)—Males, (F)—female Sprague-Dawley rats. Se$^{75}$ and Se given as selenite. Total Se$^{75} = 54 x 10^7$ c/m in 27 days.
** Administration of selenium terminated at 27 days.
*** Estimated by difference of 100 — (feces + urine + carcass, including all tissues).
**** Each group consisted of 5 animals.
***** Results are on 2 animals; others died after discontinuation of selenium administration.

account for the extreme toxicity of selenium injected subcutaneously when compared with oral administration (3).

The retained selenium in the carcass was not inert but was metabolically active, as indicated by continued elimination of selenium by urinary and gastrointestinal tracts up to termination of the experiments (3, 90, and 150 days). The respiratory elimination of selenium continued in the intraperitoneal and intragastric atoxic groups and intragastric-toxic group up to 90 and 150 days. However, in the subcutaneous group, respiratory elimination ceased three days after the final dose of Se$^{75}$ was administered.

Urinary and fecal excretion of Se$^{75}$ in the toxic group never approached that of the atoxic group even at 90 days, but respiratory elimination of selenium was above that of the atoxic group throughout the experiment. The carcass in the toxic group (killed 3 days after the final injection) contained less Se$^{75}$ than the atoxic (tracer) group.
This difference was due to high concentration of Se\textsuperscript{75} in the intestinal content and to increase in the respiratory elimination of Se\textsuperscript{75} during the experiment.

Distribution of retained Se\textsuperscript{75} in tissues. The distribution of Se\textsuperscript{75} in tissues of animals that received atoxic doses of selenium and were sacrificed at 4 and 90 days after the final dose is given in Figure 4. The selenium (7.5 \text{\textmu}g/day) was administered intragastrically, interperitoneally, and subcutaneously for 27 days. The eviscerated carcass contained the highest concentration of selenium when the results were based on the whole body regardless of the mode of administration. The highest concentration of Se\textsuperscript{75} in the internal organs was present in the liver, kidney, and testicle, when the results were based on the amount of Se\textsuperscript{75} retained by the whole organ. When the results were based per gram of tissue, the kidney, testicle, liver, blood, adrenal, carcass, and spleen, in descending order, retained the highest concentrations of Se\textsuperscript{75}. There were some variations in total retention of Se\textsuperscript{75} in the different organs after the different modes of administration, but the maximum uptake of Se\textsuperscript{75} occurred in the same organs.

Accumulation of Se\textsuperscript{75} in the lung, heart, and brain was 1 percent or less,
regardless of the mode of administration, and the gastrointestinal tract free of content was slightly over 1 percent. Distribution of Se\textsuperscript{75} at the end of 96 hours in decreasing order was the following: gastrointestinal tract > lung > heart > bone > muscle > brain.

The eviscerated carcass of animals sacrificed at 90 days contained 85 to 90 percent of the retained Se\textsuperscript{75}, and lesser amounts were found in the other organs (Fig. 4). Retention of Se\textsuperscript{75}, calculated per gram of tissue, merits some comment. The spleen and adrenals showed an apparent increase in Se\textsuperscript{75} content in 90 days. The retained Se\textsuperscript{75} in other organs decreased with time. In the spleen and adrenals, there was only slight decrease of Se\textsuperscript{75} with increase of time, suggesting that the Se\textsuperscript{75} present in these tissues was not metabolized and remained deposited in the organs. It is interesting to note that even at 90 days, the blood contained a considerable amount of Se\textsuperscript{75} regardless of the mode of administration (Fig. 4).

At the end of 90 days, the animals that received subcutaneous injections of Se\textsuperscript{75} had higher concentrations of the isotope in the skeletal muscles.
Retention of Se$^{75}$ in other organs, which are not given in the table, and the calculated percent per gram of tissue, in decreasing order, were the following: heart $>$ lung $>$ bone $>$ brain, regardless of the mode of administration.

A comparative study on retention of Se$^{75}$ after intragastric administration of atoxic (tracer) and toxic doses of selenium to male rats is indicated in Figure 5. The distribution of Se$^{75}$ in the tissues showed some differences between the two groups of animals sacrificed at 72 hours after the final dose was given. The kidney, blood, and gastrointestinal tract, which included the intestinal content, in the toxic group retained higher concentrations of Se$^{75}$ than the atoxic group; the liver with toxic doses of selenium contained less selenium than the atoxic group. In animals sacrificed 90 days after the final toxic dose, the tissues retained similar relations—that is, the organ containing more Se$^{75}$ at the end of 72 hours also showed higher Se$^{75}$ retention at the end of 90 days, with exception of the spleen and adrenals. The spleen and adrenals again indicated an apparent increase in retention of Se$^{75}$ at the end of 90 days. This presents further evidence that, in these tissues, the retained selenium was fixed and not metabolized. The eviscerated carcass at both time intervals contained the highest concentration of Se$^{75}$ when the results were calculated on the total amount retained in the animals. However, when the results were calculated per gram of tissue, the carcass contained about 2 percent per gram of tissue at the end of 72 hours and 6 to 8 percent of the retained Se$^{75}$ at the end of 90 days. These results indicate that while liver, kidney, and blood selenium decreases gradually, the selenium deposited in the carcass (skeletal muscle, bone, skin, and fur) changes at a slower rate.

