Accelerated Hepatic Haem Catabolism in the Selenium-Deficient Rat

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1. Hepatic microsomal cytochrome *P*-450 concentrations are lower in selenium-deficient rats treated with phenobarbital for 4 days than in similarly treated control rats. 2. No defect in haem synthesis was found on the basis of measurements of δ -aminolaevulinate synthase (EC 2.3.1.37), δ -aminolaevulinate dehydratase (EC 4.2.1.24) and ferrioxalase (EC 4.99.1.1) activities, and urinary excretion of δ -aminolaevulinate, porphobilinogen, uroporphyrin and coproporphyrin. 3. No defect in apo-(cytochrome *P*-450) synthesis was found by studies of [^3H]- and [^{14}C]-leucine incorporation into apo-(cytochrome *P*-450) separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. 4. An increase in haem catabolism was found. An 8-fold increase in hepatic microsomal haem oxygenase (EC 1.14.99.3) activity occurred in selenium-deficient rats after phenobarbital treatment, compared with a less than 2-fold increase in control rats. Also excretion of ^{14}CO in the breath after administration of δ -amino[5- ^{14}C]laevulinate was greater by phenobarbital-treated selenium-deficient rats than by similarly treated controls. 5. These studies demonstrate that the defective induction of cytochrome *P*-450 by phenobarbital in selenium-deficient rats is accompanied by increased haem catabolism. This could be due to increased breakdown of cytochrome *P*-450 or to catabolism of haem before it attaches to the apo-cytochrome. The role of selenium in stabilizing cytochrome *P*-450 and/or in protecting haem from breakdown remains to be determined.

Involvement of selenium with the hepatic microsomal cytochrome *P*-450 system has long been suspected (Diplock *et al.*, 1971; Siami *et al.*, 1972), but only recently has the system been investigated in detail by using selenium-deficient rats (Burk & Masters, 1975). That study showed induction of cytochrome *P*-450 by phenobarbital to be impaired in selenium deficiency.

The induction of cytochrome *P*-450 by phenobarbital involves several processes. Apo-(cytochrome *P*-450) and haem must be synthesized in increased amounts, and haem must be transported from the mitochondria to the endoplasmic reticulum and attached to the apo-cytochrome. Failure of normal phenobarbital-mediated cytochrome *P*-450 induction could be due to defects in any of these processes. Alternatively, enhanced degradation of cytochrome *P*-450 or of haem could impair induction.

We reported preliminary studies showing normal activity of δ -aminolaevulinate synthase (EC 2.3.1.37), the rate-limiting enzyme in haem synthesis, but enhanced hepatic microsomal haem oxygenase (EC 1.14.99.3) activity in selenium-deficient rats treated with phenobarbital (Correia & Burk, 1976c).

These observations suggested that increased catabolism of haem or cytochrome *P*-450 occurred, rather than diminished haem synthesis. The present paper reports the results of a more detailed examination of both the haem-synthetic pathway and haem catabolism in phenobarbital-treated selenium-deficient rats. Additionally, synthesis of apo-(cytochrome *P*-450) has been investigated in these rats. Part of this work was presented at the meeting of the Federation of American Societies of Experimental Biology in April 1976 (Correia & Burk, 1976a).

Experimental

Chemicals

δ -Aminolaevulinate, ATP, NADPH, reduced glutathione, pyridoxal 5'-phosphate, CoA and haemin hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. [1,4- ^{14}C]-Succinate, [4,5- ^3H]- and [U- ^{14}C]leucine, [^{55}Fe]-ferrous citrate and δ -amino[5- ^{14}C]laevulinate were obtained from New England Nuclear Corp., Boston, MA, U.S.A. Porphobilinogen was obtained from Porphyrin Products, Logan, UT, U.S.A.

Animals

Male Holtzman rats were used. They were given tap water and fed *ad libitum* with a semi-synthetic diet (Burk & Masters, 1975) with *Torula* yeast as a protein source and corn oil as a fat source from the time of weaning (20–21 days). The diet contained 100 i.u. of vitamin E/kg as DL- α -tocopheryl acetate. Control rats were fed on the same diet supplemented with 0.5 mg of selenium/kg as Na₂SeO₃. Selenium deficiency was verified by measurement of glutathione peroxidase (EC 1.11.1.9) in the livers of some of the rats fed on this diet (Lawrence & Burk, 1976). After 4 weeks, liver glutathione peroxidase activity measured with H₂O₂ as substrate was invariably less than 5% of control. Rats used in these experiments had been fed on the respective diets for 12 weeks or more and weighed 250–300 g. Control and selenium-deficient animals were age-matched in each experiment.

