Intestinal Absorption and Tissue Distribution of [^{14}C]Pyrroloquinoline Quinone in Mice (43219)

CARSTEN R. SMIDT,* CLIFFORD J. UNKEFER,† DAVID R. HOUCK,†,[1] AND ROBERT B. RUCKER*,[2]
Department of Nutrition, University of California, Davis, California 95616 and Isotope and Structural Chemistry Group,† Los Alamos National Laboratory, Los Alamos, New Mexico 87545*

Abstract. **Pyrroloquinoline quinone (PQQ) functions as a cofactor for prokaryotic oxidoreductases, such as methanol dehydrogenase and membrane-bound glucose dehydrogenase. In animals fed chemically defined diets, PQQ improves reproductive outcome and neonatal growth. Consequently, the present study was undertaken to determine the extent to which PQQ is absorbed by the intestine, its tissue distribution, and route of excretion. About 28 μg of PQQ (0.42 μCi/μmol), labeled with ^{14}C derived from L-tyrosine, was administered orally to Swiss-Webster mice (18–20 g) to estimate absorption. PQQ was readily absorbed (62%, range 19–89%) in the lower intestine, and was excreted by the kidneys (81% of the absorbed dose) within 24 hr. The only tissues that retained significant amounts of [^{14}C]PQQ at 24 hr were skin and kidney. For kidney, it was assumed that retention of [^{14}C]PQQ represented primarily PQQ destined for excretion. For skin, the concentration of [^{14}C]PQQ increased from 0.3% of the absorbed dose at 6 hr to 1.3% at 24 hr. Furthermore, most of the [^{14}C]PQQ in blood (>95%) was associated with the blood cell fraction, rather than plasma.** [P.S.E.B.M. 1991, Vol 197]

Pyrroloquinoline quinone (PQQ) has been shown to act as a redox-cofactor for bacterial dehydrogenases, such as methanol and glucose dehydrogenase (1–4). PQQ was also thought to function in eukaryotic systems as a cofactor for enzymes such as the copper-containing amine oxidases (5–9), choline dehydrogenase (10), and adrenal medulla dopamine-β-hydroxylase (11, 12). Although this proposal is now dismissed (9, 12), the original work, which was aimed at identifying PQQ as eukaryotic cofactor, stimulated and encouraged corroborative studies aimed at identifying putative nutritional and pharmacologic roles for PQQ (13–15). For example, PQQ supplementation has been shown to improve reproductive outcome and neonatal growth (15).

[1] Present address: Sterling Winthrop Research Institute, Columbia Turnpike, Rensselaer, NY 12144.

[2] To whom requests for reprints should be addressed at Department of Nutrition, University of California, Davis, CA 95616-8669.

Received July 19, 1990. [P.S.E.B.M. 1991, Vol 197]
Accepted December 19, 1990.

0037-9727/91/1971-0027$3.00/0

Few data are available, however, on the metabolism of PQQ in mammalian tissues. Consequently, we deemed it important to establish the extent to which PQQ is absorbed and distributed in tissues. For this purpose, PQQ, labeled with ^{14}C, was prepared for oral administration to young mice, since mice have been shown previously to respond positively to PQQ supplementation (15).

Materials and Methods

Preparation of [^{14}C]PQQ. In *Methylobacterium extorquens* AM1 (Pseudomonas AM1, American Type Culture Collection 14718), PQQ is derived from condensation of L-glutamate and L-tyrosine (16). Therefore, for the biosynthesis of [^{14}C]PQQ, [^{14}C]tyrosine (>500 μCi/μmol; New England Nuclear) was added at 0.25 mCi/liter of Pseudomonas AM1 medium to serve as a precursor for the synthesis of [^{14}C]PQQ; methanol (0.5%) served as an additional carbon source. The cells were batch cultured at 30°C until the optical density at 560 nm was 4.0, or the PQQ concentration was 400 μg/liter (see below). Cells were then removed by centrifugation (10,000*g*, 10 min).

