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REGULATION OF INTESTINAL CYTOCHROME P-450 AND HEME BY DIETARY NUTRIENTS

CRITICAL ROLE OF SELENIUM

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Abstract—The intestinal cytochrome P-450 (I-P-450)-dependent mixed function oxidase (MFO) system is regulated to a remarkable extent by various ingested xenobiotics, including drugs and carcinogens, as well as dietary nutrients. Accordingly, acute dietary iron deprivation is found to result in a marked decrease in I-P-450 content and activity. This decrease is most pronounced in the villous tip cells, the very cells committed to absorption of ingested materials. We investigated the mechanistic basis for such acute reduction and report that iron was not only required as a co-substrate for I-P-450 heme formation, but also as a regulator of two key heme-synthetic enzymes, δ-aminolevulinic acid synthetase and ferrochelatase. In addition, our studies revealed that dietary deprivation of selenium for a single day dramatically reduced I-P-450-dependent MFO activity. This prompt reduction apparently reflects impaired I-P-450 formation resulting from lowered ferrochelatase activity and consequently decreased intestinal heme availability, and was not a consequence of intracellular peroxidation presumably enhanced by concomitant lowering of the seleno-dependent glutathione peroxidase. Thus, we report the novel observation that dietary selenium also appears to be a critical modulator of intestinal cytochrome P-450-dependent metabolism of ingested drugs, carcinogens, and toxins that are absorbed by the intestinal mucosa.

The gastrointestinal tract represents a primary portal of entry for a wide variety of foreign compounds ingested deliberately as dietary constituents (natural chemicals, intentional food additives, incidental food contaminants) and drugs or, unintentionally, as toxins or environmental carcinogens. Recent studies from many laboratories including ours have demonstrated that the intestinal mucosa can convert ingested chemicals generally to their less toxic, readily excretable derivatives. In fact, several drugs and toxins have been shown to undergo extensive "first pass" metabolism in the intestinal mucosa following their oral intake [1–7]. This entails metabolism specifically in the intestinal mucosa rather than by intraluminal bacteria or in the liver.

In the intestinal mucosa, as in the liver, several of these biotransformation reactions are catalyzed by the microsomal mixed function oxidase (MFO)† system of which cytochrome P-450 and NADPH-cytochrome P-450 reductase are key components [7–15]. Previous studies from here and elsewhere have indicated the existence of a gradient in mucosal MFO activity from the duodenum to the colon that closely parallels a similar gradient in intestinal absorptive capacity [7, 15, 16]. In addition, the most mature epithelial cells at the mucosal villous tips, pivotal in absorption of intraluminal constituents, are also the richest in cytochrome P-450 content and related MFO activity. Indeed, a progressive gradient of cytochrome P-450 content and activity is observed along the height of the intestinal villus [15, 17, 18] as the crypt cell matures into a villous tip cell.

Intestinal cytochrome P-450, like its hepatic isozymes, is inducible by a wide variety of xenobiotics [7–9, 11–14, 19–21]. However, it appears to be influenced by intraluminal regulators to a greater extent than by parenteral ones. Rats switched from regular Purina lab chow known to contain natural cytochrome P-450 inducers [22], to an inducer-free diet, rapidly exhibit a marked decrease in intestinal, but not hepatic, cytochrome P-450 content and activity [7, 15]. These findings indicate that intestinal cytochrome P-450 content and function may be modulated to a large extent by the intraluminal environment. Xenobiotics occurring in this environment largely regulate intestinal cytochrome P-450 content and function. However, these variables are apparently well-maintained albeit at a reduced level in the absence of such xenobiotics, indicating that dietary nutrients may also play a pivotal regulatory role. Indeed, intestinal cytochrome P-450 has been shown to be critically dependent on amino acids, fats, vitamins and iron [7, 15, 23–25].

A prompt and marked reduction in intestinal cytochrome P-450 content and activity is observed within 24 hr of oral iron deprivation [15]. This reduction is more prominent in villous tip cells than in crypt cells and occurs without any appreciable changes in plasma iron levels, which indicates a

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† Abbreviations: MFO, mixed function oxidase; GSH, reduced glutathione; AHH, aryl hydrocarbon hydroxylase; EROD, 7-ethoxyresorufin-*O*-deethylase; ALAS, δ-aminolevulinic acid synthetase; ALAD, δ-aminolevulinic acid dehydratase; and MHO, microsomal heme oxygenase

critical and acute dependency of intestinal cytochrome P-450 on intraluminal iron. Since iron is a constituent of heme (iron-protoporphyrin IX), we investigated whether such dependency reflects a critical requirement of intraluminal iron for synthesis of the cytochrome P-450 heme moiety. We also explored whether the trace nutrient selenium, which is required for hepatic cytochrome P-450 synthesis and assembly [26, 27], is also required for intestinal cytochrome P-450 structure and function.

EXPERIMENTAL PROCEDURE

Materials

NADPH, NADH, isocitrate, isocitrate dehydrogenase, bovine serum albumin, trypsin inhibitor, Tris, glutathione, GSH reductase, dithioerythritol, δ-aminolevulinic acid, hemin, coenzyme A, pyridoxal-5-phosphate, ATP, succinate, succinyl thiokinase, EDTA, and 3,4-benzo[a]pyrene were obtained from the Sigma Chemical Co., St. Louis, MO. [1,4-^{14}C]Succinic acid (NEC-099, 35.5 mCi/mmole) and [methyl-^{3}H]thymidine (NET-027, 6.7 Ci/mmole) were obtained from the New England Nuclear Corp., Boston, MA. Sodium selenite was purchased from ICN Biochemicals, Cleveland, OH. Resorufin, 7-ethoxyresorufin and 3-OH benzo[a]pyrene were obtained from Matheson, Colemen and Bell, Norwood, OH, the Pierce Chemical Co., Rockford, IL, and the IIT Research Institute, Chicago, IL, respectively. Ion exchange columns for δ-aminolevulinic acid synthetase (ALAS) assay were custom packed by Bio-Rad, Richmond CA. Fetal calf serum was purchased from the Grand Island Biological Co., Grand Island, NY. Deuteroporphyrin was obtained from Porphyrin Products, Logan, UT.