Treated rats received 80 mg of phenobarbital sodium/kg intraperitoneally daily for 4 days. All rats were starved for 18 h after the last injection, and then stunned and decapitated. The livers were perfused *in situ* with 0.15 M-KCl and then excised and weighed.

Assays

(1) *δ -Aminolaevulinate synthase activity.* Liver homogenates in 0.1 M-pyridoxal phosphate/0.9% NaCl/0.01 M-Tris/HCl buffer, pH 7.4, were used to assay δ -aminolaevulinate synthase activity essentially by the method of Strand *et al.* (1972).

(2) *δ -Aminolaevulinate dehydratase activity (EC 4.2.1.24).* The enzyme activity was assayed in the soluble fraction (100 000g supernatant) of 50% (w/v) liver homogenate in 0.1 M-sodium phosphate buffer, pH 6.8, as described by Finelli *et al.* (1974).

(3) *Ferrochelatase activity.* Liver mitochondria were prepared as follows. Liver homogenate (25%, w/v) in 0.25 M-sucrose/0.1 M-sodium phosphate buffer, pH 7.4, was centrifuged at 1000g for 10 min and the supernatant centrifuged at 10000g for 10 min. The pellet so obtained was suspended in 0.25 M unbuffered sucrose and resedimented at 10000g for 10 min. The pellet was resuspended in 0.25 M-sucrose to a final concentration of 4–5 mg of protein/ml.

Ferrochelatase activity was determined by a modification of the method of Yoda & Israels (1972) as follows. Liver mitochondria were incubated at 37°C for 30 min in a shaking water bath, in the presence of 0.2 M-Tris/HCl, pH 7.4, 0.2 M-sucrose, 10 mM-sodium succinate, 1 mM-protoporphyrin IX- α in 0.1% Tween 80/0.2 M-Tris/HCl, pH 7.4, and [⁵⁵Fe]-ferrous citrate (1 μ Ci; New England Nuclear, NEZ-052, lot no. 6741, specific radioactivity 13.9 mCi/mg). The reaction was terminated by chilling the reaction mixture in ice and then freezing it. Appropriate zero-time and non-enzymic [⁵⁵Fe]-

haem controls were included. Washed rat erythrocytes suspended in 0.14 M-NaCl and containing known quantities of haemoglobin were added as a haem carrier. Haemin was isolated from this mixture by the method of Labbe & Nishida (1957) and dissolved in 0.2 ml of 1 M-NaOH. Then 0.2 ml of 30% H₂O₂ and 10 ml of Dimilume (Packard Instrument Co., Downers Grove, IL, U.S.A.) were added, the ⁵⁵Fe radioactivity was determined in a Beckman liquid-scintillation spectrometer (model LS-250) by using the wide ³H-iso-set module. Ferrochelatase activity was expressed as d.p.m. of ⁵⁵Fe incorporated into haem/30 min per mg of protein.

The activity of the mitochondrial marker enzyme cytochrome oxidase (EC 1.9.3.1) was determined by the method of Wharton & Tzagoloff (1967).

(4) *Microsomal haem oxygenase activity.* This was determined in the 18000g supernatant of liver and spleen homogenate, as described previously (Correia & Burk, 1976b).

(5) *Cytochrome P-450 and protein.* These were determined by the methods of Raj & Estabrook (1970) and Lowry *et al.* (1951) respectively. The protein standard was bovine serum albumin.

(6) *Determination of porphyrin precursors in urine.* Urinary δ -aminolaevulinate and porphobilinogen were determined by the method of Davis & Andelman (1967). Coproporphyrin and uroporphyrin were determined fluorimetrically as described by Schwartz *et al.* (1960).

