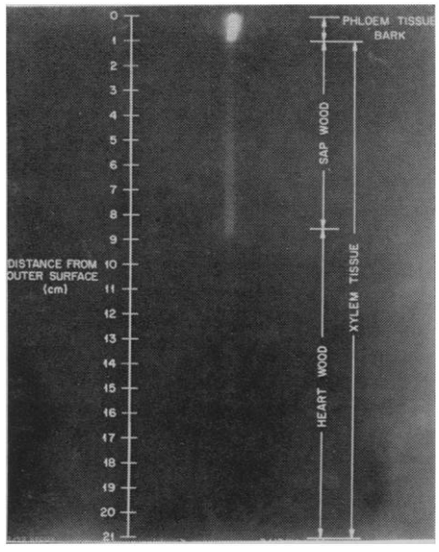

Fig. 2. Autoradiogram of core from a *Liriodendron* tree labeled with Cs^{137}. The core was taken horizontally, towards the center of the trunk.

raphy ranged from 0.65 to 0.75 with the 71 percent phenol solvent, and 0.60 to 0.70 with the butanol-acetic acid solvent. Ionic Cs^{137} yielded an R_F of 0.75 with 71 percent phenol and 0.68 with butanol-acetic acid. Only one radioactive spot was found per paper chromatogram during sap fractionation. Since the R_F values of these spots were always in the range of the R_F values for ionic Cs^{137}, and because only one active spot was found for each sap extract, the Cs^{137} in the sap appears to exist primarily in the free ionic form.

The oven-dried wood was ground to a powder with a Wiley mill. This powder was placed in glass tubes (1 cm in diameter) to form columns 15 cm long. Water was washed through the columns and collected in 25-ml fractions until a negligible amount of Cs^{137} was eluted from the wood columns as determined by counting the activity of the water fractions. Various concentrations of HCl then were washed through the columns and collected in 25-ml fractions to remove the remaining Cs^{137}; 1*N* HCl was found to be an optimum concentration for elution of Cs^{137}.

Water removed 83 to 89 percent of the total Cs^{137} remaining in the ground wood after sap removal, while 1*N* HCl was required to remove most of the residual quantity (Fig. 1). According to Noller (*5*), monovalent cationic salts of carboxylic acids are not appreciably hydrolyzed by water, while the carboxylic groups are displaced from their salts by mineral acids (HCl, for example). When the *p*H of the solvent washed through the columns dropped to *p*H 1, 70 to 80 percent of the Cs^{137} remaining after water elution was released in the first three fractions (Fig. 1).

Other columns of equal size were made with nonlabeled ground wood of various hardwood and softwood species. Twenty-five milliliters of Cs^{137} in solution with water (*p*H 5.0) (0.021 μc/ml) were washed through each column and the eluate collected at the base. All of the Cs^{137} added to these nonlabeled columns was retained by the wood.

These data suggest an ionic bonding between at least some of the Cs^{137} and any organic groups carrying a negative charge (such as carboxylic groups) and available in natural plant compounds. According to Bonner (*6*), plant compounds such as proteins, pectinic substances, polyuronide hemicelluloses, organic acids, and many others fulfill these requirements under certain conditions of *p*H.

Cores, taken horizontally and towards the center of the trunk, were obtained from trees labeled with Cs^{137}, and gross autoradiograms were made to determine the distribution of activity with respect to phloem versus xylem tissue and heartwood versus sapwood.

These autoradiograms showed that the bulk of the radioactivity is in the phloem tissue, even though some activity appears throughout the xylem tissue (Fig. 2). Also, since the Cs^{137} in the extracted sap accounted for such a small percentage of the total Cs^{137}, the bulk of the remaining activity appears to have been in the phloem, either in the sap of the sieve tubes, in the parenchyma, or in other living cells.