Animals

Adult male Sprague–Dawley rats (200–240 g, Simonsen Laboratories, Gilroy, CA) were housed in hanging stainless steel cages, in rooms maintained at 25° with a controlled 12 hr-diurnal lighting cycle, and were protected from contact with pesticides and other extraneous environmental factors known to influence cytochrome P-450-dependent MFO activity [8, 14, 21, 28].

Diets

Animals were routinely weaned from the standard laboratory chow diet onto the specific dietary regimen by feeding the semisynthetic diet deficient in the specific experimental variable for 2 days. This regimen was instituted to permit sufficient time for one complete crypt to villous tip cell transformation, sloughing of tip cells from the mucosa, and consequent expurgation of natural cytochrome P-450 inducers normally present in the laboratory chow [7, 12, 19]. The animals were then fed the specific purified inducer-free diets, deficient in, or supplemented with, iron and/or selenium. The following diets were routinely used: (i) *semisynthetic iron- and selenium-deficient* (−Fe, −Se), consisting of vitamin-free casein, 27%; corn starch, 56%; hydrogenated vegetable oil, 14%; salt mixture without added ferrous sulfate or sodium selenite, 3%; and ICN vitamin diet fortification mixture, 1% (ICN Nutritional Biochemicals, Cleveland, OH). The iron and selenium contents of this diet were determined to be below 0.1 mg and 0.005 mg per 100 g diet, respectively; (ii) *semisynthetic iron-supplemented, selenium-deficient diet* (+Fe, −Se) consisting of the above diet with 10 mg iron as ferrous sulfate added per 100 g diet; (iii) *semisynthetic iron-deficient, selenium-supplemented diet* (−Fe, +Se) consisting of −Fe, −Se diet supplemented with 0.05 mg selenium as sodium selenite per 100 g diet; (iv) *semisynthetic iron- and selenium-supplemented diets* (+Fe. +Se) consisting of −Fe, +Se diet with iron and selenium supplements as in diets (ii) and (iii). Following weaning, animals were divided into four groups, each fed *ad lib.* one of the above diets and given free access to water for a minimum of 4 days. Regardless of the dietary regimen, the animals were found to gain weight uniformly during this period.

The intracellular selenium-status of the intestinal mucosa of rats fed the above diets was assessed by determining the activity of the selenium-dependent GSH-peroxidase [29].

Intestinal cell preparations

Rats were stunned by a blow to the head, decapitated, and exsanguinated. The abdomen was opened by a midline incision, and the intestinal tract was excised from the stomach at the pyloric junction and gently pulled away from the mesentery. The small intestine was then sectioned 30 cm distal to the pylorus, flushed with ice-cold saline followed by ice-cold 0.05 M Tris buffer (pH 7.8) containing 20% glycerol and 1.15% KCl to wash off intestinal contents, and placed in the same buffer at 4°. All subsequent procedures were conducted at 4°. Immediately after intestinal excision, villous tip and crypt cells of the mucosa were separated by the differential scraping technique [15, 30]. The intestine was laid on a cold glass plate and split longitudinally along the mesenteric axis. Villous cells were removed by light scraping with the edge of a metal spatula, while crypt cells were removed from the underlying connective tissue by heavier scraping. The effectiveness of this method for isolating individual cell populations was determined by following the *in vivo* incorporation of an i.p. injection of [^{3}H]thymidine into isolated nuclear DNA as described [15]. After 1 hr,crypt cell DNA exhibited the highest radioactivity per mg nuclear protein, while at 48 hr villous tip cell DNA showed maximum radioactivity, compatible with one full crypt to tip migration cycle and in agreement with previous reports [15, 31].

Cell fraction preparation

Whole homogenate and 9000 g supernatant, cytosol (105,000 g supernatant), and microsomal (105,000 g pellet) fractions that were prepared by differential centrifugation of the following "standard" homogenate, were subsequently used for various enzyme assays; the mitochondrial fraction was prepared as described under the ferrochelatase assay. Individual cell populations from three or four rats (as indicated) were pooled, weighed and suspended in 2–7 vol. of 0.05 M Tris/20% (v/v) glycerol/1.15%KCl buffer (pH 7.8), supplemented

with trypsin inhibitor (5 mg/g wet wt of small intestine) and heparin (3 units/ml) to reduce degradation and agglutination of the MFO system [32]. Mucosal tissue was then homogenized in a glass–Teflon Potter–Elvehjem homogenizer with twenty strokes at 200 rpm. Homogenates were sedimented at 9000 *g* for 20 min at 4°, and the supernatant fraction was centrifuged at 105,000 *g* for 60 min at 4°. The pellet was collected, suspended, and finely dispersed in 0.05 M Tris/20% glycerol/1.15% KCl buffer (pH 7.8) by ten passes through a 16-gauge needle. Microsomes were repelleted by centrifugation at 105,000 *g* (60 min). This procedure usually eliminated contaminating hemoglobin from tip cells, as indicated by the absence of the characteristic 420 nm peak in the CO-reduced minus reduced difference spectrum of the final microsomal suspension. The final microsomal pellet was resuspended and homogenized in the 0.05 M Tris/20% glycerol/1.15% KCl buffer by hand in a glass–Teflon homogenizer, to yield approximately 2 mg protein/ml buffer.

Assays

Intestinal cytochrome P-450. The content of the cytochrome was determined by the method of Omura and Sato [33] which employs the CO-reduced difference spectra of microsomes in 0.5 ml matched cuvettes in an Aminco dual beam DW-2A spectrophotometer, using an extinction coefficient of 91 $mM^{-1}\,cm^{-1}$. The amount of sodium dithionite required for reducing cytochrome P-450 was controlled carefully since excess dithionite appears to cause microsomal degradation with consequent loss of spectrally detectable cytochrome. Contaminating hemoglobin interfered frequently in the spectral assay of crypt cell cytochrome P-450 and occasionally in that of the tip cell cytochrome; the spectral correction described by Kowal *et al.* [34] was then employed to circumvent this particular problem.

Aryl hydrocarbon hydroxylase (AHH). The enzyme activity was determined in the 9000 *g* supernatant fraction by the fluorescence assay [35] using 3-OH benzo[a]pyrene, one of the major metabolites [32], as the reference standard.