Determination of apo-cytochrome synthesis

Pairs of control and phenobarbital-treated rats, starved for 18 h after the last dose of phenobarbital, received intraperitoneally 50 μ Ci of L-[U-¹⁴C]leucine and 200 μ Ci of L-[4,5-³H]leucine respectively. Thus incorporation of [¹⁴C]- and [³H]-leucine into protein is used as an estimate of basal and phenobarbital-stimulated synthetic rates respectively. The rats were killed 4 h later, livers were perfused with 0.25 M unbuffered sucrose, and 50% (w/v) liver homogenates were prepared in 0.25 M-sucrose/0.1 M-phosphate buffer, pH 7.4. Equal volumes of the homogenates from each pair member were combined and microsomal fractions were prepared and washed in 0.25 M-sucrose. Portions of microsomal fractions containing 250 μ g of protein were electrophoresed by the sodium dodecyl sulphate/polyacrylamide-slab-gel method described by Siekevitz (1973). Purified cytochrome P-450 from phenobarbital-treated rat liver, kindly given by Dr. Wayne Levin and Dr. Dene Ryan of Hofmann-La Roche, Nutley, NJ, U.S.A., was used as a standard. Fig. 1 shows a gel with the standard and a microsomal sample after electrophoresis. The gels were sliced as indicated and digested in Protosol/NH₃/Liquifluor (New England Nuclear) at 50°C for 48 h (Ward *et al.*, 1970). ¹⁴C and ³H radioactivity in each slice were determined in a

Beckman liquid-scintillation spectrometer (model LS-250).

¹⁴CO in expired air

Control and phenobarbital-treated rats were starved for 18 h after the last dose of phenobarbital and were given δ -amino[5-¹⁴C]laevulinate (5 μ Ci) via the tail vein at zero time. They were then put into a CO-collection device previously described (Landaw *et al.*, 1970) and ¹⁴CO was trapped and measured.

Results

The data presented in Table 1 confirm previous observations that phenobarbital induction of hepatic microsomal cytochrome *P*-450 is impaired in selenium-deficient rats (Burk *et al.*, 1974; Burk & Masters, 1975). The cytochrome *P*-450 values are somewhat higher than those reported in the above two papers, possibly because of the different assay method used.

Since haem is required for the synthesis of cytochrome *P*-450, the haem-synthetic pathway was studied and the activities of three enzymes in this pathway are shown in Table 2. δ -Aminolaevulinate synthase, the rate-limiting enzyme, was increased by phenobarbital treatment, but activities were similar in

selenium-deficient and control rat livers. No effects of selenium status and/or phenobarbital treatment on δ -aminolaevulinate dehydratase or ferrochelatase activities were detected. Ferrochelatase is a mitochondrial inner-membrane enzyme, so its activity was also calculated on the basis of the activity of the similarly localized marker enzyme, cytochrome oxidase. Again, no effects of phenobarbital treatment or selenium status were observed when expressed in terms of activity relative to cytochrome oxidase activity (results not shown).

Urinary porphyrins and porphyrin precursors are often increased by enzyme defects in the haem-synthetic pathway. The urinary excretion of these substances is shown in Table 3. δ -Aminolaevulinate excretion by control rats was increased by phenobarbital. No such increase was observed in selenium-deficient rats, and no differences between selenium-deficient and control rats were seen. No significant effect of selenium status or phenobarbital on excretion of porphobilinogen or uroporphyrin was found. Selenium-deficient rats excreted more coproporphyrin than did controls, but owing to the large variations, it was significant only for the group not treated with phenobarbital. A few (phenobarbital-treated selenium-deficient) rats excreted large quantities of porphobilinogen, uroporphyrin and copro-

Table 1. *Effect of selenium deficiency and phenobarbital treatment on hepatic microsomal cytochrome P-450 content*
Phenobarbital treatment was with 80 mg/kg body wt. intraperitoneally daily for 4 days. Results are means $\pm$ S.E.M. for *n* experiments. *Not significantly different from untreated controls by unpaired *t* test (*P* > 0.05). **Different from phenobarbital-treated control (*P* < 0.02). †Different from untreated selenium-deficient rats (*P* < 0.02). ††Different from untreated control rats (*P* < 0.02).

Diet	Untreated		Phenobarbital-treated	
	<i>n</i>	(nmol of cytochrome <i>P</i> -450/mg of protein)	<i>n</i>	(nmol of cytochrome <i>P</i> -450/mg of protein)
Selenium-deficient	8	1.17 $\pm$ 0.12*	9	2.13 $\pm$ 0.13**†
Control	9	1.15 $\pm$ 0.09	9	2.58 $\pm$ 0.12††

Table 2. *Effect of selenium deficiency and phenobarbital treatment on some haem-synthetic enzymes*
Phenobarbital treatment was with 80 mg/kg body wt. intraperitoneally daily for 4 days. Results are means $\pm$ S.E.M. for *n* experiments. *Different from untreated selenium-deficient rats (*P* < 0.01). **Different from untreated control rats (*P* < 0.01). All other pairs on the basis of selenium deficiency or phenobarbital treatment not significantly different (*P* > 0.05).