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Selenoamino Acids: Decrease of Radiation Damage to Amino Acids and Proteins

Abstract. *Selenomethionine and selenocystine protect amino acid and protein systems from radiation damage. Selenoamino acids are more powerful protectors than the analogous sulfur amino acids and other known —SH protectors. Free-radical scavenger and repair mechanisms by which selenoamino acids react with induced free radicals may be the key reactions in the biological function of selenium.*

The role of sulfhydryl compounds in reducing radiation damage has been extensively studied. These compounds are known to function by mechanisms (*1*) including, free radical scavenger effects, repair of damage sites, and capacity to form mixed disulfides. Electron paramagnetic resonance studies on solid amino acids, peptides, and proteins indicate that radiation-induced unpaired electrons finally localize on sulfur atoms (*2–5*). Gordy and Miyagawa (*2*) suggested that unpaired electrons can migrate through certain segments of polypeptide chains of proteins. Furthermore, Henriksen *et al.* (*5*) showed an intermolecular transfer of unpaired electrons from protein molecules to sulfur protectors. Collectively, this evidence suggests that the ideal radiation protector is a molecule which can release and accept electrons and hydrogen atoms easily without itself becoming dissociated. If this is the case, a selenoamino acid would be a better protector than the analogous sulfur amino acids because the ionization potential and bond energy of selenium compounds are smaller than that of sulfur compounds (*6*), and also because selenium is more metallic and has unique oxidation-reduction properties (*7*).

The biological function of selenium as a trace nutrient can be ascribed to

Table 1. Decrease of radiation damage to amino acids by selenomethionine.

Amino acid	$D½$ in 10^6 rad		
	+ None	+ Methionine	+ Selenomethionine
Alanine	8.0	45 (2.3)*	59 (2.9)
Leucine	7.6	22 (2.6)	24 (3.9)
Arginine	3.6	21 (2.0)	25 (4.9)
Serine	3.6	16 (1.8)	25 (5.0)
Histidine	3.5	3.7 (3.0)	14 (6.7)
Phenylalanine	3.3	3.0 (3.0)	6.6 (5.3)

* Figures in parentheses indicate $D½$ of protector.

the unique oxidation-reduction properties of compounds like the selenoamino acids and selenoproteins (*8*). Lipid antioxidant properties of selenoamino acids and proteins have been described (*8, 9*). Other mechanisms, especially the free radical scavenger effects and repair to damage sites, are probably of great importance in its biological function and are evaluated in this research.

The experiments described here are designed to test the effects and mechanisms of selenoamino acids, by comparison with analogous sulfur amino acids, in reducing radiation damage in chemical systems consisting of aqueous amino acids and proteins. As a simple test system, binary amino acid solutions (0.01*M* amino acid + 0.01*M* methionine or selenomethionine) were chosen and the solution was adjusted to *p*H 7 by addition of HCl or NaOH. After γ-radiation by Co^{60} over a wide dose range (up to 10^7 rad) at 28°C under anaerobic conditions, the amino acids were analyzed by quantitative paper chromatography (*10*). Controls of single amino acid solutions (0.02*M*, *p*H 7) were radiated at the same time. From graphs of the log of the amino acid retention plotted against the radiation dose, the doses that destroy 50 percent of the initial amino acids in the systems (half destruction dose, $D_{1/2}$) were obtained. Half destruction doses are summarized in Table 1.

Methionine offers almost no protection to the amino acids, phenylalanine and histidine, which are easily destroyed by radiation, but it does offer considerable protection to the amino acids, relatively resistant to radiation. In contrast, selenomethionine protects both the labile and stable amino acids against radiation as shown by the higher $D_{1/2}$ values. Furthermore the $D_{1/2}$ for selenomethionine compared to the $D_{1/2}$ for methionine shows that selenomethionine is more stable. The high resistance to radiation damage of selenomethionine when it is radiated alone is also interesting. The $D_{1/2}$ value of 0.01*M* and 0.02*M* selenomethionine solutions are 3.6×10^6 and 5.6×10^6 rad, whereas those of 0.01*M* and 0.02*M* methionine are 1.4×10^6 and 3.6×10^6 rad.

Protection by methionine and selenomethionine can be attributed mainly to the free radical scavenger and the repair mechanisms. The first case involves the competitive reaction between the protector and the test amino acid for the free radicals from water. These radicals, • OH, • H, and • OOH, react with the protector more readily than with the test amino acid, probably because of the greater stability of protector-radical intermediates.