7-Ethoxyresorufin-O-deethylase (EROD). The enzyme activity was assayed by a modification of the fluorometric methods described [36, 37]. The reaction mixture (final volume, 1 ml) consisted of 0.05 M Tris buffer (pH 7.4) containing $MgCl_2$ (5 mM), $MnCl_2$ (5 mM), NADPH (0.5 mM), NADH (0.5 mM), isocitrate (6 mM), isocitrate dehydrogenase (0.02 units), bovine serum albumin (0.8 mg), and microsomal protein (0.2 to 0.5 mg). After 3 min of preincubation in a shaking water bath at 37°, reactions were started with 7-ethoxyresorufin (1 nmole) and terminated at 10 min with 1.0 ml of ice-cold acetone [37]. Assay blanks consisted of incubation mixtures in which the reaction was terminated with ice-cold acetone immediately after substrate addition, followed by a 10 min incubation at 37°. The presence of microsomes in the reaction mixture reduced the fluorescence-contribution of 7-ethoxyresorufin, apparently by binding it. A standard curve (10–100 pmoles of resorufin) was run, therefore, in the presence of microsomes and 7-ethoxyresorufin (1 nmole), and the mixtures were subsequently treated exactly like assay blanks. After acetone addition, samples were vortexed and centrifuged for 15 min at 2000 rpm in an ICN centrifuge, and the supernatant fractions were analyzed in an Aminco Bowman spectrofluorometer, with excitation wavelength set at 530 nm and emission wavelength at 580 nm. Reaction rates were expressed as pmoles resorufin formed per mg microsomal protein per min. Using this procedure, reactions were found to be linear for up to 15 min and 1.0 mg protein.

δ-Aminolevulinic acid synthetase (ALAS). The activity of the enzyme was determined following dilution (1:1, v/v) of the "standard" homogenate (50%, v/v), prepared as described above, with 0.1 M Tris/0.9% NaCl buffer, containing pyridoxal phosphate (0.1 mM) at pH 7.4. The method of Strand *et al.* [38], as previously modified [27], was essentially followed except that water was used to wash the Dowex-1-acetate column before the final elution of the pyrroles with methanol/acetic acid.

δ-Aminolevulinic acid dehydratase (ALAD). ALAD activity was monitored in intestinal cytosolic fractions obtained from cell homogenates (20%, v/v), as reported [39].

Ferrochelatase. Cell fractions obtained from the intestinal mucosa (30 cm segment distal to the pylorus) were pooled from four rats and homogenized in 7 vol. of 0.05 M Tris/0.25 M sucrose containing EDTA (1 mM), pH 8.2, and supplemented with trypsin inhibitor and heparin (as described earlier), in a glass–Teflon homogenizer. Mitochondria were isolated, and their ferrochelatase was solubilized according to the procedure described by Cole *et al.* [40]. The enzyme activity was assayed also by the method of Cole *et al.* [40] except for the following modifications: deuteroporphyrin was used instead of mesoporphyrin since the enzyme activity with this substrate was 3-fold greater [41]; the substrate concentration of the dilute porphyrin solution was determined spectrally at 398 nm [42] using a millimolar extinction coefficient of 433 cm^{-1}; sodium succinate (1 mM) was included in the reaction mixture to ensure anaerobiosis [43]; and round bottom glass tubes (12 ml) containing the reaction mixtures were initially bubbled with N_2, capped with rubber caps, and continuously gassed with N_2 through 23-gauge needles during the assay. Control reactions included both iron and enzyme blanks. After preincubation at 37° with gassing for 5 min, reactions were started with $FeSO_4$ (30 μM Fe, final concentration) by injection through the rubber caps. The reactions were incubated anaerobically for 20 min at 37° in a shaking water bath and terminated by addition of iodoacetamide (46.5 mM, final concentration). The reduced minus oxidized difference spectrum of the pyridine hemochromogen of deuteroheme so formed was recorded by an Aminco DW-2 spectrophotometer, and its content was calculated from the peak-trough spectral difference obtained between 545 and 530.5 nm using an extinction coefficient of 15.3 $mM^{-1}\,cm^{-1}$ [41].

Microsomal heme oxygenase (MHO). Intestinal mucosal cells were homogenized by the "standard" procedure described, except that fetal calf serum (5%, v/v) was included in the homogenizing buffer. The enzyme activity was monitored by a modification

Table 1. Effects of dietary iron and selenium on intestinal cytochrome P-450 mixed function oxidase system*

Diet	I-P-450† (pmoles/mg protein)	AHH‡ (pmoles/mg protein/15 min)	EROD† (pmoles/mg protein/min)
+Fe, +Se			
Tips	23.18 ± 9.5	11.68 ± 3.7	21.00 ± 4.4
Crypts	2.21 ± 0.4§	2.56 ± 0.5§	3.20 ± 0.8§
−Fe, +Se			
Tips	9.13 ± 5.8‖	3.17 ± 0.7‖	5.16 ± 1.2‖
Crypts	2.63 ± 1.3	1.91 ± 0.6§¶	1.35 ± 0.1§¶
+Fe, −Se			
Tips	16.90 ± 5.1	6.20 ± 1.0	5.95 ± 1.1‖
Crypts	4.00 ± 0.7§	1.60 ± 0.3§	1.17 ± 0.4§¶
−Fe, −Se			
Tips	8.40 ± 4.5‖**	2.60 ± 0.3‖**	1.64 ± 0.1‖††
Crypts	2.30 ± 0.5	1.11 ± 0.3§¶	0.80 ± 0.2§‡‡

* All values are mean ± S.E.M.; each N represents a pool of mucosal tissue from four rats.
† Microsomes, N = 4–5.
‡ 9000 *g* Supernatant, N = 4–5.
§ $P < 0.05$ vs corresponding villous tips.
‖ $P < 0.05$ vs +Fe, +Se control tips.
¶ $P < 0.05$ vs +Fe, +Se crypts.
** $P < 0.05$ vs +Fe, −Se tips.
†† $P < 0.02$ vs +Fe, −Se and −Fe, +Se tips.
‡‡ $P < 0.05$ vs −Fe, +Se crypts.

of the procedure previously described [44]. Incubation mixtures (1 ml) contained microsomes (1.5 to 2 mg protein), liver cytosol (1.5 mg protein, as source of biliverdin reductase) and methemalbumin (32 μM). Methemalbumin was prepared as a heme (2.6 mM) solution in 0.1 M Tris–HCl, NaCl (5 mM) and 0.67% bovine serum albumin, pH 7.4, as previously reported [45]. The mixtures were incubated in cuvettes maintained at 37° and the reaction was monitored spectrally at 468 nm in an Aminco DW-2 spectrophotometer following initiation with NADPH (0.5 mM). An extinction coefficient of 60 $mM^{-1} cm^{-1}$ was used to calculate the bilirubin (nmoles) formed per mg microsomal protein per 10 min [44].