Placental transmission. Placental transmission and retention of Se$^{75}$ with tracer and toxic doses of selenium in pregnant rats are indicated in Table 3. Higher concentrations of Se$^{75}$ were retained in the kidney, red blood cells, whole blood, gastrointestinal tract, placentas, amniotic fluid, and carcass in the toxic group than in the group that received the tracer doses of selenium. The liver of the toxic group contained less Se$^{75}$ than the liver of the tracer group. Although the amniotic fluid and the placentas of the toxic group contained twice as much Se$^{75}$ as the tracer group, the fetuses in the toxic group contained somewhat less Se$^{75}$ than in the tracer group. Two animals in the toxic group had premature pups six hours after administration of the toxic dose, approximately 19 days of their pregnancy. Whether the higher concentration of selenium in the placenta or amniotic fluid was responsible for the premature births cannot be ascertained from the present data.
### TABLE 3—Placental Transmission and Retention of Se$^{75}$ in Tissues of Pregnant Rats After Intragastric Administration of Selenium to Rats for 6 Days

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Tracer dose*</th>
<th>Toxic dose (Se + Se$^{75}$)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>percent***</td>
<td>percent per gram</td>
</tr>
<tr>
<td>Liver</td>
<td>23.3</td>
<td>19.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.4</td>
<td>9.9</td>
</tr>
<tr>
<td>Uterus</td>
<td>2.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Placenta</td>
<td>5.4</td>
<td>9.7</td>
</tr>
<tr>
<td>Fetuses</td>
<td>14.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>3.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Whole blood</td>
<td>3.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Red-blood cells</td>
<td>3.1</td>
<td>15.8</td>
</tr>
<tr>
<td>Plasma</td>
<td>3.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Eviscerated carcass</td>
<td>31.4</td>
<td>35.3</td>
</tr>
</tbody>
</table>

* 1.8 x 10$^5$ c/m Se$^{75}$ (7.5 μg Se)/day for 6 days. Sacrificed 24 hrs. after last dose.
** Se$^{75}$ (1.8 x 10$^5$ c/m) and 2 mg Se (Na$_2$SeO$_3$)/kg body weight.
*** Se$^{75}$ retained (eviscerated carcass + blood + internal organs + uterus and its contents = 100.

### TABLE 4—Se$^{75}$ Excretion and Retention in Pregnant Rats (including Fetuses)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Tracer dose*</th>
<th>Toxic dose (Se + Se$^{75}$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent of administered Se$^{75}$ recovered</td>
<td></td>
</tr>
<tr>
<td>Urine (7 days)</td>
<td>34.4</td>
<td>14.8</td>
</tr>
<tr>
<td>Feces (7 days)</td>
<td>22.4</td>
<td>21.6</td>
</tr>
<tr>
<td>Carcass (Total animal)</td>
<td>15.9**</td>
<td>28.8**</td>
</tr>
<tr>
<td>Respiratory tract***</td>
<td>27.3</td>
<td>34.8</td>
</tr>
</tbody>
</table>

* Tracer dose: 1.8 x 10$^5$ c/m Se$^{75}$ (7.5 μg Se)/day. Toxic dose: 2 mg Se (Na$_2$SeO$_3$)/kg/day + Se$^{75}$ (1.8 x 10$^5$ c/m). Selenium was given I. G. for 6 days.
** Including the intestinal content.
*** Estimated by difference of 100 — (urine + feces + carcass).
Retention and excretion of Se\(^{78}\) in pregnant rats receiving tracer and toxic doses of selenium are summarized in Table 4. The urinary excretion of Se\(^{78}\) in the toxic group is less than that of the tracer group. The two groups excreted about the same amount of Se\(^{78}\) in the feces. However, the intestinal content was not removed and it is included with the carcass of both groups. The retained selenium in the intestinal content of the toxic group was four to five times greater than that of the tracer group. This difference is evident from the data on the carcass of the toxic group, which contained almost twice the amount of Se\(^{78}\) as the atoxic (tracer) group when the intestinal content was not removed.

Respiratory elimination of Se\(^{78}\) was higher in the toxic group than in the group that received the tracer dose. The urinary and fecal excretion of Se\(^{78}\) by the non-pregnant tracer group of females was higher (Table 2) than by the pregnant tracer group (Table 4). Respiratory elimination of Se\(^{78}\) was more than 20 percent in the pregnant toxic group compared with the group that received tracer doses of selenium.

**DISCUSSION**

The studies on selenium metabolism indicate that the mode of administration of trace amounts of selenium had no marked influence on the rate of excretions except after single-dose administration. When selenium in the body pool was increased within the toxic range, the usual metabolic pattern of selenium excretion was reversed, and the main pathway of Se\(^{78}\) elimination was by the gastrointestinal tract instead of the urinary tract. The maximum excretion of Se\(^{78}\) in animals receiving repeated tracer doses was within 24 hours, while in the toxic group the maximum excretion of Se\(^{78}\) was delayed to 48 or 72 hours. There was continued excretion of Se\(^{78}\) by the urinary and gastrointestinal tracts up to 150 days (Table 2).