Diet	Phenobarbital	δ -Aminolaevulinate synthase		δ -Aminolaevulinate dehydratase		Ferrochelatase	
		<i>n</i>	(pmol/30 min per mg of protein)	<i>n</i>	(nmol of porphobilinogen formed/30 min per mg of protein)	<i>n</i>	(d.p.m. of [⁵⁵ Fe]-haem formed/30 min per mg of protein)
Selenium-deficient	—	6	631 $\pm$ 81	4	8.5 $\pm$ 0.7	4	1516 $\pm$ 175
	+	9	969 $\pm$ 75*	4	7.4 $\pm$ 0.3	4	2088 $\pm$ 515
Control	—	8	678 $\pm$ 90	4	9.7 $\pm$ 1.4	4	2083 $\pm$ 367
	+	9	973 $\pm$ 31**	4	9.2 $\pm$ 1.1	4	1744 $\pm$ 393

Table 3. *Effect of selenium deficiency and phenobarbital treatment on urinary excretion of porphyrins and porphyrin precursors*
 Phenobarbital treatment was with 80mg/kg body wt. intraperitoneally for 4 days; there were six or more animals in each group. Urine was collected for the 24h after the last injection. Results are means $\pm$ S.E.M. *Different from untreated control rats ($P < 0.03$); **different from untreated control rats. All other pairs on the basis of selenium deficiency or phenobarbital treatment not significantly different ($P > 0.05$).

Diet	Phenobarbital	Excretion ($\mu\text{g}/24\text{h}$)			
		δ -Aminolaevulinate	Porphobilinogen	Uroporphyrin	Coproporphyrin
Selenium-deficient	—	105 $\pm$ 11.0	2.3 $\pm$ 1.5	1.0 $\pm$ 0.1	12.9 $\pm$ 1.2**
	+	108 $\pm$ 17.3	34.1 $\pm$ 28.6	3.2 $\pm$ 1.8	29.4 $\pm$ 16.4
Control	—	78.3 $\pm$ 9.4	8.8 $\pm$ 6.5	0.6 $\pm$ 0.1	3.5 $\pm$ 0.7
	+	136 $\pm$ 20.6*	5.7 $\pm$ 1.7	0.8 $\pm$ 0.2	4.6 $\pm$ 0.9

porphyrin. This led to much higher average excretions in this group, but also to large variations which precluded statistically significant differences.

The synthesis of apo-(cytochrome *P*-450) was investigated by a double-labelling technique described in the Experimental section. This technique allows an estimate of an average synthesis rate of all apoprotein forms of cytochrome *P*-450. It appears to be the best current means of obtaining such an estimate, since purification of cytochrome *P*-450 is very difficult. Fig. 1 shows sodium dodecyl sulphate/polyacrylamide gels of purified cytochrome *P*-450 and of microsomal fraction. Gels were sliced so that slice C, corresponding to the standard apo-cytochrome electrophoretic bands, contained the cytochrome *P*-450 proteins. Table 4 illustrates the results of the incorporation of labelled leucine *de novo* into the gel slice containing liver microsomal cytochrome *P*-450 apoprotein. It demonstrates a greater phenobarbital-mediated increase in the incorporation of the label ($[^3\text{H}]$ leucine) into the proteins of slice C in selenium-deficient rats than in controls. Since apo-(cytochrome *P*-450) makes up most of the slice-C protein, this suggests that the phenobarbital-mediated increase in apo-(cytochrome *P*-450) synthesis not only is unimpaired, but is even greater in selenium-deficient rats than in controls. Furthermore labelling of gel slice C with $[^{14}\text{C}]$ leucine was comparable in untreated selenium-deficient and control rats.