Other assays

Microsomal heme content was monitored by the reduced minus oxidized difference (557 − 575 nm) spectrum by the pyridine-hemochromogen method, employing an extinction coefficient of 34.7 $mM^{-1} cm^{-1}$ [46]. Cytochrome b_5 concentration was determined spectrally by the NADH reduced minus oxidized difference between 424 and 409 nm, using an extinction coefficient of 185 $mM^{-1} cm^{-1}$ [47]. NADPH–cytochrome P-450 reductase activity was determined by the method of Williams and Kamin [48], using cytochrome *c* as the external electron acceptor. Glutathione (GSH) peroxidase activity was monitored in the cytosolic fraction as described by Lawrence and Burk [29]. Protein was assayed by the method of Lowry *et al.* [49].

Statistical analysis

All experiments utilized pair fed animals, of identical weight, from the same commercial shipment, with comparisons made between rats fed diets supplemented in iron, selenium, or both, and the corresponding diets deficient in one of these dietary variables. Statistical analysis employed Student's paired *t*-test for differences between means of the paired sample data. The data of Figs. 1–3 are expressed as percent of mean values obtained in the rats fed the control (+Fe, +Se) diet.

RESULTS

Dependence of intestinal cytochrome P-450 content and function on dietary iron and/or selenium

In accordance with previous findings [15,17,18], a tip to crypt descending gradient in cytochromes P-450 and b_5, NADPH–cytochrome P-450 reductase activity and cytochrome P-450-dependent AHH and EROD activities was observed along the height of the intestinal mucosal villus. Such a gradient was not observed in microsomal heme content, possibly because of variable contamination of crypt cell preparations with hemoglobin and/or myoglobin derived from the underlying tissues, which are apparently resistant to removal by the routine washing procedures. Consistent with this possibility, spectral measurements revealed a large 420 nm peak in crypt cell microsomes following CO binding.

In confirmation of previous findings [15], deprivation of intraluminal iron by feeding rats a purified semisynthetic iron-deficient diet significantly lowered intestinal cytochrome P-450 content and its associated AHH and EROD activities (Table 1). This reduction was selectively observed in the mature epithelial cells situated at the villous tip.

That dietary selenium was critically required to maintain cytochrome P-450 content and activity in these cells was indicated by the finding that its acute deprivation for a period as short as 1 day (Table 1, Fig. 1) also lowered cytochrome P-450 content and activity albeit to a lesser extent than that observed

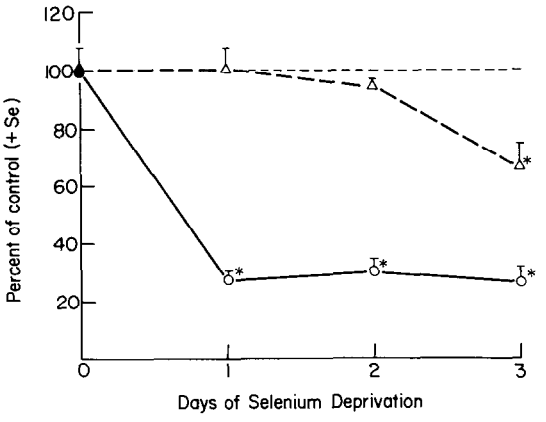

Fig. 1. Time course of the effect of dietary selenium deficiency on intestinal villous tip EROD (○) and GSH-peroxidase (△) activities. Rats were fed the control (+Fe, +Se) semisynthetic diet for 5 days prior to switching to the selenium-deficient (+Fe, −Se) diet. In each separate experiment, mucosal tissue pooled from three pair fed rats was prepared as described (Experimental Procedure). Results are the mean ± S.E. from three to four experiments and are expressed as percent of the values from control fed (+Fe, +Se) rats. Control values for EROD (●) and GSH-peroxidase (▲) activities were 21.9 ± 0.8 pmoles/mg microsomal protein/min (N = 4) and 9.4 ± 0.4 nmoles/mg cytosolic protein/min (N = 4) respectively. Key: (*) values were significantly different ($P < 0.05$) from pair fed control (+Fe, +Se) values.

following iron deprivation and in spite of adequate dietary iron. Due to the high variability routinely encountered in assays of intestinal enzymes, only alterations in EROD activity following acute selenium deprivation were found to be statistically significant (Table 1). That intestinal AHH and EROD activities are critically dependent on both iron and selenium was further confirmed by their dramatic reduction following combined dietary deficiency of both elements (Table 1). These findings not only confirm the critical requirement of iron for intestinal cytochrome P-450 dependent MFOs but reveal a heretofore unknown acute dependence on dietary selenium for their structural maintenance and function.

In addition, acute dietary deprivation of either iron or selenium failed to affect villous tip microsomal cytochrome b_5 content or the activity of the usually rate-limiting enzyme in microsomal cytochrome P-450-dependent oxidations, NADPH–cytochrome P-450 (*c*) reductase (Fig. 2). Essentially similar findings were obtained in crypt cells (not shown). Moreover, combined dietary deficiency of both elements produced no observable changes in these microsomal components in either cell population. This indicated that the impairment of the intestinal MFO activity was due primarily to decreased cytochrome P-450 content.

Effects of acute dietary iron and/or selenium deficiency on intestinal heme metabolism

To investigate whether the observed lowering of the intestinal cytochrome P-450 content and activity in rats acutely deprived of intraluminal iron was due to impaired synthesis of the prosthetic heme (ferriprotoporphyrin IX) moiety of the cytochrome, we examined intestinal microsomal heme content and the activity of three enzymes in the heme synthetic pathway: mitochondrial ALAS, the rate-limiting enzyme; cytoplasmic ALAD; and mitochondrial ferrochelatase, the terminal enzyme which actually inserts the iron into the porphyrin moiety and often constitutes a secondary rate-limiting step. Furthermore, to exclude the possibilty that accelerated heme degradation was not responsible for the observed microsomal cytochrome P-450 reduction in the intestinal mucosa, the activity of MHO, the rate-limiting enzyme in heme degradation, was also determined.