Results reported by earlier investigators on selenium excretion by the urinary, respiratory, and gastrointestinal tracts showed considerable variation (8, 9). More recent studies indicate that excretion and retention of injected selenium (selenite) can be altered by arsenic and cadmium, age, and weight of the animals as well as by modification of the diets (10). Administration of arsenite increased elimination of selenium via the gut, while cadmium reduced elimination and increased retention of selenium in the body. Arsenite increased the gastrointestinal excretion of selenium more than twofold. Olson *et al.* (11) reported that the urinary and gastrointestinal elimination of 5 ppm selenium (selenate) at 13 to 16 days was 51 and 12 percent, respectively, and arsanilic acid (0.015%) had no effect on selenium excretion by the gastrointestinal and urinary tract.
Comparing results of the above investigators with present studies indicates that, if the results are evaluated on the basis of the dose of selenium given to the animals, these discrepancies can be resolved. Higher urinary excretion of selenium occurs when the dose of selenium is at a level that does not produce toxic effects in the animals. This is evident from the present results as well as from the results reported by Olson et al. (11). If the dose of selenium is increased to subacute toxic levels (that is, 1.5, 2.0, or 2.5 mg Se/kg, depending on the sex and age of the animals) and if the administration of selenium is continued for a long period (Fig. 1), then the gastrointestinal excretion of selenium exceeds that of the urinary tract. If the experiment is of shorter duration (Table 1; Fig. 2), then both the urinary and fecal selenium excretion in the toxic group is greatly reduced.

Ganther and Baumann (10) presented similar data after intramuscular administration of 2 mg Se/kg. They reported that the gastrointestinal tract and feces contained from 6.6 to 7.6 percent, and the urinary excretion was 9.8 to 14.1 percent of the injected selenite after intramuscular injections of selenium. If the selenium was reduced to 1.5 mg/kg, the intestinal content and the feces contained 8.9 to 16.1 percent, and the urinary excretion was from 12.2 to 15.4 percent of the dose when the animals were given the same diet. If arsenite and selenite were injected simultaneously, there was a threefold increase in the gastrointestinal excretion of selenium; with cadmium and selenite, both the urinary and fecal excretion of selenium was depressed.

The results appear to be conflicting as to the amount of selenium eliminated by the respiratory tract (8, 9). The results presented by Ganther and Baumann (10) indicate that the diet as well as the administration of arsenic or cadmium with selenium would greatly influence the volatile selenium compound(s) eliminated by the respiratory tract.

From the results presented by Olson et al. (11), it appears that not only the diet or arsenic or cadmium administration have considerable effect on selenium elimination by the respiratory tract (10), but the selenium compound(s) injected or fed to the animals as well as the amount of selenium given determines the amount of selenium eliminated by the respiratory tract. Elimination of selenium by the respiratory tract was higher after the selenite (22-25%) injection than after the selenate (11-16%) injection when the amount of selenium given was 2.0 mg Se/kg of body weight. If the dose of selenium was decreased to 0.016 mg/kg (as selenite), only 1.1 percent of the administered dose was eliminated by the respiratory tract in six hours. When seleniferous wheat was fed to the animals, the respiratory elimination of selenium
was on the average of 1.8 percent of the intake.

In the experiments reported here, rats received repeated doses of selenite-Se⁷⁵ (2.0 or 2.5 mg Se/kg) and exhaled between 27 and 35% of the Se⁷⁵ by the respiratory tract. These results are in agreement with the results reported by Ganther and Baumann (10) when they fed crude diets to the animals, and also with the results of Olson et al. (11) when the amounts of selenium were comparable to those used in the present experiment.

Respiratory excretion of selenium after administration of repeated atoxic doses was between 20 and 25 percent in the various experimental groups that were sacrificed 72 hours after the final dose. If the animals were permitted to survive 90 or 150 days, there was further excretion by the respiratory tract, except in the subcutaneous group. These results are higher than those reported by Olson et al. (11) using microgram quantities, but they terminated their experiments at six hours after a single injection of selenite. The present results are based on repeated administrations of selenium (selenite) and on longer duration of the experiments, which may account for the variation.

Results in the present studies as well as in studies by Ganther and Baumann (10) and by Olson et al. (11) on elimination of selenium were based on the amount of Se⁷⁵ excreted, when consider-
as selenotaurocholic acid (7). The presence of high concentrations of selenium in the gastrointestinal tract may have been partially due to the reduced motility of the organs, increased destruction of the erythrocytes which contain large amounts of selenium, and subsequent degradation of the hemoglobin product(s) which appear in the bile. These results suggest that all these factors may be responsible for the high concentration of Se\textsuperscript{75} in the gastrointestinal tract during selenium intoxication. In vitro studies indicated that addition of 2 ppm selenium as selenite to the substrate reduced intestinal motility, and 8 ppm completely inhibited the gastrointestinal activity (12). Intestinal stasis as well as kidney damage has been associated with acute, subacute, and chronic selenosis (13).

Retention of Se\textsuperscript{75} in the carcass was higher after repeated subcutaneous administration than when selenium was administered by other routes. The animals that received chronic toxic doses of selenium and were sacrificed 90 days after the final dose retained less selenium in the carcass and liver than animals that received tracer doses. However, the kidney contained more Se\textsuperscript{75} than that of the atoxic group.

Pathological changes characteristic of chronic selenosis were observed in the liver, testicles, and spleen (13) in animals injected with 2 or 2.5 mg Se/kg. The retained Se\textsuperscript{75} in the animals was metabolically active in the toxic as well as in the atoxic groups in all organs (with the exception of the spleen and adrenals), since selenium gradually decreased with time (90 and 150 days) in the tissues. The rate of decrease from the carcass (muscle, bone, skin, and fur) was slower than from the other tissues.

