Biochemical Pharmacology, Vol. 28, pp. 313–319.

0006-2952/79/0115-0313 $02.00/0

HEPATIC DRUG METABOLISM AFTER PHENOBARBITAL AND DIPHENYLHYDANTOIN ADMINISTRATION IN THE RAT—INFLUENCE OF VITAMIN D_3 STATUS*

MARIELLE GASCON-BARRÉ†, MICHEL G. CÔTE‡ and JULES BRODEUR‡§

Département de pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

(*Received* 6 *December* 1977; *accepted* 23 *May* 1978)

Abstract—Measurements of aniline hydroxylation, aminopyrine *N*-demethylation and cytochrome P-450 content after a 3-week treatment with phenobarbital (PB), diphenylhydantoin (DPH) or a combination of the two drugs were undertaken during normal vitamin D status (D+) and vitamin D deficiency (D−) with or without vitamin D_3 (D_3) supplementation. Serum calcium concentrations were reduced after D deprivation but responded by a significant increase toward normal values to a single pharmacological dose of D_3. Serum phosphorus concentrations were also slightly raised by the supplementation. Even in the presence of higher cytochrome P-450 content in D− rats, aniline hydroxylase and aminopyrine *N*-demethylase activities were lower in D− than in D+ animals. These two enzymatic parameters, as well as cytochrome P-450 content, were increased by anticonvulsant (ACV) drug treatment regardless of the D nutritional status. The *in vivo* hexobarbital sleeping time was shortened by ACV drugs but the sleeping time tended to be longer in D− than in D+ rats. Supplementation with 1000 I.U. of D_3 lowered aniline hydroxylase activity both in D+ and D− animals; the supplementation had no effect on aminopyrine *N*-demethylase activity in D+ animals but had an inhibitory effect after PB and a stimulatory effect after DPH treatment in D− animals. Cholecalciferol supplementation lowered cytochrome P-450 content toward normal values in D− rats while it had no effect in D+ animals. These observations suggest that (1) PB and DPH pretreatment do not alter the normal response of serum calcium and phosphorus to a single pharmacological dose of D_3; (2) in a state of vitamin D deficiency accompanied by hypocalcemia, the inducing capacity of PB and DPH on the liver mixed function oxidase system is not lost; (3) under certain circumstances, vitamin D_3 can influence the catalytic activity of the mono-oxygenase complex; (4) cytochrome P-450 is influenced by vitamin D deficiency and/or changes in extracellular calcium but the forms induced by PB and DPH may not necessarily be the ones specifically involved in vitamin D metabolism.

Much interest has arisen during the past few decades about nutrient–drug relationships. The influence of chronic anticonvulsant (ACV) drug therapy on vitamin D (D, cholecalciferol) metabolism was first suggested by reports from Germany [1, 2] of drug-induced osteomalacia among the epileptic population. Since that time epidemiological and clinical reports which tend to confirm these early observations have been published [3–13]; fundamental studies have also been undertaken to investigate the relationship between ACV drugs and vitamin D handling [14–18].

Under normal physiological conditions, vitamin D is hydroxylated first in the liver in position 25 and then in the kidney in position 1. In man the 25-hydroxy metabolite [25(OH)D] is the most abundant circulating form of the vitamin with normal serum concentrations ranging between 25 and 35 ng ml^{-1} [19, 20], while the 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] is considered to be the active hormone with serum concentrations reported to vary between 2.1 and 4.5 ng dl^{-1} [21]. The involvement of cytochrome P-450 in cholecalciferol 25-hydroxylation, first considered doubtful [22], now appears more certain [23] and the reaction is reported to be carried out in liver microsomes [22] as well as liver mitochondria [23]. *In vivo*, phenobarbital (PB) and diphenylhydantoin (DPH) alter the hepatic metabolism of vitamin D by increasing the production of the active hepatic metabolite as well as accelerating the appearance of inactive polar metabolites [14, 16, 18].

Epileptic patients suffering from florid rickets, osteomalacia or other more subtle signs of vitamin D deficiency already present an abnormal vitamin D status; no reports exist on the characterization of the hepatic mixed function oxidase system (MFO) during vitamin D deficiency or supplementation. This characterization is thought to be important since many ACV drugs are known to induce MFO activity and, although the exact nature of vitamin D 25-hydroxylation is still unclear, ACV drugs and/or vitamin D nutritional status are strongly suspected to influence the reaction. Measurements of aniline hydroxylase and aminopyrine *N*-demethylase activity as well as of amounts of cytochrome P-450 as indicators of the metabolic capacity of the MFO after a 3-week treatment with two of the most widely used ACV drugs, PB and DPH, were undertaken, therefore, during vitamin D_3 deficiency with or without D_3 supplementation. We now report that both ACV drugs and vitamin D_3 status influence the activity of the liver MFO and the amount of cytochrome P-450 in the rat.