Intestinal villous tip cells in control (+Fe,+Se) rats were found to exhibit lower ALAS activity than corresponding crypt cells (Table 2). Acute iron deficiency significantly lowered this activity in both intestinal tip and crypt cells. ALAD activities were comparable in both intestinal mucosal cell types in control rats, and acute iron deficiency failed to affect them substantially (Table 2). Acute dietary iron deficiency lowered ferrochelatase activity (in spite of deliberately optimized iron concentrations in the assay mixture), indicating that iron may be necessary for maintenance of this enzyme (Table 2). In contrast to the effects of prolonged iron deficiency [44], acute iron deficiency only minimally stimulated MHO activity in villous tip cells but apparently did not affect it in crypt cells. These studies indicate that dietary iron is required for the functional activity of at least two key heme-synthetic enzymes in the intestinal villous tip cells, in addition to its well-recognized vital role as a substrate in heme synthesis.

Given the novel finding that dietary selenium is critically required for maintenance of cytochrome P-450 and its dependent MFO activity, we investigated its role in intestinal heme metabolism. Acute dietary selenium deficiency *per se* had no effect on

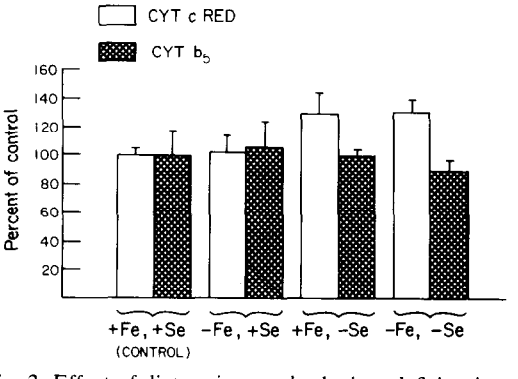

Fig. 2. Effect of dietary iron and selenium deficiencies on microsomal NADPH–cytochrome P-450 (*c*) reductase (CYT *c* RED) activity and cytochrome b_5 (CYT b_5) content of villous tip cells. The experimental protocol is described in Experimental Procedure. Results are mean ± S.E. and are expressed as percent of values from pair fed control (+Fe, +Se) rats. Control values for P-450 reductase activity and cytochrome b_5 content were 32.0 ± 2.9 nmoles cytochrome *c* reduced/mg protein/min and 25.0 ± 5.0 pmoles/mg protein, respectively. No statistically significant differences were found.

Table 2. Effect of dietary iron on intestinal heme metabolism*

Diet	Heme† (pmoles/mg protein)	ALAS‡ (pmoles/mg protein/30 min)	ALAD§ (nmoles/mg protein/hr)	Ferrochelatase‖ (nmoles/mg protein/20 min)	MHO† (pmoles/mg protein/min)
+Fe, +Se					
Tips	128.0 ± 9.3	83.47 ± 12.1	3.38 ± 0.5	9.02 ± 1.1	332.3 ± 46.9
Crypts	125.0 ± 14.6	238.50 ± 65.1¶	4.32 ± 0.4	20.79 ± 3.7**	153.7 ± 24.2**
−Fe, +Se					
Tips	105.0 ± 5.5††	45.41 ± 12.1††	4.23 ± 0.5	4.69 ± 0.6††	379.8 ± 18.5††
Crypts	114.1 ± 15.5	152.99 ± 46.4¶‡‡	4.02 ±0.3	19.43 ± 2.5**	152.7 ± 25.5¶
+Fe, −Se					
Tips	99.2 ± 6.5††	86.48 ± 9.9	4.19 ± 0.5	5.65 ± 1.1††	209.9 ± 43.8
Crypts	69.1 ± 10.2¶‡‡	195.58 ± 19.0¶	6.25 ± 0.9¶	12.92 ± 2.6¶‡‡	98.2 ± 13.7‡‡
−Fe, −Se					
Tips	93.3 ± 5.7††	56.01 ± 8.1††§§	6.09 ± 0.3††§§		339.4 ± 107.2
Crypts	76.9 ± 8.3¶‡‡	166.78 ± 19.2¶‡‡‖‖	6.69 ± 0.2		81.3 ± 25.1¶

* All values are mean ± S.E.M.; each N represents a pool of mucosal tissue from four rats.
† Microsomes, N = 5.
‡ Whole homogenate, N = 8.
§ 105,000 *g* Supernatant fraction, N = 4.
‖ Solubilized mitochondria, N = 5.
¶ $P < 0.05$ vs corresponding tips.
** $P < 0.005$ vs corresponding tips.
†† $P < 0.05$ vs +Fe, +Se tips.
‡‡ $P < 0.05$ vs +Fe, +Se crypts.
§§ $P < 0.05$ vs +Fe, −Se tips.
‖‖ $P < 0.005$ vs +Fe, −Se crypts.

ALAS and ALAD activities in either cell type, but it significantly reduced their ferrochelatase activities (Table 2). Thus, this reduction might have been responsible for the observed decrease in microsomal heme content (Table 2) and consequently in intestinal cytochrome P-450 content and its associated MFO activity in rats deprived of both iron and selenium (Table 1). Dietary selenium deficiency appeared to lower MHO activity in villous tip cells which would reflect decreased rather than increased heme catabolism but this finding was not statistically significant. On the other hand, it significantly lowered MHO activity in crypt cells (Table 2). Such a reduction may reflect decreased microsomal heme content produced by acute selenium-deficiency in these cells.

