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-

treated heart digest was determined by the method of Good *et al.* (*10*).

The metabolic CO_2 was determined as the difference between the CO_2 content of the system after perfusion, and the previously determined CO_2 content of the system at the start of perfusion. The CO_2 content in both cases was determined from the volume of alkaline perfusate and the CO_2 content of an aliquot measured manometrically (Van Slyke). Portions of the final alkaline perfusate were added to liquid scintillation vials containing 10 ml of dioxane gel (*11*); separate samples of the final perfusate were acidified to *p*H 1, warmed, and shaken before the addition of dioxane gel. The samples were counted and the counts converted to disintegrations per minute. The $C^{14}O_2$ was calculated from the difference in counts of the basic and acidified samples.

Table 1. The radioactivity in the various lipid fractions of the heart. Results are expressed as total disintegrations per minute per gram wet weight of heart ventricle in each fraction. Means ± 1 standard error are shown. Recovery of DPM was the sum of DPM in the respective lipid fractions. The fraction which moved most rapidly (solvent front) consisted mainly of cholesterol esters.

	Phospho-lipids	Cholesterol	Free fatty acids	Triglyceride	Solvent front	Recovery
			Four hearts, perfused for 10 minutes only			
	26,800	5,220	11,600	155,000	4,860	204,000
	20,600	6,660	8,830	175,000	6,630	218,000
	36,900	6,120	11,700	200,000	6,690	261,000
	18,400	13,700	10,600	177,000	9,650	229,000
Mean	25,700	7,920	10,700	177,000	6,960	228,000
S.E.	4,480	1,940	674	9,110	991	12,200
			Four hearts perfused for an additional 60 minutes			
	31,900	4,740	10,600	130,000	817	179,000
	34,800	3,940	7,070	151,000	2,450	200,000
	25,500	2,270	2,790	132,000	1,500	166,000
	25,400	1,620	1,790	87,300	777	117,000
Mean	29,400	3,140	6,070	125,000	1,390	165,000
S.E.	2,370	723	1,870	13,500	392	17,500

Table 2. The radioactivity in the various lipid fractions of the heart. Results are expressed as the means of the percentage of the total counts of the heart lipid in each fraction, ± standard error. The percentage recovery of total disintegrations per minute was calculated by comparing the total counts scraped from the chromatoplate to the counts in a sample of the lipid extract pipetted directly into a scintillation vial.

Phospho-lipids	Cholesterol	Free fatty acids	Triglyceride	Solvent front	Recovery
		Perfused for 10 minutes only			
11.2 ± 1.5	3.5 ± 0.8	4.7 ± 0.4	77.5 ± 1.0	3.0 ± 0.4	98.1 ± 2.5
		Perfused for an additional 60 minutes			
18.1 ± 1.3	1.8 ± 0.3	3.5 ± 0.9	75.8 ± 1.4	0.8 ± 0.2	94.7 ± 4.4

Table 3. The quantity of each lipid fraction from perfused rat hearts. Units are expressed per gram wet weight of ventricle.

	Phospholipids (μmole)	Cholesterol (μmole)	Free fatty acids (μequiv.)	Triglycerides (μmole)	Total lipids (mg)
		Perfused for 10 minutes only			
	30.4	2.77	13.08	2.20	30.3
	30.5	3.04	13.44	2.84	32.4
	33.2	2.98	12.89	2.16	32.8
	30.6	2.87	11.33	3.09	31.8
Mean	31.2	2.91	12.68	2.57	31.8
S.E.	0.7	0.06	0.47	0.23	0.5
		Perfused for an additional 60 minutes			
	27.7	3.40	11.62	2.08	32.2
	29.6	3.36	12.41	1.97	27.8
	27.5	3.03	12.17	2.13	26.9
	22.0	3.10	10.19	2.32	28.0
Mean	26.7	3.22	11.60	2.12	28.7
S.E.	1.6	0.09	0.50	0.07	1.2

Table 4. The specific activity (disintegrations per minute per μmole or per μequivalent) of each lipid fraction from perfused rat hearts.