The second case concerns reactions after the test amino acid becomes a radical by loss of a hydrogen atom or an electron. Repair mechanisms would include irreversible transfer of a hydrogen atom or an electron from the protector to the test amino acid radical forming a protector radical as the original test amino acid molecule forms again. Most probably, the induced protector radicals through the scavenger and repair mechanisms degrade into stable products, but it is also possible that they react again with a free electron or a free hydrogen atom to form the original protector molecules.

The possibility that these protection reactions will take place is dependent entirely upon the thermodynamic quantities of the free radical reactions; in this case, protection mechanisms may apply only to the test amino acids whose radical intermediates are labile compared to those of the protector. However, if the free radical intermediates of the test amino acid and the protector have similar stability, presumably thermodynamic quantities for both reactions are quite similar, so that $D_{1/2}$ of the test amino acid is almost equal to that of the protector, whereupon protection is not found. In this case, free radicals from water react evenly with the test amino acid and the protector; also the reverse reactions such as the transfer of a hydrogen atom or an electron from the test amino acid molecule to the protector radical as well as the transfer from the protector molecule to the test amino acid radical are favorable. This is best shown in the case of histidine and phenylalanine. The higher $D_{1/2}$ for selenomethionine must be ascribed to catalytic self-repair mechanisms. Probably the radical intermediates of selenomethionine are very stable and readily react with a hydrogen atom or an electron, thereby returning to the original molecule.

Table 2. Decrease of radiation damage to enzyme by selenoamino acids.

Protector	$D½$ (10^5 rad)	
	Alcohol dehydrogenase	Ribonuclease
None	0.3	1.0
Methionine	0.5 (1.8)*	2.9 (1.8)
Selenomethionine	2.6 (4.5)	9.7 (4.9)
Cysteine	2.1 (3.2)	14.7 (3.4)
AET	0.6	5.6
MEA	1.6	10.3
Cystine†	0.6	2.6
Selenocystine†	1.9	4.9

* Figures in parentheses indicate $D½$ of protector. † 3×10^{-4} molar.

In enzyme test systems, neutral solutions of yeast alcohol dehydrogenase (ADH) and ribonuclease (0.1 percent protein by weight + 0.001*M* protector) were radiated at 22°C as previously described. As a comparison, several known sulfhydryl protectors, L-cysteine, 2 - mercaptoethylamine - hydrochloride (MEA), and 2-aminoethylisothiuronium bromide-hydrobromide (AET) were also tested. Enzymatic activities were assayed spectrophotometrically (*11*) and destruction of some protectors was determined by quantitative paper chromatography.

Half-destruction doses of the enzymes and protectors are summarized in Table 2. The selenoamino acids, selenomethionine and selenocystine, are much better protectors than the analogous sulfur amino acids. Comparison with methionine indicates that selenomethionine has 5.2 and 3.3 times the protective activity, and selenocystine has 3.2 and 1.9 times the activity of cystine for ADH and ribonuclease, respectively. Differences in the protective effects depend on the lability of the enzymes. When subjected to radiation, the dehydrogenase (ADH) is 3.3 times more labile than ribonuclease; therefore, scavenger and repair effects by methionine are less effective in the ADH system than in the ribonuclease system. Nevertheless, for ADH, selenomethionine gives better protection than cysteine

which is known to be one of the best protectors.

The evidence presented here indicates that selenoamino acids are more powerful protectors against radiation than analogous sulfur amino acids and other known sulfhydryl protectors. Furthermore, these powerful protector effects of selenoamino acids in reducing free radical damage through free radical scavenger and repair mechanisms may be the key reactions in the biological function of selenium.