Role of dietary selenium in regulation of intestinal cytochrome P-450-dependent MFO activity

Selenium is the prosthetic moiety of GSH-peroxidase [50, 51], an enzyme critical in detoxification of organic and inorganic peroxides [29, 52, 53]. It was conceivable, therefore, that acute dietary selenium deprivation could, by impairing the functional activity of intestinal GSH-peroxidase, result in accumulation of toxic peroxides, capable of intracellular membrane damage and hence cytochrome P-450 inactivation [54, 55]. Indeed, such acute selenium deprivation significantly reduced the selenium-dependent GSH-peroxidase activity of rat villous tip cells to 60% of basal values (Table 3). Feeding of rats with a selenium deficient diet for 2 weeks has been reported to reduce their intestinal GSH-peroxidase activity [56]. Our findings indicate that this reduction occurs much more rapidly, i.e. within just 3 days of dietary selenium deprivation. To determine whether such reduction was responsible for the observed impairment of cytochrome P-450-dependent MFO activity (Table 1), rats acutely deprived of dietary selenium were re-fed the selenium-supplemented diet (+Fe, +Se diet, i.v.) for 0, 1, 2 or 3 days, and the restoration of GSH-peroxidase activity was correlated with reversal of impairment of EROD activity in villous tip cells (Fig. 3). Selenium resupplementation for even a single day reversed villous tip EROD activity to control values without significant restoration of GSH-peroxidase activity (Fig. 3). These findings, coupled with the results depicted in Fig. 1, indicate that the critical role of dietary selenium in maintenance of intestinal cytochrome P-450 structure and function is independent of its function as the prosthetic moiety of GSH-peroxidase.

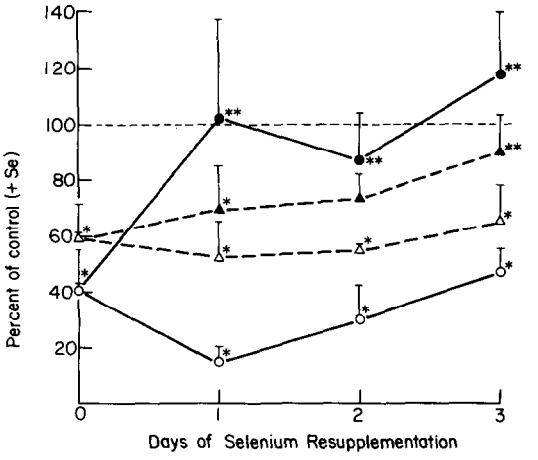

Fig. 3. Time course of dietary selenium resupplementation effects on intestinal villous tip EROD (——) and GSH-peroxidase (– – –) activities. For each experiment, rats were fed the selenium-deficient (+Fe, −Se) semisynthetic diet (open symbols) for 4 days, at which time rats were switched to the selenium-supplemented (+Fe, +Se) diet (closed symbols); additional rats were pair fed as controls (+Fe, +Se; 100% levels) throughout. In each separate experiment, mucosal tissue pooled from three rats was prepared as described (Experimental Procedure). Results are the mean ± S.E. from three experiments. Key: (○) mucosal EROD activity of rats fed the selenium-deficient or (●) selenium-resupplemented diets; (△) mucosal GSH-peroxidase activity of rats fed the selenium-deficient or (▲) selenium-resupplemented diets. Control (+Fe, +Se) values for EROD and GSH-peroxidase activities were 10.6 ± 1.1 pmoles/mg microsomal protein/min and 13.3 ± 1.3 nmoles/mg cytosolic protein/min respectively. A single asterisk (*) indicates values significantly different from control (+Fe, +Se) fed values ($P < 0.05$). A double asterisk (**) indicates values significantly different from selenium-deficient (+Fe, −Se) fed values ($P < 0.05$).

Table 3. Effects of dietary iron and selenium deficiencies on intestinal villous tip cell glutathione peroxidase*

Diet	GSH-peroxidase† (nmoles NADPH/mg protein/min)
+Fe, +Se	9.49 ± 0.4
−Fe, +Se	12.15 ± 0.8‡
+Fe, −Se	6.10 ± 0.6§

* All values are mean ± S.E.M., N = 5; each N represents a pool of mucosal tissue from three rats.
† 105,000 *g* Supernatant, H_2O_2 substrate.
‡ $P < 0.02$ vs +Fe, +Se diet.
§ $P < 0.001$ vs +Fe, +Se diet.

DISCUSSION

The above findings confirm previously reported observations that intraluminal iron deprivation for a period as short as 48 hr results in a markedly reduced microsomal cytochrome P-450 content and its dependent MFO activity in the rat intestinal mucosa [15]. This reduction was again observed to be most pronounced in the villous tip cells, i.e. the very site of iron absorption. Maintenance of intestinal villous cytochrome P-450 structure and function in rats, thus, is critically dependent on intraluminal iron. Our findings indicate that iron is essential for formation of cytochrome P-450 heme moiety in intestinal villous tip cells, not only directly by providing the prosthetic moiety for insertion into protoporphyrin IX as suspected, but more importantly by modulating the intestinal activity of ferrochelatase, the key enzyme catalyzing such insertion. Moreover, acute intraluminal iron deprivation also reduced intestinal ALAS activity, indicating a regulatory role of iron at this rate-limiting step.

Muller-Eberhard and co-workers [57, 58] have reported similar reduced activity of hepatic ALAS and ferrochelatase in rats fed a low iron diet for a 10- to 30-day period following weaning. In agreement with those reports [57, 58], our findings also suggest that (i) iron is possibly not a repressor of intestinal ALAS since its acute deprivation *reduced* rather than enhanced ALAS activity; and (ii) the lowering of intracellular heme in the intestinal mucosa that was produced by dietary iron deprivation did not appear to derepress the enzyme, as would be expected normally [59, 60], following heme depletion. Accordingly, demonstration of such classical derepression of the intestinal ALAS may require the presence of intraluminal iron. Thus, the regulation of intestinal heme-synthesizing enzymes appears to be mechanistically similar to that of the corresponding hepatic isozymes. However, in contrast to findings in the liver [57, 58], reduction of heme-synthesizing activity in the intestinal mucosa was associated with a marked fall in villous tip cytochrome P-450 content and activity. These findings indicate that the villous tip hemoprotein, unlike its hepatic isozyme, is critically dependent on heme synthesized *de novo* and *in situ* from intraluminal iron. This is not surprising since the enterocyte appears to enrich itself in cytochrome P-450 during its upward migration to the villous tip [15, 17, 18]. On the other hand, under the same conditions of acute dietary iron deprivation, crypt cells were capable of maintaining their cytochrome P-450 content, thus resembling the hepatocyte in this aspect [57]. It is noteworthy that such iron-dependent modulation of intestinal villous tip heme and hemoprotein formation, in concert with intestinal cell turnover kinetics, was extremely rapid, unlike that observed in the liver [58].

