BIOLOGICAL HANDLING OF ^{75}Se-DISELENODIBUTYRIC ACID

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The need for gamma-labeled nutrients to trace absorption, distribution and excretion during life has stimulated our interest in lipids which contain radioselenium. More specifically, we have begun to study monoselenofatty acids. An intermediate in the synthesis of a number of these substances is diselenodibutyric acid (which contains a diselenide bond). Since the biological handling of diselenide compounds has not been adequately documented, we have studied the metabolism of diselenodibutyric acid, and preliminary results are presented in this communication.

SYNTHESIS AND PHYSICAL PROPERTIES

Diselenodibutyric acid has been described in the literature (*1*) and can be synthesized by a number of procedures. Because of the availability of particular starting materials, we chose a two-step synthesis using ω-bromobutyric acid ethyl ester.

$Br\ (CH_2)_3\ COO\ C_2H_5$
ω-bromobutyric acid ethyl ester

$\xrightarrow{K_2SeSO_3}$ $Se\ (CH_2)_3\ COO\ C_2H_5$
$Se\ (CH_2)_3\ COO\ C_2H_5$
diselenodibutyric acid ethyl ester

$\xrightarrow[\text{Saponification}]{KOH}$ $Se\ (CH_2)_3\ COOH$
$Se\ (CH_2)_3\ COOH$
diselenodibutyric acid

The material was a yellow solid that melted at 87°C. Ascending chromatography, using Whatman No. 1 paper and butanol:acetic acid:water (4:1:1 by volume), revealed a single spot at R_f 0.91; the spot was located by ultraviolet absorption, by its reaction with bromcresol green and by the detection of radioactivity when labeled material was used.

The radiolabeled substance was synthesized using $K_2{}^{75}SeSO_3$. The first production had a specific activity of 41 μCi/mg compound or 88 μCi/mg Se. The appearance, melting point and chromatographic behavior were identical to nonlabeled diselenodibutyric acid.

MATERIALS AND METHODS

The ^{75}Se-diselenodibutyric acid was studied for possible transport against a concentration gradient. The compound was dissolved ($10^{-5}M$) in pH 7.4 Krebs-bicarbonate buffer and placed at the same concentration on both sides of everted intestinal sacs (mice, rats and hamsters) which were prepared by the method of Wilson and Wiseman (*2*). The outside volume was 5 ml and the inside volume was 1 ml.

Incubation was carried out at 37°C for 1 hr. At the end of incubation, the inner and outer fluids were centrifuged, counted and compared with standards. The gut sacs were blotted and weighed. From the disappearance of radioactivity, entry into the tissues was determined. Radioactivity per unit weight of tissue could then be calculated. The inner and outer fluids as well as standards were chromatographed.

Adult dogs were anesthetized with sodium pentobarbital, and the bladder catheterized. ^{75}Se-diselenodibutyric acid was administered intravenously (40–200 μCi) and frequent blood samples were drawn. The dogs were placed under a Picker Dynapix scanner, and multiple scans were made over the first 2 hr to trace the distribution of the radiolabel. Periodically urine was collected, counted and compared with an aliquot of the standard. Other aliquots were chromatographed in butanol:acetic acid:water (4:1:1 by volume). Experiments were performed in other dogs prepared similarly to those above, in which an oral tube was introduced into the stomach or duodenum. The ^{75}Se-diselenodibutyric acid was administered through the tube into the stomach or upper small intestine, and blood samples obtained. In one experiment, the gastroduodenal junction was ligated so that radiolabeled compound in the stomach could not pass into the small intestine.

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Seven days after intravenous injection in one dog, the animal was sacrified, the organs weighed and aliquots removed for counting.

Other dogs were counted at a distance of 4 ft from the uncollimated surface of the Dynapix scanner and compared with standards to assess approximately the whole-body retention of ^{75}Se.