Phospholipids	Cholesterol	Free fatty acids	Triglycerides
	Perfused for 10 minutes only		
818 ± 115	2720 ± 684	849 ± 65	70400 ± 7820
	Perfused for an additional 60 minutes		
1102 ± 59	960 ± 198	514 ± 157	59700 ± 8120

Table 1 shows that 228,000 ± 12,200 disintegrations per minute, or 21.8 ± 1.5 percent of the injected palmitate-1-C^{14} was recovered in heart lipids. Results in Table 2 show that 77.5 ± 1.0 percent of the total disintegrations per minute in heart lipids was present as triglyceride and 11.2 ± 1.5 percent as phospholipid. Hearts after perfusion contained 2.6 ± 1.1 μmole of glycogen per gram of wet weight of tissue.

A comparison of the results required the assumption that the hearts perfused for the additional 60 minutes at the beginning of perfusion were comparable to those that were perfused for 10 minutes only. Support for this assumption was the close agreement of the total disintegrations per minute per gram wet weight of heart in those perfused for 10 minutes only (Table 2). During the 60-minute perfusion period 193 ± 29 μmole of CO_2 per gram wet weight was formed. The $C^{14}O_2$, with 44,918 ± 2780 disintegrations per minute, accounted for 72 percent of the decrease in heart lipid C^{14} during perfusion. The production of $C^{14}O_2$ and diminution of total lipid radioactivity was direct evidence of the oxidation of carbon derived from endogenous heart lipids.

It is of interest to consider the metabolic CO_2 produced in relation to the net changes in lipid content of hearts (Tables 3 and 4). The glycogen content after the 10-minute perfusion period, as a potential source of CO_2, was negligible. If it is assumed that each mole of phospholipid contained two fatty acid moieties having an average chain length of 16 carbon atoms, and that the fatty acids were completely oxidized to CO_2, the net decrease in phospholipid could have accounted for 144 μmole of CO_2, or 75 percent of the total CO_2.

It can be seen that the specific activity of phospholipid increased (Table 4), as did the percentage of label in the phospholipid fraction (Table 2); the specific activity of triglycerides tended to decrease. These changes are consistent with the hypothesis that fatty acids are extracted by the heart cells and initially incorporated into triglyceride, primarily within the cytoplasm;

subsequently, fatty acids are transferred to phospholipid, primarily within mitochondria, prior to oxidation by way of the citric acid cycle. The significance of the changes in free fatty acid, cholesterol esters, and cholesterol is not clear.

These results provide the first direct evidence for oxidation of endogenous lipid by the perfused rat heart.

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Gibberellins: Their Effect on Antheridium Formation in Fern Gametophytes

Abstract. *Gametophytes of the fern* Anemia phyllitidis *respond to seven different gibberellins (A_1, A_3, A_4, A_5, A_7, A_8, and A_9) by forming antheridia. Gibberellin A_7 is active at concentrations as low as 5×10^{-10} g/ml. Species from two of the three other genera in the family Schizaeaceae respond similarly to gibberellin A_3. In contrast, nearly 40 species from 7 other families of ferns do not develop antheridia when supplied with gibberellin.*

In a series of three papers, Döpp (*1*) demonstrated that the bracken fern, *Pteridium aquilinum* (L.) Kuhn, secreted a biologically active substance which caused newly germinated gametophytes of bracken fern to form male reproductive organs, or antheridia. These distinctive reproductive structures are formed from single cells of the gametophyte by a series of unequal cell divisions (*2*). As shown in Fig. 1, each division is followed by striking differentiaton of the two daughter cells. The natural substance, which could initiate this series of divisions, was obtained by Döpp in aqueous extracts of the gametophytes or in the medium upon which gametophytes had been cultured for several weeks.

Although this substance was organ-specific, it was not species-specific, in that its presence in culture media led to the formation of antheridia in *Dryopteris filix-mas* (L.) Schott as well as in the bracken fern. Furthermore, aqueous extracts of *Dryopteris* elicited antheridia in *Pteridium*. In each instance, the ferns produced antheridia under conditions in which no antheridia were formed in extract-free control cultures. The response of more than 50 species of ferns from 8 major families was summarized by Voeller (*3*). Two additional antheridium-inducing substances, or antheridogens have been found in other families of ferns (*4*).