More intriguing, however, is our finding that intestinal cytochrome P-450 was also critically dependent on intracellular selenium. Acute deprivation of intraluminal selenium for a single day (Fig. 1) resulted in a marked fall in intestinal cytochrome P-450-dependent EROD activity. This fall was apparently solely associated with reduced cytochrome P-450 content, since intestinal NADPH-cytochrome P-450 (*c*) reductase activity was unaltered even after a more rigorous dietary regimen of selenium deprivation (Fig. 2). Moreover, GSH-peroxidase activity also remained unchanged at this time. Oral selenium deprivation for a further 2-day period lowered GSH-peroxidase to 70% of its basal level, but it did not reduce EROD activity further. Resupplementation of dietary selenium for a single day dramatically restored EROD activity to normal levels without significant concurrent restoration of GSH-peroxidase activity (Fig. 3). Thus, this effect of selenium on intestinal cytochrome P-450-dependent EROD activity appears to be independent of its well-recognized prosthetic function in GSH-peroxidase [50, 51]. That is, selenium did not appear to maintain intestinal cytochrome P-450 by protecting it from inactivation by potentially pernicious intracellular peroxides that might have accumulated as a consequence of impaired GSH-peroxidase activity.

The finding of a prompt fall in the functional activity of intestinal cytochrome P-450 following acute dietary selenium deprivation (Fig. 1), and its dramatic reversal on selenium resupplementation (Fig. 3) in rats fed inducer free diets, confirms not only that the constitutive form of intestinal cytochrome P-450 exhibits dynamic turnover characteristics (as indeed suggested by studies of dietary iron deprivation and resupplementation [15]) but, also, that it is critically dependent on intracellular selenium. A similar requirement has been demonstrated recently for the *de novo* synthesis of a phenobarbital inducible species of hepatic cytochrome P-450 in primary non-proliferating cultures of adult rat hepatocytes [61]. In contrast, maintenance of the constitutive form of hepatic cytochrome P-450, with relatively slow turnover characteristics, was not dependent on selenium [61, 62]. Parallel findings of intracellular selenium requirements in cultured hepatocytes and intestinal villous tip cells indicate that cells actively synthesizing cytochrome P-450 are critically dependent on exogenously supplied selenium. Such acute dependence of intestinal cytochrome P-450 on exogenous selenium is in contrast to that of the hepatic isozymes and their dependent MFO activity in rats, which are unaffected even by more rigorous dietary deprivation, i.e. selenium-withdrawal instituted postweaning and subsequent maintenance on a selenium-deficient diet for 8–12 weeks [26, 27]. Thus, the intestinal cell apparently derives its selenium supplement solely from the diet, whereas the liver may derive its requirement from the whole body stores of selenium. This is consistent with the widely recognized fact that induction of selenium deficiency in most tissues of the rat is an impossible feat [63].

These studies thus suggest that rapidly regenerating tissues such as the intestinal mucosa exhibit inordinately high selenium turnover characteristics. If selenium is also required for maintenance of cytochrome P-450 content of such tissues, then acute selenium deprivation may greatly impair their abilities to detoxify potential carcinogens and other noxious xenobiotics. In addition, acute deprivation of selenium by reducing GSH-peroxidase activity may considerably reduce the potential of the intestinal mucosa (and possibly other rapidly turning over tissues) to detoxify notoriously toxic organic (lipid) peroxides and hydroperoxides, thereby enhancing the intracellular peroxidative potential of the tissue and promoting a scenario for chemical carcinogenesis.

In summary, we report that short-term deficiencies of nutrients such as iron and selenium rapidly and profoundly affected intestinal cytochrome P-450 content and function. Of the two intestinal mucosal cell populations, the villous tips appeared by far to be more responsive and/or sensitive to such intraluminal influences than are the cells in the crypts of Lieberkuhn. Thus, dietary constituents appear to affect significantly xenobiotic metabolizing enzymes in the very cells of the intestinal mucosa which are committed to their absorption. This finding is of profound clinical significance given the impressive growing list of commonly used drugs in the therapeutic arsenal, whose biodisposition entails a predominant intestinal "first pass" [1–7]. Alteration of the intestinal "first pass" of any such drug might severely hamper its delivery to pharmacological sites and thereby modify

its effectiveness. On the other hand, impaired intestinal detoxification of other ingested xenobiotics, either intentionally supplemented (food additives) or naturally occurring (carcinogens) in the diet, may constitute a decisive risk factor in environmentally induced gastrointestinal carcinogenesis.