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Oxidation of Carbon-14-Labeled Endogenous Lipids by Isolated Perfused Rat Heart

Abstract. *Lipids of the rat heart were labeled with carbon-14 in vivo. The production of $C^{14}O_2$ during the subsequent perfusion of these glycogen-depleted hearts with buffer, but without added substrate, provided direct evidence of the oxidation of endogenous heart lipids. The net decrease in phospholipid content alone could account for 75 percent of the total metabolic CO_2 formed.*

The isolated rat heart perfused with bicarbonate buffer containing C^{14}-labeled substrates is particularly useful for studying intermediary metabolism of heart muscle (*1*). Several observations in this laboratory (*2*) have suggested that endogenous noncarbohydrate moieties were an important source of two-carbon units for oxidation. First, hearts depleted of glycogen continued to beat and actively form CO_2 when perfused with buffer without added substrate. Second, the $C^{14}O_2$ produced by glycogen-depleted hearts perfused with buffer containing 5 m*M* glucose-U-C^{14} or 0.5 m*M* palmitate-1-C^{14} accounted for less than 50 percent of total respiratory CO_2. The results of this study demonstrate the oxidation of endogenous lipids by hearts perfused with buffer without added substrate. The observations suggest that fatty acids derived from phospholipids, and to a lesser extent triglyceride, were the major source of carbon for oxidation.

Fed, male albino rats, weighing between 200 and 250 g, were anesthetized with ether. After a midline incision, the superior mesenteric artery and the hepatic portal system were ligated according to the method of Borgstrom and Olivecrona (*3*); this functional hepatectomy produced a twofold increase in the uptake of C^{14}-labeled fatty acids by heart muscle. Then 0.5 ml of rat serum, containing 0.36 μc of Na-palmitate-1-C^{14}, was injected into the inferior vena cava. The rats were kept lightly anesthetized for 30 minutes and then killed by decapitation. All hearts were perfused at 37°C in an open system with modified (*1*) Krebs-Henseleit bicarbonate buffer, with no added substrate, for 10 minutes to wash out any radioactivity present in the extracellular fluid. A stream of gas, consisting of 95 percent O_2 and 5 percent CO_2, was bubbled through the perfusate. After removal of adipose and connective tissues, the ventricles of four hearts were placed in four tubes containing 10 ml of $CHCl_3$: CH_3OH (2 : 1, vol/vol). The ventricles of three other hearts were digested in 30 percent KOH for the determination of glycogen content. Another group of four hearts was perfused for 60 minutes at 37°C with buffer without added substrate in a closed, recirculated system (*1*) previously equilibrated with 95 percent O_2 : 5 percent CO_2. After 60 minutes, the CO_2 in the gas phase of the closed system was trapped in the liquid phase by adding 2 ml of 5*N* NaOH to the perfusate and circulating by way of a heart bypass for 10 minutes. The final perfusate was collected under mineral oil, and the ventricles were placed in the mixture of $CHCl_3$ and CH_3OH. The wet weight of the ventricles ranged from 624 to 875 mg.

Hearts that had been placed in the mixture of $CHCl_3$ and CH_3OH were minced and homogenized. Lipids were extracted from the homogenate by the method of Folch *et al.* (*4*). Samples of the lipid extract were fractionated by means of thin-layer chromatography using Silica Gel G (*5*) as the adsorbent, and a mixture of petroleum ether, diethyl ether, and acetic acid (90 : 10 : 1) as the eluant. Fractions were located by means of iodine vapor and identified by means of reference standards. The fractions identified were phospholipids, cholesterol, free fatty acids, triglycerides, and a fraction at the solvent front, which contains cholesterol esters.

After allowing the iodine color to fade overnight, the fractions, together with the adsorbent, were scraped from the glass plate into a liquid scintillation vial containing 2 ml of ethanol, which served to elute the lipids. Ten milliliters of toluene, containing 4.0 g of PPO (2,5 diphenyloxazole) and 100 mg of dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene] per liter, were added. Samples were counted in a liquid scintillation spectrometer for 10 minutes. Net counts in samples containing triglyceride were more than 3500, and those containing phospholipid were more than 800 per 10 minutes; in other fractions there were 150 to 430 counts per 10 minutes. Background count was 120 per 10 minutes. Counts were corrected to disintegrations per minute by means of a toluene-C^{14} internal standard.

Phospholipids in the lipid extract were determined by the method of Sperry (*6*), cholesterol by the method of Forbes (*7*), triglycerides by the method of Van Handel (*8*), and free fatty acids by the method of Dole (*9*). The glycogen content of the KOH-