Placental transmission of selenium has been reported in rats and cats by Westfall \textit{et al.} (14) from seleniferous grains (organic selenium) and by use of inorganic selenium. The fetuses of animals fed seleniferous grain stored more selenium than those receiving inorganic selenium in the diet. The present studies indicate not only that selenium passes through the placenta, but also that the growing fetus withdraws a considerable amount of selenium from the maternal tissues as indicated by decreased selenium retention in the eviscerated carcass. The accumulation of Se\textsuperscript{75} in erythrocytes of pregnant animals was higher with toxic doses of selenium than with tracer doses of selenium.

Female reproductive organs retained comparatively less selenium than the male reproductive organs. Accumulation of selenium in the testicles (Fig. 4) merits consideration. It is well known that selenium can produce malformations in chicks (15) and congenital malformations in lambs (16). The production of malformations in rats by selenium was unsuccessful (14; Rosenfeld, unpublished results). In
all studies, only the female rats received selenium, and selenium-free males were used for breeding. The results in these experiments suggest that studies related to mammalian malformations with selenium should include selenized males as well as females.

The experiments with rats indicated that a low level of selenium had no effect on spermatogenesis and on the viability of sperms for as long as eight months, but impaired reproduction in the females (17) suggests that oogenesis is more susceptible to the effects of selenium than spermatogenesis. The accumulation of selenium in the male reproductive organ may produce sperm abnormalities which may be an important factor in congenital malformations in mammals.

**SUMMARY**

Selenium excretion and retention in tissues were studied in groups of rats by the use of tracer amounts (7.5 μg Se) of selenite-Se$^{78}$. The selenium was given intraperitoneally, intragastrically, and subcutaneously. Data on elimination of selenium indicate that total elimination following repeated administrations (by various routes) does not differ significantly, but the amount excreted by the kidney, gut, and lung shows some differences. The main mode of elimination with atoxic (tracer) doses of selenium is the urinary tract, since over 40 percent of the injected dose is excreted in the urine and about one-half or less is excreted by the gastrointestinal tract and lung. Selenium was eliminated by the urinary, gastrointestinal, and respiratory tracts up to 150 days. Respiratory elimination ceased after three days after subcutaneous injections.

Administration of repeated subacute toxic doses (2.5 Se mg/kg) increased selenium retention in the blood and kidney, and large amounts of Se$^{75}$ were present in the intestinal content. Repeated subacute toxic doses of selenium reversed the route of elimination as indicated by the decreased rate of urinary excretion and increased excretion of Se$^{75}$ in the feces and by the lung. Se$^{75}$ retained by the tissues is metabolically active (with exception of the spleen and adrenals) since the selenium content decreased with time.

Placental transmission of Se$^{75}$ and distribution of Se$^{75}$ in the fetuses and maternal tissues after repeated administration of subacute toxic and tracer doses of selenium indicate transport of selenium from the carcass to the embryos. Urinary excretion of Se$^{75}$ in the atoxic pregnant group was slightly decreased with significant decrease in urinary Se$^{75}$ excretion in the toxic group as compared with the non-pregnant females. Pregnancy increased the elimination of selenium by the respiratory tract in both groups, but this increase was higher in the group that
received the subacute toxic doses. Subacute toxic doses of selenium increased the Se\textsuperscript{75} content of the red blood cells, placenta, and amniotic fluid, but the fetuses contained slightly less Se\textsuperscript{75} than the group that received the tracer doses.

**Technical assistance by:**
Thomas Averett
Craig Graham

**REFERENCES**

Part V
Metabolism of Selenium in Sheep

By I. Rosenfeld and H. F. Eppson

Investigations dealing with elimination and distribution of selenium in tissues of livestock are limited and the results reported are divergent.

Rosenfeld and Beath (1) studied the tissue distribution and urinary excretion of selenium by sheep using high, medium, and low-protein diets in conjunction with administrations of 15, 20, and 30 mg of selenium per day (sodium selenite) for various time intervals. The results indicated that, when 15 mg of selenium was given to the animals, 23 to 36.9 percent of the daily dose was excreted in the urine. If the dose was increased to 30 mg of selenium, the urinary excretion in the survival groups decreased to 4.7 and 8.3 percent of the dose. These results are in accordance with the findings of the preceding report on rats with subacute toxic selenosis (2).

Cousins and Cairney (3) reported that after oral doses of 5 mg of selenite to sheep, 40 percent of the dose was excreted in the feces in 72 hours. Butler and Peterson (4) reported that when tracer doses (12 μg of selenium (H₂Se⁷⁵O₃) were introduced into the rumen of sheep, 51 percent was excreted in the feces over a 72-hour period. Peterson and Spedding (5) reported on the excretion of Se⁷⁵ by sheep that had been fed red clover containing Se⁷⁵ selenium (6.1 μg) which was predominantly incorporated in plant protein(s). They reported that the sheep eliminated 53.8 percent of the dose in the feces in 99 hours, while the urinary excretion was 1.78 and 2.29 percent, respectively. Buescher et al. (6) reported that the main pathway of selenium elimination in swine was the gastrointestinal tract.

These differences not only were due to the different species of animal used, but there were also differences in diets and mode of administration; in one study (5) there were differences in the selenium compounds administered.

The present studies were primarily initiated to investigate the biosynthesis of seleno-compounds by ruminants. Biosynthesis of selenocystine and selenomethionine in the rumen of sheep as indicated by the presence of Se⁷⁵-selenocystine and -methionine in wool (7) and the separation of selenotaurocholic acid from bile (8) have been reported in earlier publications.

The present report deals with the distribution of selenium in tissues of two animals (sheep) that received chronic toxic doses of Se⁷⁵-selenite in.
travenously and orally for various time intervals. Studies on the excretion of selenium in feces and urine and on retention in tissues after repeated administration of Se$^{75}$-selenite is presented.