Next, the culture broth (pH 6.8–7.0) was passed over a DEAE Sephadex-A25 column, 10-ml bed volume/1.0 liter of broth. PQQ bound to resin was eluted with the stepwise addition (20 ml each) of NaCl in 5

m*M* potassium phosphate buffer (pH 7) at 0.1, 0.2, 0.5, or 1.0 *M* NaCl. Fractions that contained PQQ (UV absorbing fractions with a λ_{max} of 248 nm) were pooled and passed over a reverse-phase column (0.5 g Baker spe C_{18}). The spent fraction was adjusted to pH 2 with 1 *M* HCl and applied to a second C_{18} column; at pH 2, PQQ binds to C_{18}. After 3–4 column washes with 10 m*M* HCl, PQQ was eluted upon the addition of 50% aqueous methanol (v/v in 10 m*M* HCl).

Radiochemical purity of PQQ was also assessed by high-performance liquid chromatography a C_{18}-reverse phase column (5 μm). A two-phase buffer system was used, which contained 3.5 m*M* tetrabutylammonium phosphate in 2.0 m*M* potassium phosphate, pH 6.5 (Buffer A); and tetrahydrofuran:water, 50:50 (v/v) (Buffer B). A gradient of 96% Buffer A, 4% Buffer B to 80% Buffer A, 20% Buffer B was effected and the eluant monitored by UV absorbance (248 nm) and fluorescence detection (16). Peaks corresponding to PQQ were collected for liquid scintillation counting and further characterization (16).

Administration of [^{14}C]PQQ. Ten male Swiss-Webster mice (Charles River Laboratories, Wilmington, MA) weighing 18–20 g were fed a chemically defined, amino acid-based diet (15) that was estimated to contain no more than 200 ng/g PQQ (15, 17). The diet was fed for a 7-day period prior to the administration of radiolabeled PQQ.

For oral administration, the specific activity of PQQ was 0.42 μCi/μmol and each animal received about 0.1 μmol of PQQ (about 80,000 dpm, corresponding to 28 μg PQQ). The isotopic PQQ was dissolved in saline buffered with 5 m*M* potassium phosphate (pH 7.0) and administered orally using a gavage needle designed for small rodents and mice (Perfectum, 24-gauge, 1 inch; Popper & Sons, Inc., New York, NY). Animals were then placed immediately into metabolic cages (100 × 200 cm) equipped for collection of excreta and expired CO_2, which was trapped in ethanolamine. To reduce spillage during ^{14}C study, the diet was provided as agarose gel cubes prepared by dissolving agarose at 1% (w/v) into a mixture of the chemically defined diet and H_2O (1:4, w/v).

After 6 and 24 hr, five animals each were killed by a carbon dioxide overdose, and blood was obtained by cardiac puncture and treated with potassium ethylenediaminetetraacetic acid as anticoagulant. Red blood cells were separated from plasma by centrifugation (1000*g*; 20 min). Liver, kidneys, adrenals, spleen, heart, lungs, brain, and skin were removed intact. The intestine was divided into three parts (duodenum, jejunum, and ileum), each of which was washed with saline, and the washings were combined with cecum and colon.

Skin samples were shaved, freed of subcutaneous fat, and briefly washed in saline solution. The remaining carcass was autoclaved in 1 volume of 16% acetic acid for 1 hr, and then homogenized. Aliquots of the tissues (0.4 g), feces, and urine were solubilized in TS-1 (Research Products International Corp., Mount Prospect, IL), decolorized with hydrogen peroxide, and counted after 3 days in EcoLite scintillation fluid (ICN Biomedicals, Inc., Irvine, CA) using a Packard Tri-Carb liquid (model 2600) scintillation spectrometer).

Chemical Detection of PQQ. PQQ-like substances were estimated by the nitroblue tetrazolium redox reaction described by Paz *et al.* (17). In particular, aliquots of skin, diet, and fecal material were analyzed for PQQ-like reacting substances in supernatant fractions obtained by first homogenizing 100- to 200-mg samples (1:10 w/v) into sodium phosphate buffer (0.005 *M*, pH 7.0). Following centrifugation, portions of the resulting supernatant fractions were analyzed as originally described (17), except that 0.02 *M* sodium borate and 0.01 *M* EDTA acid were also present in assays (*cf.* Results and Discussion).