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REFERENCES

1. P. A. Routledge and D. G. Shand, *A. Rev. Pharmac. Toxic.* **19**, 447 (1979).
2. W. A. Colburn, I. Bekersky, B. H. Min, B, J. Hodshon and W. A. Garland, *Res, Commun. Chem. Path. Pharmac.* **27**, 73 (1980).
3. B. Kaye and D. W. Roberts, *Xenobiotica* **10**, 97 (1980).
4. H. K. Seitz, M. A. Korsten and C. S. Lieber, *Life Sci.* **25**, 1443 (1979).
5. E. J. Pantuck, K. C. Hsiao and S. A. Kaplan, *J. Pharmac. exp. Ther.* **191**, 45 (1974).
6. R. E. Poore and R. A. Neal, *Toxic. appl. Pharmac.* **23**, 759 (1972).
7. L. W. Wattenberg, J. L. Leong and P. J. Strand, *Cancer Res.* **22**, 1120 (1962).
8. R. M. Welch, J. Cavallito and A. Loh, *Toxic. appl. Pharmac.* **23**, 749 (1972).
9. N. G. Zampaglione and G. J. Mannering, *J. Pharmac. exp. Ther.* **185**, 676 (1973).
10. S. J. Stohs, R. C. Grafstrom, M. D. Burke and S. Orrenius, *Drug Metab. Dispos.* **4**, 517 (1976).
11. C. Lehrmann, V. Ullrich and W. Rummel, *Naunyn-Schmiedeberg's Archs. Pharmac.* **276**, 89 (1973).
12. E. J. Pantuck, K. Hsiao, R. Kuntzman and A. Conney, *Science* **187**, 744 (1975).
13. R. S. Chhabra, R. J. Pohl and J. R. Fouts, *Drug Metab. Dispos.* **2**, 443 (1974).
14. E. Hietanen, M. Laitinen, M. Lang and H. Vainio, *Pharmacology* **13**, 287 (1975).
15. H. Hoensch, C. H. Woo, S. B. Raffin and R. Schmid, *Gastroenterology* **70**, 1063 (1976).
16. V. Ullrich and P. Weber, *Biochem. Pharmac.* **23**, 3309 (1974).
17. J. R. Dawson and J. W. Bridges, *Biochem. Pharmac.* **30**, 2415 (1981).
18. F. Hartman, R. Owen and D. M. Bissell, *Am. J. Physiol.* **242**, G147 (1982).
19. W. D. Loub, L. W. Wattenberg and D. W. Davis, *J. natn. Cancer Inst.* **54**, 985 (1975).
20. K. C. Hsiao, E. J. Pantuck, L. W. Wattenberg, R. Kuntzman, M. Jacobson and A. H. Conney, *Fedn. Proc.* **34**, 742 (1975).
21. B. G. Lake, R. Hopkins, J. Chakraborty, J. W. Bridges and D. V. W. Parke, *Drug Metab. Dispos.* **1**, 342 (1973).
22. L. W. Wattenberg, *Toxic. appl. Pharmac.* **23**, 741 (1972).
23. T. E. Edes, S. K. Clinton, C. R. Truex and W. J. Visek, *Proc. Soc. exp. Biol. Med.* **162**, 71 (1979).
24. R. P. Clement, P. J. Tofilon and W. N. Piper, *Nutr. Cancer* **3**, 63 (1981).
25. M. Laitinen, *Gen. Pharm.* **7**, 263 (1976).
26. R. F. Burk and M. A. Correia, *Biochem. J.* **168**, 105 (1977).
27. M. A. Correia and R. F. Burk, *J. biol. Chem.* **253**, 6203 (1978).
28. P. Uotila and J. Marniemi, *Biochem. Pharmac.* **25**, 2323 (1976).
29. R. A. Lawrence and R. F. Burk, *Biochem. biophys. Res. Commun.* **71**, 952 (1976).
30. J. M. Dietschy and M. D. Siperstein, *J. clin. Invest.* **44**, 1311 (1965).
31. A. R. Imondi, M. E. Balis and M. Lipkin, *Expl. Cell Res.* **58**, 323 (1969).
32. S. J. Stohs, R. C. Grafstrom, M. D. Burke, P. W. Moldeus and S. G. Orrenius, *Archs. Biochem. Biophys.* **177**, 105 (1976).
33. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
34. J. Kowal, E. R. Simpson and R. W. Estabrook, *J. biol. Chem.* **245**, 2438 (1970).
35. A. P. Alvares, G. Schilling, W. Levin and R. Kuntzman, *Biochem. biophys. Res. Commun.* **29**, 521 (1967).
36. M. D. Burke and R. T. Mayer, *Drug Metab. Dispos.* **2**, 583 (1974).
37. K. M. McCormack, S. Z. Cagen, D. E. Rickert, J. E. Gibson and J. G. Dent, *Drug Metab. Dispos.* **7**, 252 (1979).
38. L. J. Strand, A. L. Swanson, J. Mannering, S. Branch and H. S. Marver, *Analyt. Biochem.* **47**, 457 (1972).
39. V. N. Finelli, L. Murthy, W. B. Peirano and H. G. Petering, *Biochem. biophys. Res. Commun.* **60**, 1418 (1974).
40. S. P. C. Cole, E. J. Vavasour and G. S. Marks, *Biochem. Pharmac.* **28**, 3533 (1979).
41. R. J. Porra and O. T. G. Jones, *Biochem. J.* **87**, 186 (1963).
42. J-H. Fuhrhop and K. M. Smith, in *Porphyrins and Metalloporphyrins* (Ed. K. M. Smith), p. 784. Elsevier, Amsterdam (1975).
43. M. S. Jones and O. T. G. Jones, *Biochem. J.* **113**, 507 (1969).
44. S. B. Raffin, C. H. Woo, K. T. Roost, D. C. Price and R. Schmid, *J. clin. Invest.* **54**, 1344 (1974).
45. M. A. Correia and R. Schmid, *Biochem. biophys. Res. Commun.* **65**, 1378 (1975).
46. K. G. Paul, H. Theorell and A. Akeson, *Acta chem. scand.* **7**, 1284 (1953).
47. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
48. C. H. Williams, Jr. and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
49. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
50. S. H. Oh, H. E. Ganther and W. G. Hoekstra, *Biochemistry* **13**, 1825 (1974).
51. D. G. Hafeman, R. A. Sunde and W. G. Hoekstra, *J. Nutr.* **104**, 580 (1974).
52. A. L. Tappel, *Fedn. Proc.* **24**, 73 (1965).
53. W. G. Hoekstra, *Fedn. Proc.* **34**, 2083 (1975).
54. E. G. Hrycay and P. J. O'Brien, *Archs Biochem. Biophys.* **147**, 14 (1971).
55. R. Tenhunen, H. S. Marver and R. Schmid, *J. Lab. clin. Med.* **75**, 410 (1970).
56. K. Reddy and A. L. Tappel, *J. Nutr.* **104**, 1069 (1978).
57. I. N. H. White, J. A. White, H. H. Liem and U. Muller-Eberhard, *Biochem. Pharmac.* **27**, 865 (1978).
58. H. H. Liem, A. Smith and U. Muller-Eberhard, *Biochem. Pharmac.* **28**, 1753 (1979).
59. F. De Matteis, in *Enzyme Induction* (Ed. D. V. Parke), pp. 185–205. Plenum Press, New York (1975).
60. U. A. Meyer and H. S. Marver, *Science* **171**, 64 (1971).
61. S. Newman and P. S. Guzelian, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2922 (1982).
62. R. F. Burk and B. S. S. Masters, *Archs. Biochem. Biophys.* **170**, 124 (1975).
63. R. F. Burk, *Wld. Rev. Nutr. Diet.* **30**, 88 (1978).