During these studies it became apparent that the method used for selenium analysis showed considerable variation when different tissues or excreta were analyzed for their selenium content. These discrepancies in selenium recoveries are discussed.

**METHODS**

Two healthy wethers weighing 70 and 75 kg were used. The animal with biliary fistula was used to determine the selenium content of bile. It was injected with 0.2 mg Se + Se$^{75}$ (selenite)/kg of body weight intravenously for six alternate days. The experiment was terminated 48 hours after the final injection. Each injection contained 3 mc of Se$^{75}$.

The same amount of selenium was given orally for 60 days to the animal used for selenium-excretion studies. The animal received 4 mc of Se$^{75}$ three times a week during 46 days and then 4 mc of Se$^{75}$ daily for 10 days. The animal was killed four days after the final dose. During the experiment, the sheep was kept in a metabolism cage. Urine and feces were collected at 24-hour intervals until termination of the experiment. Se$^{75}$ content of urine and air-dried feces was determined at 24, 48, 72, and 96-hour intervals after administration of the isotope as indicated in the results. Total selenium determination in feces and urine was determined at selected time intervals.

The Se$^{75}$ content of the blood was checked at the beginning of the experiment and at intervals as indicated in the data.

At termination, various tissues (2 to 5 g) were homogenized with a glass-tissue homogenizer and total Se$^{75}$ was determined. The homogenized tissues were treated with 10 percent trichloroacetic acid (1:9) and protein precipitated. The precipitate contained the protein-bound Se$^{75}$, the supernatant contained the acid-soluble Se$^{75}$. The precipitated proteins were washed three times with 3 volumes of 5 percent TCA, followed by alcohol and ether; in this manner the tissue proteins were purified. Selenium present in the proteins was considered to be bound within the proteins. The acid-soluble proteins contained free Se$^{75}$ in unknown chemical combination(s).

Protein fractionation in one animal was carried out with 85 percent alcohol in addition to 10 percent TCA. Purification of tissue proteins was carried out in the same manner as described with trichloroacetic acid. The results by the two methods of protein separation are indicated in the data.

Se$^{75}$ content in various dilutions of urine, feces, and tissues was determined
in triplicate aliquots in a well-type Scintillation detector. Total selenium was determined according to the method of Klein described by Trelease and Beath (9). The excretion data of Se$^{75}$ are expressed as the percent of the dose eliminated in feces, urine, and bile. The total blood, plasma, and red-blood-cell volumes were calculated as follow: whole blood, 80 ml/kg of body weight; plasma, 45 ml/kg; and red-blood cell, 35 ml/kg. The Se$^{75}$ content of the various fractions of blood was expressed as the percent of injected dose retained in the blood and its components. Results of tissue Se$^{75}$ are expressed as percent retained in the tissues.

**RESULTS**

Elimination of Se$^{75}$ by sheep receiving subtoxic doses of selenium is indicated in Table 1. The results indicate that the maximum urinary excretion occurs at 24 hours, and in the feces and bile at 48 hours.

If the administration of selenium was repeated each day for five days, daily excretion of Se$^{75}$ in urine and feces increased, and high concentrations of selenium were present in the excreta up to 72 hours after final dose (Table 2).

**TABLE 1—Rate of Se$^{75}$ Excretion by Sheep**

<table>
<thead>
<tr>
<th>Total Se$^{75}$ administered</th>
<th>Interval after each dose</th>
<th>Percent of dose recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>95 mc*</td>
<td>0 - 24 hrs.</td>
<td>22.0 (24)</td>
</tr>
<tr>
<td></td>
<td>24 - 48 hrs.</td>
<td>11.9 (14)</td>
</tr>
<tr>
<td></td>
<td>48 - 72 hrs.</td>
<td>4.5 (10)</td>
</tr>
<tr>
<td></td>
<td>72 - 96 hrs.</td>
<td>2.5 (7)</td>
</tr>
<tr>
<td><strong>Total Se$^{75}$ excreted</strong></td>
<td>60 days</td>
<td>38.5</td>
</tr>
</tbody>
</table>

*Dose administered 4 mc 3 times/week. Duration of experiment, 60 days.

**TABLE 2—Excretion of Se$^{75}$ in Urine and Feces After Five Successive Daily Administrations of Se$^{75}$**

<table>
<thead>
<tr>
<th>Intervals after final dose</th>
<th>Percent of dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>24</td>
<td>41.9</td>
</tr>
<tr>
<td>48</td>
<td>22.0</td>
</tr>
<tr>
<td>72</td>
<td>7.7</td>
</tr>
<tr>
<td>96</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Percentage of the daily dose excreted after oral administration of selenium.
The data indicate that, when the selenium intake is increased by continued daily intake, the urinary and fecal excretions showed corresponding increase. In sheep, as in laboratory animals, the apparent main pathways of selenium elimination are the gastrointestinal and urinary tracts. When subtoxic doses of selenium are administered to rats, elimination of selenium by the urinary tract is almost twice that by the gastrointestinal tract; with subacute toxic doses the mode of elimination is reversed (2). In sheep with chronic selenosis, urinary elimination is only slightly higher than fecal excretion when selenium excretion is studied for an extended time.