Chemical analysis (*5*, *6*) of pure, isolated antheridogen A, as the hormone is named, showed that the substance is of low molecular weight, and contains a carboxyl group and one unsaturated carbon-carbon bond. The pK_a of the acid is about 5.0. The properties of antheridogen A suggest that, among the chief groups of naturally occurring plant growth substances, it most resembles the gibberellins. Moreover, gibberellin A_3 has quite striking androecium- (male flower) promoting properties, when applied to various flowering plants (*7*). The possibility that the antheridogens are gibberellin-like substances is also interesting in that Kato *et al.* (*8*) reported the detection of a gibberellin-like substance in ferns, although in sporophytes rather than gametophytes.

The results of tests of the activity of gibberellin A_3 upon *Pteridium aquilinum* and *Onoclea sensibilis* L., the latter being particularly responsive to the antheridogen, have been consistently negative (*3*). The substance was tested over a wide range of concentrations, both in the presence and absence of such other growth substances as indole-3-acetic acid and 6-furfurylaminopurine (kinetin). Six other gibberellins, A_1, A_4, A_5, A_7, A_8, and A_9, showed no effect upon antheridium induction in *Onoclea* (*3*). A slight effect of gibberellin A_3 upon both antheridium and archegonium induction in *Aspidium oreopteris* was reported by Witsch and Rintelen (*9*). Evidently, the effect was not organ-specific, nor did these workers detect enhancement of the formation of reproductive organs in *Polypodium vulgare* L. or in *Pteridium aquilinum* grown on gibberellin-containing media.

Table 1. The response of species of the Schizaeaceae to gibberellin A_3, at a concentration of 5×10^{-5} g/ml.

Species	Gibberellin	Controls
Schizaea pusilla Pursh (*19*)	+	+
Lygodium japonicum (Thunb.) Sw. (*20*)	+	−
Anemia hirsuta (L.) Sw. (*21*)	+	−
A. pastinacaria Moritz	+	−
A. oblongifolia (Cav.) Sw.	+	−
A. tomentosa var. *mexicana* (Presl) Mickel	+	−
A. tomentosa var. anthriscifolia (Schrad.) Mickel*	+	−
A. jaliscana Maxon	+	−
A. rotundifolia Schrader (*20*)	+	−
A. phyllitidis (L.) Sw. (*22*)	+	−
A. phyllitidis (*20*)	+	−
Mohria caffrorum (L.) Desv. (*23*)	+	−

*Collected in South America by Lichtenstein.

In contrast with the slight, or complete lack of response of the species of fern prothallia tested, *Anemia phyllitidis* (L.) Sw. responds strikingly to gibberellin. Schraudolf (*10*) showed that 100 percent of the gametophytes of this member of the family Schizaeaceae produced antheridia when grown with gibberellin A_3 at concentrations of 5×10^{-6} g/ml, or higher. Indeed, 70 to 80 percent of the gametophytes bore antheridia when grown on media with a gibberellin concentration of

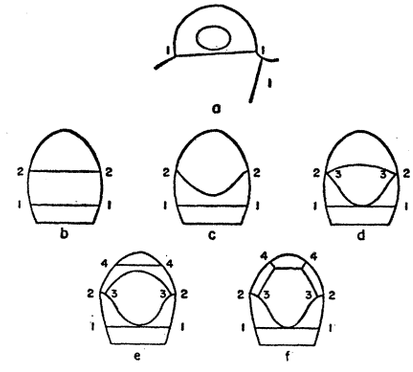

Fig. 1. Diagrammatic representation of the formation of an antheridium [after Davie (*2*)]. (*a*) The antheridial initial, showing a nucleus at its center. The initial is cut off from its parent cell by wall one (1). (*b*) The two-celled stage. (*c*) Depression of cell wall two (2). (*d*) Delimitation of the gametogenic, central region. (*e*) Formation of the "cap" cell and the donut-shaped "collar," or ring cell. (*f*) A mature antheridium.