In addition to counting, whole-blood samples were separated into red cells and plasma by centrifugation and saline washing. The separated samples were counted individually. Plasma proteins were precipitated by adding one-third volume of 30% trichloracetic acid, followed by centrifugation and washing with saline. The precipitate was then shaken with approximately 10 times its bulk of a saturated solution of $NaHSO_3$. After centrifugation, the supernatant and precipitate were counted separately. Such experiments were repeated with rats 1 hr after introduction of 10 μCi of ^{75}Se-diselenodibutyric acid into the stomach and 7 days later.

RESULTS

When ^{75}Se-diselenodibutyric acid was initially present at the same concentration in the fluids bathing the mucosal and serosal sides of everted intestinal sacs (mice, rats, hamsters), there was no transport against a concentration gradient. However, significant radioactivity disappeared from the bathing fluids and could be detected in the gut wall after washing with saline. The final ^{75}Se ratio, tissue-to-bathing solution, was over 4:1. Therefore the compound or its products accumulated in the gut wall.

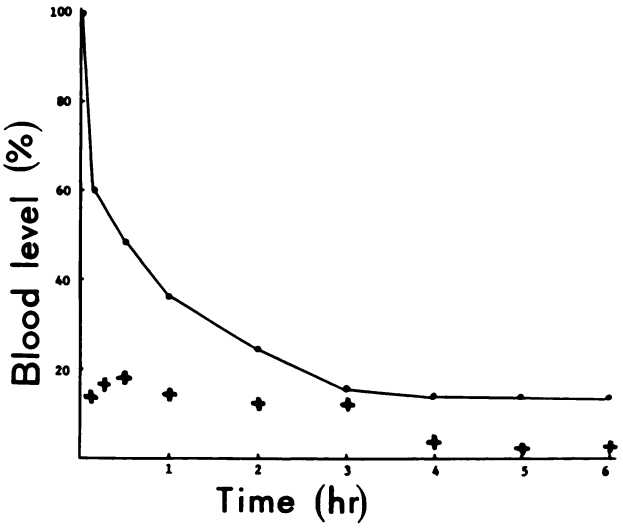

FIG. 1. Blood level of radioactivity in dog given about 40 μCi of ^{75}Se-diselenodibutyric acid through gastric tube (crosses). One week later, after drawing a blood sample to correct for small residual radioactivity, compound was given i.v. (points and line).

TABLE 1. DISTRIBUTION OF ^{75}Se IN ORGANS 1 WEEK AFTER THE INTRAVENOUS INJECTION OF 40 μCi ^{75}Se-DISELENODIBUTYRIC ACID IN ADULT DOG*

	Counts per organ		Counts per gram
Liver	290×10^4	Left kidney	268×10^3
Left kidney	76×10^4	Right kidney	242×10^3
Right kidney	67×10^4	Liver	76×10^3
Spleen	62×10^4	Lung, spleen,	
Pancreas	55×10^4	jejunum	$25–37 \times 10^3$

* Histologic examination revealed no gross defects in liver, kidneys or urinary bladder.

In the dog immediately after intravenous injection of ^{75}Se-diselenodibutyric acid Dynapix scans revealed a wide distribution of the radiolabel. Within 10 min, as the radioactivity in the blood fell, distinct localization in the kidneys and liver could be observed. Radioactivity in the urine appeared within minutes. Chromatography of urine revealed that the major peak of radioactivity correspond to the injected compound.

Rapid disappearance of radiolabel from the bloodstream of the dog occurred after intravenous injection of ^{75}Se-diselenodibutyric acid (Fig. 1). Disappearance of half of the compound injected into the blood occurred in about 30 min; the entire curve was more complex than a single exponential expression. After the first 6 hr, the remaining radioactivity in the blood decreased to half of its value in about 6 days. When ^{75}Se-diselenodibutyric acid was administered by tube into the stomach or duodenum, rapid entry into the bloodstream (and excretion into the urine) was noted (Fig. 1). This rapid absorption into the blood was almost solely a function of duodenal activity since it was nearly abolished in the dog when ^{75}Se-diselenodibutyric acid was introduced into the stomach after gastroduodenal ligation.