Selenium content of whole blood and its components is indicated in Fig. 1 at 2, 46, and 53 days after beginning of the experiment. It is evident that, with continued intake of selenium, there is continued accumulation of selenium in the blood. The plasma contains higher concentrations of selenium than the red blood cells 24 hours after the selenium intake, but the selenium content of plasma decreases rapidly while the selenium content of red-blood cells increases and the rate of decrease from the red blood cells is slower than that from the plasma.

Distribution and binding of Se\(^{75}\) in tissues and tissue fractions after six intravenous injections in one animal and after 60 days of oral administration of selenium in another are indicated in Tables 3 and 4. All tissues, after intravenous injection, contain more selenium than after oral administration except the liver. In the liver, with continued intake of selenium, the accumulation is greater than in any other organ. Retention of Se\(^{75}\) was slightly higher in the kidney than in the liver of sheep that received six injections of selenium.

The most evident differences are found between the results of six-day intravenous and 60-day oral administration of selenium as to the amount of selenium bound with the proteins in the various tissues. In the six-day experiment over 80 percent of Se\(^{75}\) was bound to the proteins of whole blood and liver and lesser amounts in other tissues (Table 3). In the kidney, heart, skeletal muscles, and gastrointestinal tract, a high percentage of Se\(^{75}\) was present in the alcohol and acid-soluble fractions. If the animal had been sacrificed at 72 or 96 hours after final injection, the soluble Se\(^{75}\) would have decreased. However, these differences in the acid-soluble fractions of these tissues persisted, as indicated in Table 4.

The results of the animal sacrificed 96 hours after the final dose following 60 days of selenium administration indicated that most of the selenium present in the tissues was bound with proteins (Table 4). However, the heart, skeletal muscle, rumen, reticulum, abomasum, and kidney contained 21.0,
FIG. 1—Two days (●): percent of Se⁷⁵ retained in sheep blood in 24 hours after a single oral administration of Se⁷⁵ preceded by one dose of selenite (non-radioactive). Forty-six days—x: the animal received 4 mc of Se⁷⁵ three times per week for 6 weeks. Blood was taken 24, 48 and 72 hours after the last dose of Se⁷⁵. Fifty-three days—O: the blood sample differs from that of the 46 days in that the animal received an additional 4 mc of Se⁷⁵ per day for 5 days. Blood was taken 24, 48, 72 and 96 hours after the final dose of Se⁷⁵ was given.
### TABLE 3—Distribution and Binding of Se\textsuperscript{75} in Tissues and Tissue Fractions\textsuperscript{*}

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Percent of activity</th>
<th>Percent of Se\textsuperscript{75} present in TCA**</th>
<th>Alcohol***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>precipitate</td>
<td>soluble</td>
</tr>
<tr>
<td>Lung</td>
<td>8.3</td>
<td>78.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Heart</td>
<td>4.8</td>
<td>70.4</td>
<td>31.2</td>
</tr>
<tr>
<td>Liver</td>
<td>23.0</td>
<td>81.5</td>
<td>18.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>24.8</td>
<td>53.4</td>
<td>47.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>11.7</td>
<td>91.1</td>
<td>9.2</td>
</tr>
<tr>
<td>Abomasum</td>
<td>6.8</td>
<td>81.8</td>
<td>19.2</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>5.4</td>
<td>74.7</td>
<td>26.1</td>
</tr>
<tr>
<td>Blood</td>
<td>8.0</td>
<td>83.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.9</td>
<td>44.1</td>
<td>58.3</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cartilage</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 3 mc of Se\textsuperscript{75}/day was injected intravenously to sheep for 6 days. The animal was sacrificed 48 hours after the final injection.

** 10 percent trichloroacetic.

*** 85 percent alcohol.

### TABLE 4—Distribution and Binding of Se\textsuperscript{75} in Tissues and Tissue Fractions\textsuperscript{*}

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Se\textsuperscript{75} retained</th>
<th>Percent of Se\textsuperscript{75} in TCA**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein-bound</td>
</tr>
<tr>
<td>Whole blood</td>
<td>4.1</td>
<td>90.3</td>
</tr>
<tr>
<td>Heart</td>
<td>4.6</td>
<td>76.5</td>
</tr>
<tr>
<td>Lung</td>
<td>4.5</td>
<td>90.3</td>
</tr>
<tr>
<td>Liver</td>
<td>40.9</td>
<td>93.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>20.0</td>
<td>83.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.1</td>
<td>86.6</td>
</tr>
<tr>
<td>Adrenal</td>
<td>2.2</td>
<td>82.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.7</td>
<td>74.7</td>
</tr>
<tr>
<td>Tongue and trachea</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Epithelium (esophagus)</td>
<td>1.2</td>
<td>92.8</td>
</tr>
<tr>
<td>Rumen</td>
<td>1.7</td>
<td>68.4</td>
</tr>
<tr>
<td>Reticulum</td>
<td>1.2</td>
<td>73.0</td>
</tr>
<tr>
<td>Abomasum</td>
<td>1.8</td>
<td>84.8</td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.1</td>
<td>91.6</td>
</tr>
<tr>
<td>Skin (hair roots)</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

* Animal received Se\textsuperscript{75} orally for 60 days and was sacrificed 96 hours after the final dose of Se\textsuperscript{75} was given.