Estimation of Absorption and Statistics. The percentage of absorption was estimated by dividing the total amount of [^{14}C]PQQ administered into the sum of ^{14}C retained in tissues plus ^{14}C in urine and carbon dioxide, but excluding ^{14}C in the gastrointestinal tract. Incorporation into tissues was expressed as the percentage of the estimated value for absorbed ^{14}C as PQQ. Differences between the 6- and 24-hr collections were assessed using Student's *t* test.

Results and Discussion

The purity and selected properties of [^{14}C]PQQ are described in Figure 1. The gavage of [^{14}C]PQQ was accepted without distress or regurgitation, except for one animal (Table I, number 10). On the average, each animal received about 80,000 dpm or 28 μg of PQQ (Table I). Since mice normally consume 3–4 g of dry food/day, the dose is equivalent to 6–10 mg of PQQ/kg diet, but in this case consumed as a single bolus.

After 6 hr, 3.3% of the label was absorbed, compared with 62.0% (range, 19–89%) after 24 hr. PQQ concentrations remaining in the intestinal lumen estimated chemically agreed with the calculated dose that was not absorbed within 10–20% (Table I). The assay described by Paz *et al.* (17) estimates PQQ, in picomole amounts, whereas interfering substances, such as ascorbic acids and most naturally occurring orthoquinones (9, 15), promote formazan formation in nano- to micromole amounts in assays. The addition of 0.02 *M* borate and chelating agents such as EDTA further eliminated interference by enediols and transition metals. For example, relative to PQQ, 0.02 *M* borate ne-