Haem catabolism was assessed by measurement of activities in liver and spleen of microsomal haem oxygenase, the rate-limiting enzyme in haem degradation. Table 5 shows that phenobarbital treatment caused an 8-fold rise in this activity in livers from selenium-deficient rats. In control animals only a slight rise caused by phenobarbital was observed in hepatic microsomal haem oxygenase activity. In contrast, no significant differences were observed in the microsomal haem oxygenase activities in untreated selenium-deficient and control rats. Microsomal haem oxygenase activity in control spleens (Table 5) decreased after phenobarbital treatment. It

Fig. 1. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of microsomal fraction*

After electrophoresis as described in the Experimental section, gels were sliced as indicated in the Figure. (a) Cytochrome *P*-450 purified from phenobarbital-treated rat liver microsomal fraction; (b) whole microsomal preparation. The results in Table 4 correspond to the slices as indicated.

remained unchanged in spleens from selenium-deficient rats.

Fig. 2 shows excretion of ^{14}CO in breath after prelabelling of the haem by injection of δ -amino $[^{14}\text{C}]$ -

Table 4. *Effect of selenium deficiency and phenobarbital treatment on incorporation of labelled leucine into microsomal proteins*

Values are total d.p.m. of [³H]- or [¹⁴C]-leucine per slice; all values are means±s.e.m. for three rats. Phenobarbital treatment was with 80mg/kg body wt. intraperitoneally daily for 4 days. *Different from phenobarbital-treated controls (*P* < 0.03); no other significant differences when selenium-deficient and control rats compared.

Gel slice (Fig. 1)	Selenium-deficient			Control		
	Phenobarbital (d.p.m. of ³ H)	No phenobarbital (d.p.m. of ¹⁴ C)	³ H/ ¹⁴ C	Phenobarbital (d.p.m. of ³ H)	No phenobarbital (d.p.m. of ¹⁴ C)	³ H/ ¹⁴ C
A	1060±219	277±35	3.8	815±71	291±46	2.8
B	1510±361	383±48	3.9	1040±68	366±43	2.9
C	5920±162*	865±75	6.9	3730±600	867±80	4.3
D	874±67	271±38	3.2	556±33	237±9	2.3
E	487±81	205±38	2.4	657±76	198±23	3.3

Table 5. *Effect of selenium deficiency and phenobarbital treatment on microsomal haem oxygenase activity in 18000g supernatant*

Phenobarbital treatment was with 80mg/kg body wt. intraperitoneally daily for 4 days. Results are means±s.e.m. for the numbers of rats given in parentheses. *Different from untreated selenium-deficient rats (*P* < 0.03). **Different from untreated control rats (*P* < 0.03). ***Different from phenobarbital-treated control rats (*P* < 0.03). †Different from untreated control rats (*P* < 0.03). ††Different from untreated control rats (*P* < 0.03). All other pairs not significantly different (*P* > 0.05).

Diet	Phenobarbital	Haem oxygenase	
		Liver	Spleen
Selenium-deficient	—	0.15±0.03 (5)	0.51±0.05 (6)†
	+	1.19±0.12 (6)*,***	0.50±0.03 (8)
Control	—	0.12±0.03 (4)	0.69±0.05 (6)
	+	0.21±0.01 (4)**	0.38±0.06 (6)††

Fig. 2. ¹⁴CO expired by selenium-deficient and control rats with and without phenobarbital treatment after δ -amino[¹⁴C]-laevulinate injection

See the Experimental section for details. ○, Selenium-deficient rats; ●, controls. Rats were studied in pairs, and owing to uncontrollable factors may only be compared with pair-mates (Bissell & Hammaker, 1976). (a) shows results from a pair of untreated rats and (b) shows results from a pair of phenobarbital-treated rats. Experiments were done twice, with similar results each time.

laevulinate. This correlates with [^{14}C]bilirubin excretion and represents the oxidation of the ^{14}C -labelled α -methene bridge of the haem moiety by haem oxygenase, followed by its cleavage (Landaw *et al.*, 1970). In untreated rats (Fig. 2a) there was no effect of selenium deficiency on ^{14}CO production. In contrast, ^{14}CO excretion was greater by selenium-deficient rats than by controls after phenobarbital treatment (Fig. 2b).

Discussion

Impairment of haem synthesis or enhancement of haem catabolism by various agents results in diminished cytochrome *P*-450 concentrations and/or impairment of its induction by phenobarbital (De Matteis, 1975; Maxwell & Meyer, 1976). Thus selenium deficiency could exert its effect on cytochrome *P*-450 through either or both of these mechanisms. Mackinnon & Simon (1976) postulated that hepatic haem synthesis is defective in selenium deficiency, but we found no evidence of such a defect. The haem-synthetic-pathway enzymes studied here had similar activities in selenium-deficient and in control rat livers. We previously reported that there was no effect of selenium status on incorporation of δ -amino[^{14}C]laevulinate *in vitro* into hepatic haem (Correia & Burk, 1976c), which suggests that the enzyme activities that were not measured individually were not diminished.