** The data on alcohol precipitation are not included since the results were similar to those with TCA separation.
25.5, 33, 26.9, 12.7, and 15.4 percent of selenium, respectively, in the acid-soluble fraction. Although high percentages of the retained Se\(^{75}\) were bound in these tissue proteins, high concentrations of Se\(^{75}\) were present in the acid-soluble fraction when compared with the acid-soluble fraction of the lung, liver, and blood. The data also suggest that the difference in distribution and accumulation of selenium in the tissues may be due to the degree of binding of selenium in the tissues. Soluble fractions of the tissues may have a greater degree of freedom for excretion or deposition in the tissue(s).

Purification of liver protein, until the nitrogen content of the protein was 16.0 percent and there was no further loss in radioactivity, indicated that 1 mg of protein contained 23 \(\mu\)g of methionine and \(8.3 \times 10^7\) c/m Se\(^{75}\). Although the amount of selenium that replaced sulfur in the liver protein was limited, these results indicate that the ruminant can and does substitute selenium compounds for sulfur compounds in the synthesis of proteins.

Tables 5 and 6 indicate the percentage of error in the method used for selenium analysis. The recovery of selenium seems to vary with the different tissues used. Nearly 100 percent recovery can be obtained from some tissues while in other tissues the recovery of selenium is very low.

### TABLE 5—Selenium (Se\(^{75}\)) Recovered in Distillate*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Percent of Se(^{75}) recovered in distillate</th>
<th>Se ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various parts of Gastrointestinal tract (6)</td>
<td>98.9 - 102</td>
<td>0.2</td>
</tr>
<tr>
<td>Kidney (2)</td>
<td>98.9 , 99.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Lung</td>
<td>75.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Liver (2)</td>
<td>98.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Heart</td>
<td>89.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Spleen</td>
<td>80.0</td>
<td>0.47</td>
</tr>
<tr>
<td>Muscle</td>
<td>75.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Wool</td>
<td>66.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Skin</td>
<td>63.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Brain (2)</td>
<td>57 , 70.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* From tissues of sheep (60-day experiment).

( ) indicates number of analyses.
In tissues, such as the lung or blood that probably contained selenium in a volatile form, the recovery is low. This is indicated in blood in Table 6, where the loss during oxidation and distillation was over 30 percent. The loss in feces probably was due to the long duration of heating necessary for oxidation. Almost 90 percent recovery was obtained in feces when the oxidation was carried out with a reflux condenser and the final oxidation was carried out with perchloric acid.

The presence of lipids and waxes (brain, skin, and wool) reduces recovery of selenium (Table 5). A considerable amount of selenium was enclosed in the distilled fat globules as well as in the residual waxes and lipids. These results indicate that higher selenium content of a number of tissues reported in the literature may be due to the suitability of the tissues for selenium analysis. Low selenium values may have been due to loss by volatilization and interference of substances present in tissues.

## DISCUSSION

The present study, which compares the continuous selenium elimination by the urinary and gastrointestinal tracts of sheep during long duration of selenium intake, is the only study at the present time on livestock. Results on rats are more numerous and were discussed by Rosenfeld and Beath (10) and in the preceding paper (Part IV) (2). The results on sheep indicate that urinary excretion of selenium is less than was reported in small animals, and fecal excretion is slightly higher. It also indicates that, if the animal receives selenium each day, there is corresponding increase in the urinary and fecal excretion of selenium up to 72 hours; however, at 96 hours the selenium excretion in urine and feces is about the same as that excreted by ani-
mals that received selenium at less frequent intervals, suggesting that after 72 hours the selenium that is eliminated reflects a constant rate of selenium metabolism "bound" to the tissues.

The results reported on fecal elimination of selenium by administration of 5 mg selenite by Cousins and Cairney (3) and the results obtained by administration of tracer doses (12 μg) of selenium by Butler and Peterson (4) when compared with our results indicate that in chronic selenosis, selenium is eliminated not only by the gastrointestinal tract but also by the urinary tract. These pathways of excretion of selenium play an important role in its metabolism. It is difficult to accept the statement made by Butler and Peterson (11) that the main route of elimination in sheep of ingested selenite or seleno-amino acids is by the gastrointestinal tract but may also by urinary tract. That statement may be valid when tracer doses of selenium are given or when selenium is bound to proteins in the forage consumed by the sheep (5).

On the basis of present results of urinary and fecal selenium excretion and of earlier reports on the urinary excretion of selenium by sheep (1), by swine (6), and by cattle (12), as well as the elimination of selenium by the respiratory tract by rats and dogs (10), it is fallacious to state that the main pathway of elimination in sheep is by the gastrointestinal tract. Further metabolic studies with various amounts of selenium administered to livestock would be of considerable value in clarifying these discrepancies among the different species of animals.

Although no studies on respiratory elimination of selenium by livestock were carried out, our experience with sheep and cattle that have ingested seleniferous vegetation or have received selenium orally or intravenously indicates that they exhale volatile selenium, which can be detected by the odor of the breath of the animal(s).

Retention and accumulation of selenium in tissues indicate that tissues differ in their ability to accumulate selenium and their affinity to bind selenium with proteins. After selenium injection or intake, the organs of excretion (kidney and gastrointestinal tract) and the muscles (cardiac and skeletal) contain large quantities of selenium in a soluble form (Table 3). Even in chronic selenosis, the above organs bind lesser amounts of selenium (between 75 and 80 percent, with proteins than with other tissues (Table 4). The liver, an organ that plays the most important part in protein synthesis, has the highest concentration of selenium bound within its proteins. Selenium is present in blood proteins, and the protein-bound selenium increases as administration of selenium is continued. This increase is due to the slower turnover rate of selenium in red blood cells and the binding of selenium in the blood proteins.
The substitution of selenium for sulfur in biosynthetic processes has been postulated by a number of investigators (10). Data have been summarized by McConnell (13) to indicate that this substitution does occur in monogastric animals. If inorganic selenium compounds are utilized in such biosynthetic processes for synthesis of seleno-amino acids in monogastric animals, this would indicate deviation in the metabolic utilization of selenium compared with inorganic sulfur compounds. There is no evidence to indicate that monogastric animals synthesize any sulfur amino acids from inorganic sulfur compounds. Selenium injected in monogastric animals is attached to the proteins (14, 15, 16), but it is doubtful that this is a true substitution of seleno-amino acids in the protein molecules.