*This work was supported by the Medical Research Council of Canada (MRC Group grant in drug toxicology)

†Recipient of a Studentship from "Le Ministère de l'Education" of the Province of Quebec. Mailing address: Centre de Recherches Cliniques, Hôpital Saint-Luc, 1058 rue St.-Denis, Montréal, Québec H2X 3J4, Canada. Send reprint requests to this address.

‡Members of the MRC Group in Drug Toxicology.

§Present address: Director, Département d'hygiène des milieux, Faculté de Médecine, Université de Montréal, Montréal, Québec H3C 3J7, Canada.

METHODS

Animals. For each experiment 12-day-old male Sprague–Dawley rats (Canadian Breeding Farm and Laboratories, St-Constant, Quebec) were ordered with nursing females and were deprived of vitamin D upon their arrival. At weaning the animals were housed individually in hanging stainless steel wire cages and fed a vitamin D-deficient diet (D-) containing 1.09% calcium and 0.9% phosphorus [24]. The non-deficient animals (D+) were fed the same diet but received 75 I.U. of vitamin D_3 (Mann Swartz, Orangeburg, NY), by gavage three times a week, while the D- animals received the vehicle only, 1,2-propanediol (Baker Chemical Co., Phillisburg, NJ). At the same time animals from both the D+ and D− groups were also randomly assigned to four drug treatments and for 3 weeks they received a daily gavage of (a) the drug solvent (saline–1,2-propanediol–95% ethanol, 5:4:1) for the control group; (b) 5,5′-diphenylhydantoin (DPH) (Eastman Organic Chemical, Rochester, NY) at a dose of 50 mg kg^{-1}; (c) sodium phenobarbital (PB) (British Drug Houses, Canada, Ltd., Montréal, Québec) at a dose of 50 mg kg^{-1}; or (d) the combination of the two ACV drugs (PB–DPH); the last drug treatments were given 26 hr before the enzymatic assays, at which time, in similarily treated animals, no drugs could be detected in the plasma. Forty-eight hr before death half of the animals belonging to the eight subgroups mentioned above received 1000 I.U. of cholecalciferol whereas the other half was given the vehicle only 1,2-propanediol.

Enzymatic assays. The animals were fasted for a period of 16 hr prior to death and killed by exsanguination. The livers were excised, washed and homogenized at 4° in 4 vol. of 0.15 M KCl using a Potter–Elvehjem type glass-Teflon homogenizer. The homogenate was centrifuged in an International centrifuge, model B-60, with an A-169 rotor, and enzymatic assays were carried out on the 9000 *g* supernatant *N*-demethylase activity was determined by measuring 4-aminoantipyrine formation according to the method described by Mazël [25] using a Unicam SP 8000 spectrophotometer, at 540 nm. The rate of aniline hydroxylation was determined by measuring the quantity of *p*-aminophenol formed [26] at 640 nm on a Coleman Jr. II colorimeter, model 620. All incubations were carried out for a period of 30 min at 37° in a Dubnoff incubator at 120 oscillations/min. The linearity of the reactions was checked carefully and found to be present over the period of time studied. Cytochrome P-450 was determined on the 105,000 *g* pellet resuspended in 0.15 M KCl and diluted in 0.1 M phosphate buffer, pH 7.4 [27]. Spectra were recorded with a Unicam SP 8000, dual beam spectrophotometer. Protein determinations were made according to the method of Lowry *et al.* [28].

Calcium and phosphorus determination. Calcium was determined according to the method of Gindler and King [29] and phosphorus determinations were made according to the colorimetric method of Natelson [30].

Hexobarbital sleeping time. D-deficient and non-deficient animals were used for this experiment and received the same drug pretreatment as described previously. A single i.p. injection of sodium hexobarbital (Winthrop, Toronto) at a dose of 120 mg kg^{-1} was made and the time elapsed between the loss and return of the righting reflex was calculated.