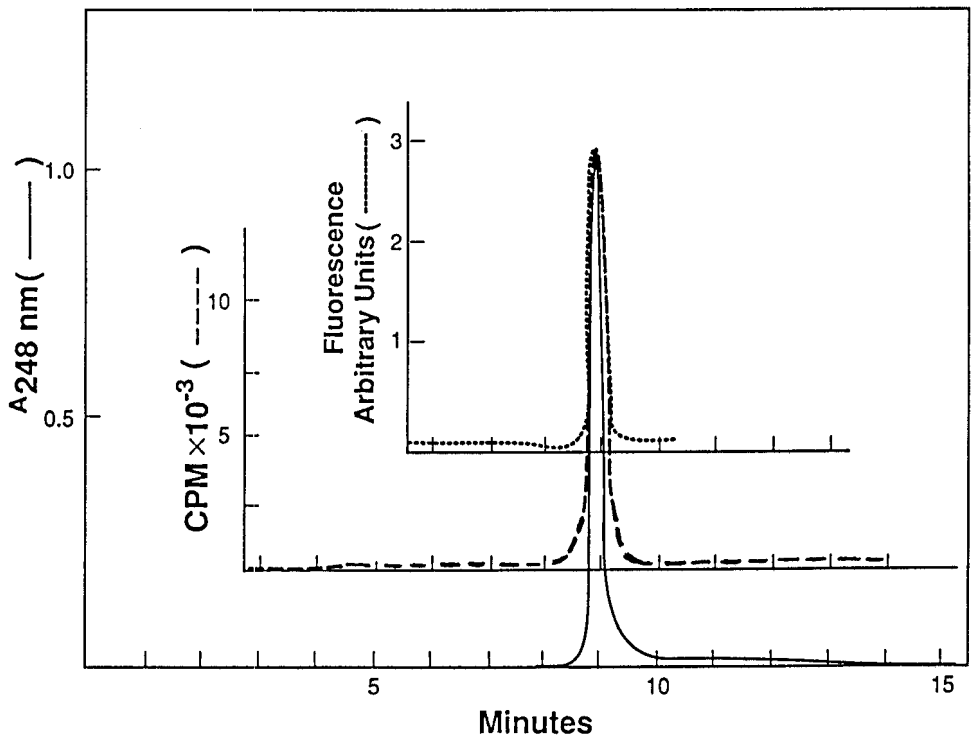

Figure 1. Radiochemically labeled PQQ. A culture medium of *M. extorquens* AM1 was the source of biosynthetic PQQ. The isolation of PQQ from 1 liter of liquid culture was as follows. After centrifugation (10,000*g* for 10 min), the culture broth (pH 6.8–7.0) was passed over DEAE Sephadex-A25. Fractions that contained PQQ (UV absorbing fractions λ_{max} 248 nm) were pooled and applied to a reversed-phase column (0.5 g, Baker spe C_{18}; see text for details). [^{14}C]PQQ was monitored by estimating radioactivity as ^{14}C (– – –), by estimating UV absorption at 248 nm (——), and by fluorescence (· · ·). Essentially all (>95%) of the radioactivity applied to the column was recovered as a single peak.

Table I. Absorption and Tissue Distribution of ^{14}C-Labeled PQQ

Time (hr)	Mice no.	Body weight (g)	Gavage (dpm)	Absorption		Urine (% of absorbed)	Liver (% of absorbed)	Red blood cell (% of absorbed)	Skin (% of absorbed)
				%	As μPQQ				
6	1	25.9	94,358	4.2	1.4	89.7	3.3	4.0	0.4
	2	26.5	103,368	4.4	1.6	29.9	3.9	6.7	0.5
	3	21.7	78,183	2.4	0.7	69.1	5.0	12.3	0.0
	4	23.1	63,100	4.6	1.0	83.5	2.6	10.2	0.0
	5	23.1	115,549	1.1	0.5	38.5	12.3	19.8	0.6
Means			90,912[a]	3.3[b]	1.0[b]	62.1[a]	5.4[a]	10.6[b]	0.3[a]
24	6	19.4	53,907	67.6	12.9	71.9	2.3	0.8	1.8
	7	24.8	67,108	19.4	4.6	94.3	0.9	2.6	0.3
	8	23.8	101,698	88.7	31.9	61.9	1.8	0.3	2.0
	9	22.1	92,802	68.5	22.5	83.0	1.1	0.5	2.1
	10	24.0	18,251	66.1	4.3	95.8	1.3	1.7	0.5
Means			66,753[a]	62.0[b]	15.2[a]	81.4[a]	1.5[a]	1.2[b]	1.3[a]

[a] Significantly different at $P < 0.05$, 6 vs 24 hr.
[b] Significantly different at $P < 0.01$, 6 vs 24 hr.

gates the signal from 100 to 1000 *M* excess of L-ascorbic acid.[3]

[3] A more detailed description of the PQQ assay is in preparation. For the work here the method described by Paz *et al.* (17) was followed as described, but sodium borate and EDTA were included in assays (see text). In contrast to a recent report (18), we have found that the assay has general applicability when carefully performed and controlled. Dopamine, 6-hydroxydopa, ascorbic acid, and tryptophan degradation products do not interefere when samples are appropriately diluted or separated using relatively simple chromatographic protocols.

It was also observed that undigested agarose was not visibly present in the colon at the 6-hr point, but was observed in the colon at 24 hr. The agarose served as a marker that aided in concluding PQQ was absorbed in the lower gut. Since a large single dose of PQQ was administered, it was also inferred that absorption was relatively efficient. This fulfills an obvious and important requirement, if PQQ-like compounds are to be

considered metabolically or nutritionally important in animals.

Urinary excretion of PQQ appeared to be the major route of elimination of absorbed PQQ. Furthermore, excretion via the kidneys were relatively efficient, e.g., 62.1% of the dose was excreted at 6 hr and 81.4% of the absorbed PQQ was excreted in urine after 24 hr. Though the rapid movement from intestine to kidney may not reflect normal or physiologic excretion kinetics, because of the excessive dose administered, PQQ is efficiently absorbed. The observations also corroborate the findings of Watanabe *et al.* (13), who found that in rats PQQ injected intraperitoneally is excreted rapidly by the kidneys. No PQQ was detected as expired carbon dioxide.