Evidence of microbial biosynthesis in the rumen of sheep from inorganic sulfur compounds of sulfur amino acids has been presented (17); isolation of Se$_{75}$-selenocystine and -selenomethionine from wool protein (7) and of Se$_{75}$-seleno-taurocholic acid from the bile of sheep (8) has been reported. These results indicate that in microbial biosynthesis in the rumen, inorganic sulfur and selenium compounds are utilized for the synthesis of amino acids and other organic compounds. The purified liver proteins of sheep contained Se$_{75}$. Although the amino acids from the liver proteins have not been isolated up to the present, purification and separation of the protein indicates that the selenium compound(s) is present in the protein molecules.

In all the above studies, the isolated proteins contained large amounts of selenium; but with purification of the isolated organic complex, only a fraction of the original selenium was retained in the purified proteins and isolated amino acids. This would indicate that selenium is present in proteins in two forms: one is attached or “bound” in an unstable form on or to the protein molecule, while the other is present as a substituted seleno-amino acid in the protein molecules. By recognizing this unstable combination of selenium with proteins, the results obtained (13, 14, 15, 16,) on monogastric animals would offer some explanation of the presence of selenium in the tissue protein of these animals.

Both forms of selenium-protein binding are present in ruminants. In isolated proteins there is a limited substituted seleno-amino acid for sulfur amino acid. The biosynthesis of sulfur amino acids always exceeds that of seleno-amino acids in a living animal. This is due to the fact that the number of sulfur atoms available for synthesis always exceed those of selenium atoms, since the extreme toxicity of selenium limits the increase of selenium in the animal.

The inadequacy of the method for selenium analysis of biological as well
as other material has been recognized as indicated by the number of modifications suggested by various investigators (10).

Gorsuch (18) studied the effect of variations of oxidation conditions and oxidation mixtures on the recovery of selenium by the use of \( \text{Se}^{75} \). He reported that the oxidation mixture used for destruction of organic matter and the rate of oxidation had considerable influence on the recovery of selenium by distillation. Kelleher and Johnson (19) reported that the usual method of separating selenium from organic samples gave very low and erratic recoveries when tested at low levels. They reported favorable recoveries by the use of isotope-dilution techniques to compensate for the unavoidable loss that occurred during analysis.

Our results indicate that not only the selenium content but also the tissue oxidized will influence the recovery of selenium. The presence of lipids or waxes which interfere with selenium recovery must be considered in oxidation of selenium-containing tissues. The recovery of selenium in the blood, heart, spleen, and feces may be related directly to the form of selenium present in these organs and excreta. There is considerable evidence that blood contains a considerable amount of dimethyl selenide, which boils at 55° C. (10). \( \text{Se}^{75} \) can be used to check the accuracy of the method of determination.

**SUMMARY**

The metabolism of selenium was studied in sheep by short-term intravenous and long-term oral selenium \( (\text{Na}_2\text{Se}^{75}\text{O}_3 + \text{Na}_2\text{SeO}_3) \) administration.

Following intravenous administration of 3 mc of \( \text{Se}^{75} \), 5.5 percent of the injected dose was present in the bile in 24 hours and 8.4 percent in 48 hours.

Following oral administration of chronic doses of selenium tri-weekly for 46 days and then daily for 10 days, 38.5 percent of the dose of \( \text{Se}^{75} \) was excreted in the urine and 30.9 percent in the feces. The maximum urinary excretion occurred at 24 hours, while the maximum fecal excretion occurred at 48 hours after \( \text{Se}^{75} \) was given. When the administration of selenium was repeated daily for 10 days, there was considerable increase of selenium excretion in urine and feces at 24, 48, and 72 hours. At 96 hours the urine and feces contained almost the same amount of \( \text{Se}^{75} \) as when the administration was given at less frequent intervals.

The tissues contained more \( \text{Se}^{75} \) when the selenium was given intravenously for six days and the animal was killed 48 hours after the final dose, than when the \( \text{Se}^{75} \) was given for 60 days and the animal was sacrificed 96 hours after the final dose.
A higher percentage of Se\textsuperscript{75} was retained by tissues in the soluble proteins after intravenous administration, while a lower percentage of Se\textsuperscript{75} was present in the soluble proteins of tissues of sheep that received selenium orally for 60 days.

In both groups, proteins of the gastrointestinal tract and kidney, and the skeletal and cardiac muscles, contained less bound Se\textsuperscript{75} than the other tissues. The maximum protein-bound Se\textsuperscript{75} was present in the liver and the blood.

Data presented indicate that the present method used for selenium determination introduces a considerable margin of error in some tissues. The loss of selenium and the accuracy of the method can be checked by the use of Se\textsuperscript{75}.

**REFERENCES**

2. Rosenfeld, I., Part IV this bulletin, 1964.

_Agricultural Experiment Station_

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_Laramie_

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