In two dogs, a mean of 50% of the administered radioactivity appeared in the urine by the end of the first day. In a dog sacrificed on the seventh day, most radioactivity was present in the liver and kidneys although lesser activity was present in nearly all organs (Table 1).

Ninety percent of the radioactivity present in the bloodstream 1 hr after intragastric introduction of ^{75}Se-diselenodibutyric acid into the dog or rat was in the plasma. Of the plasma radioactivity, 80% precipitated when trichloracetic acid was added. After the precipitate was washed with saline, most (>80%) of its residual radioactivity became soluble when it was shaken with a saturated solution of

$NaHSO_3$ (but not with saline). However, blood samples drawn from the rat or dog 3–7 days later revealed radioactivity in precipitated plasma proteins that largely did not become solubilized when shaken in an $NaHSO_3$ solution.

DISCUSSION

Prior to these experiments with ^{75}Se-diselenodibutyric acid, we tried to predict the biological handling of the compound. The three predictions were as follows:

1. Since the first preparation had a low specific activity (41 μCi/mg compound), toxicity might occur from the selenium.

2. The compound, being a dicarboxylic acid, would enter the liver and perhaps fat depots.

3. The diselenide bond (—Se—Se—) might exchange with —S—S—, —SH or other linkages.

The first prediction turned out to be incorrect. In the doses used in rats and dogs, there was no toxicity apparent during the week of close observation. We do not propose human studies, however, until a specific activity of at least 100-fold greater is achieved. The reason for the lack of toxicity with the low-specific-activity compound may be related in part to the rapid urinary excretion and in part to the formation of nontoxic linkages within the body.

The second prediction, of the sites of localization, was only partly correct. There was no significant accumulation in fat depots. Hepatic uptake occurred as expected while the rapid renal uptake and excretion of ^{75}Se-diselenodibutyric acid was not expected. Entry of this compound into the liver and kidneys somewhat mimics the distribution of chlormerodrin labeled with radiomercury. ^{75}Se could be found in nearly all tissues following administration of ^{75}Se-diselenodibutyric acid; in addition, the radiolabeled compound entered (or bound to) intestinal sacs *in vitro*. However, at a concentration of $10^{-5}M$ there was no net transport across the sacs when the concentration was initially the same on both sides.

Following administration of ^{75}Se-diselenodibutyric acid into the small intestine, radioactivity appeared quite rapidly in the bloodstream. The majority of the radioactivity was precipitated with the plasma proteins, but it resolubilized when it was shaken with sodium bisulfite (*3*). This "sulfitolysis" suggests that linkages such as —Se—S— were broken. Hence, the third prediction, of the interaction of the diselenide linkage with —S—S— or other moieties, is likely correct. Additional data are required about the specificity of this event.

In addition to the interest in ^{75}Se-diselenodibutyric acid as a diselenide with intriguing biological properties, the compound will be of use as an intermediate in the synthesis of monoselenofatty acids.

SUMMARY

Radiolabeled ^{75}Se-diselenodibutyric acid was synthesized and compared with the nonlabeled compound. It is rapidly absorbed from the small gut of the dog and rodents *in vivo*. However, the compound does not undergo net transport across the gut wall *in vitro* when the concentration is initially the same on both sides. There is an accumulation within the gut wall. When given intravenously, radiolabel rapidly appears in the urine (about one-half is excreted in 1 day in the dog, and approximately 90% is eliminated by 1 week). In addition to renal localization, the compound shows considerable hepatic uptake; these findings partially resemble the distribution of chlormerodrin and suggest binding to sulfhydryl groups. Initial plasma radioactivity is largely precipitated by trichloracetic acid, indicating protein binding. However, a major portion of the initial protein-bound radiolabel is freed by treatment with sodium bisulfite, suggesting that much of the binding was of the form —Se—S—. After several days, remaining plasma radioactivity that precipitated with trichloracetic acid did not solubilize when shaken with sodium bisulfite. ^{75}Se-diselenodibutyric acid, in addition to its possible use in following renal and hepatic binding and possible sulfide-selenide interchange, forms the starting point for the synthesis of a series of monoselenofatty acids.

ACKNOWLEDGMENT

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