The liver retained only a small percentage of the absorbed PQQ, i.e., 5.4% after 6 hr and 1.5% after 24 hr. Of interest, the majority of [^{14}C]PQQ in blood was associated with the blood cell fraction (95–97%) at both 6 and 24 hr. At 6 hr, the blood cell fraction constituted about 10% of the absorbed label, which was diminished to 1.2% at 24 hr (Tables I and II). Comparing the time course of distribution of label in the tissues (Table I), most tissues retained some of the absorbed PQQ. The largest variation in retention occurred in the kidney, which mostly likely reflects variation in the passage from kidney to bladder.

In skin, there was a 4-fold increase in the percentage of absorbed label after 24 hr. The accumulation of radioactivity in skin also corresponded to a net increase in the content of PQQ in skin, i.e., in phosphate-buffered extracts of skin samples. Values for PQQ were increased significantly from 308 ± 36 ng/g at 6 hr to 389 ± 61 ng/g ($P < 0.05$); 80 ng is equivalent to about 1% of the absorbed dose as [^{14}C]PQQ (Table I). The adrenal gland and brain did not incorporate significant amounts of radioactivity.

This work was undertaken as an extension of a previous observation that PQQ may be of nutritional importance (15). Whether or not the nutritional responses are due specifically to PQQ or a phenomenon associated with antioxidant function(s) or redox cycling is currently under investigation. Our original proposal that the dietary response to PQQ is due primarily to its role as a vitamin-like cofactor has been questioned (9). Indeed, it is now clear that in copper-containing amine oxidases, the cofactor is peptidyl trihydroxyphenylalanine, 6-hydroxydopa (9), and that DOPA-β-hydroxylase contains no PQQ (12). Consequently, whether or not PQQ functions nutritionally as a cofactor, as a probiotic, or merely contributes pharmacologically to novel redox reactions remains to be resolved.

Nevertheless, the work herein demonstrates that when PQQ is found as a part of the food chain, it is absorbed in the lower intestine. Moreover, PQQ associates with blood cells and accumulates in skin, a site where PQQ has been shown to influence the maturation of collagen and accumulation of lysyl oxidase (15).

Table II. Distribution of ^{14}C Label in Tissues[a]

Tissue	% Absorbed dpm in tissue			
	6 hr		24 hr	
	Mean	Range	Mean	Range
Blood plasma	0.4	0.0–1.7	0.1	0.0–0.2
Kidneys	3.5	1.5–9.9	10.7	0.4–29.4
Spleen	0.3	0.1–1.3	0.0	0.0–0.1
Heart[a]	0.2	0.1–0.5	0.0	0.0–0.0
Lungs[a]	0.4	0.2–0.9	0.1	0.0–0.1
Brain	0.2	0.0–0.6	0.0	0.0–0.1
Adrenals	0.0	0.0–0.0	0.0	0.0–0.0
Remaining carcass	16.5	0.0–56.1	3.7	1.2–7.5
Expired carbon dioxide	0.0	0.0–0.0	0.0	0.0–0.0

[a] Means for the 6-hr values are significantly different from those at 24 hr, $P < 0.05$. At 6 hr, 1% of the absorbed dose was equal to approximately 30 dpm, i.e., 90,912 dpm (total dose) × 0.033 ÷ 100% (Table I). Samples were counted for periods sufficient to ensure analytical counting precision of ±0.1 to 0.2%. At 24 hr, 1% of the absorbed dose is the equivalent of approximately 414 dpm, i.e., 66,753 dpm × 0.62 ÷ 100% (Table I).

This work was supported by NIH Grants AM-25358 and HL-15916 and United States Department of Agriculture Grant 89-37200-4429.

The preparation of [^{14}C]PQQ was supported by the Department of Energy, Division of Biological Energy Research.

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