Further evidence that haem synthesis was not directly impaired by selenium deficiency was the lack of consistently increased urinary excretion of haem precursors by selenium-deficient rats. Some selenium-deficient rats did excrete large amounts of haem precursors after phenobarbital treatment, however, and subsequent studies have suggested a cause for this. δ -Aminolaevulinate synthase is normally the rate-limiting enzyme of the haem-synthetic pathway. The induction of this enzyme 4 h after a single dose of phenobarbital is much greater in selenium-deficient rat livers than in controls (M. A. Correia & R. F. Burk, unpublished work). Under these conditions other steps in the pathway could become rate-limiting and excretion of precursors could occur. In the present experiments, δ -aminolaevulinate synthase activity was measured 24 h after the last dose of phenobarbital, by which time no effect of selenium deficiency on δ -aminolaevulinate synthase activity could be detected.

We found strong evidence for increased hepatic haem catabolism in selenium-deficient phenobarbital-treated rats. Hepatic microsomal haem oxygenase activity in this group was 6–10-fold that found in the other groups. Microsomal haem oxygenase catalyses the conversion of haem into biliverdin, CO and Fe^{2+} and serves as the rate-limiting step in haem catabolism (Tenhunen *et al.*, 1969). Its activity fluctuates

depending on haem load under certain conditions, and increases in its activity are thought to indicate stimulated rates of haem catabolism (Bissell *et al.*, 1974; Bissell & Hammaker, 1976). Furthermore the greater ^{14}CO excretion in phenobarbital-treated selenium-deficient rats than in similarly treated control rats (Fig. 2b) after δ -amino[5- ^{14}C]laevulinate injection also indicates increased haem breakdown in this group. Since no increase of microsomal haem oxygenase activity occurred in the spleen, it seems likely that the increase in haem catabolism is confined to the liver.

Neither haem oxygenase activity nor ^{14}CO excretion was significantly elevated here in untreated selenium-deficient rats as compared with the controls. However, in subsequent studies with rats fed on the special diets for a longer period of time, hepatic haem oxygenase activity was found to be higher in selenium-deficient rats than in controls, indicating that a defect exists which is independent of phenobarbital (R. F. Burk & M. A. Correia, unpublished work). This sheds some light on the possible role of phenobarbital in the present findings. It is well known that phenobarbital causes a marked increase in haem synthesis. In a normal rat the haem produced after phenobarbital injection is utilized for cytochrome *P*-450 synthesis. However, it appears that in the selenium-deficient liver this utilization is defective and haem is catabolized. Thus the role of phenobarbital as observed here seems to be to aggravate an existing defect in haem metabolism by increasing the production of haem.

The finding of markedly increased haem catabolism and normal or increased synthesis under these conditions could be due to any of several processes. Diminished apo-cytochrome synthesis could result in higher concentrations of 'free' haem and thus in increased haem catabolism. However, the data in Table 4 suggest that synthesis of the apo-cytochrome(s) is increased by selenium deficiency, rather than decreased by it. The possibility still remains that the apo-cytochrome synthesized in selenium deficiency is defective and does not bind haem adequately.

The only well-defined biochemical function of selenium in animals is as a constituent of the enzyme glutathione peroxidase (EC 1.11.1.9) (Oh *et al.*, 1974). It is not resolved at present whether selenium exerts its effect on haem metabolism through this enzyme, although our preliminary data suggest that it does not (Correia & Burk, 1976b). Cytochrome *P*-450 can be destroyed by lipid hydroperoxides (Mannering *et al.*, 1975), and it is possible that accumulation of these substances could occur in the absence of glutathione peroxidase activity and cause enhanced cytochrome *P*-450 breakdown with consequent release of haem and induction of microsomal haem oxygenase.

Since the increased haem catabolism observed in selenium-deficient rats is minimal except under

conditions of increased haem synthesis and occurs within hours of a stimulation of haem synthesis, it seems most likely that selenium may serve in haem transport or facilitate its attachment to the apo-cytochrome. Little is known about these processes in the liver cell and more studies will be required to establish whether selenium plays a role in them.

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