Statistical analysis. Results for enzymatic activities and cytochrome P-450 content are expressed as activity for total liver/100 g body weight. All *in vitro* experiments were planned and analyzed according to a 2 × 4 factorial analysis of variance [31]. This statistical approach makes it possible to analyze: (1) the influence of a vitamin D supplemented vs a non-supplemented regimen (factor A), (2) the influence of various ACV drugs administered (factor B), and (3) the possible interaction between vitamin D supplementation and ACV drug treatments. The experiment on hexobarbital sleeping time was analyzed according to a one-way analysis of variance because of the uneven number of animals in each group; comparisons between non-supplemented D− and D+ animals (Table 1) were treated according to the two-tailed Student's *t*-test.

RESULTS

Table 1 presents the comparative values between D-deficient and D-normal animals for body weight,

Table 1. Influence of vitamin D_3 status and drug treatment on the activity of the MFO and cytochrome P-450 content*

Drug treatment†	Body weight (g)	Liver wt (g/100 g)	Microsomial proteins‡ (mg/100 g)	Cytochrome P-450‡ (nmolcs/100 g)	Aminopyrine *N*-demethylase‡ (μg/100 g)	Aniline hydroxylase‡ (μg/100 g)
Control	D−§ 109.6 ± 8.4‖	3.1 ± 0.12	74.8 ± 6.5	32.6 ± 2.2¶	99.0 ± 16.7**	363.4 ± 29.1
	D+§ 146.5 ± 5.6	3.3 ± 0.09	72.0 ± 2.4	21.3 ± 0.6	161.9 ± 21.5	371.0 ± 18.0
Diphenylhydantoin	D− 120.5 ± 4.7‖	3.2 ± 0.10	91.9 ± 4.4**	52.7 ± 2.8¶	225.0 ± 24.2‖	446.6 ± 24.4**
	D+ 154.4 ± 8.6	3.4 ± 0.17	77.3 ± 4.6	32.9 ± 2.6	363.1 ± 34.0	536.8 ± 38.8
Phenobarbital	D− 116.1 ± 7.1**	4.3 ± 0.06	150.5 ± 5.3	179.6 ± 2.8‖	674.2 ± 13.0‖	748.3 ± 23.1**
	D+ 141.5 ± 5.6	4.5 ± 0.16	134.6 ± 8.8	118.9 ± 11.8	791.6 ± 33.5	990.6 ± 43.7
Diphenylhydantoin + phenobarbital	D− 135.4 ± 10.8	4.8 ± 0.13	173.1 + 5.1**	183.4 ± 9.2‖	789.2 ± 56.1	794.6 ± 52.6**
	D+ 144.0 ± 2.5	4.7 ± 0.13	149.7 ± 7.7	134.2 ± 11.7	910.4 ± 68.2	1052.0 ± 25.0

*Each value represents the mean ± S. E. M.; n = five animals per group.
†Treatment lasted for a period of 3 weeks; all drugs were administered daily at a dose of 50 mg kg^{-1}.
‡Expressed as per total liver/100 g body weight 30 min.
§D− = vitamin D deficient animals and D+ = vitamin D normal animals.
**Significance of difference between D− and D+ animals, $P < 0.05$.
‖Significance of difference between D− and D+ animals, $P < 0.01$.
¶Significance of difference between D− and D+ animals, $P < 0.001$.

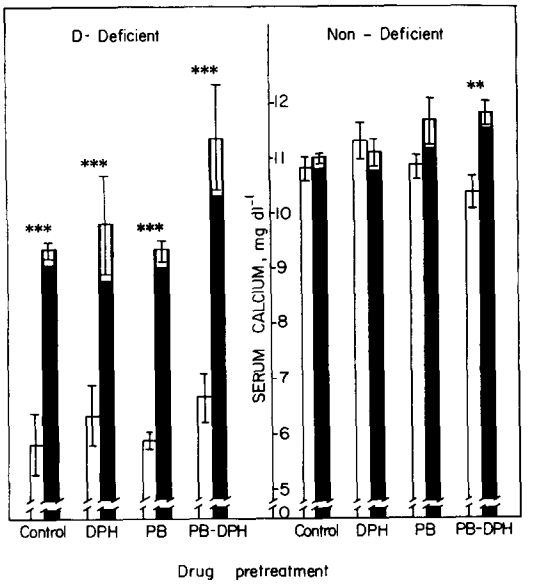

Fig. 1. Influence of a 3-week drug pretreatment and vitamin D_3 nutritional status on the concentration of serum calcium. Key: (■) 1000 I.U. D_3 *per os*, 48 hr before death; and (□) 1,2-propanediol *per os*, 48 hr before death: Each value represents the mean ± S. E. M.; n = five animals per group. Statistically significant from animals receiving 1,2-propanediol only: (**) $P < 0.01$, and (***) $P < 0.001$.

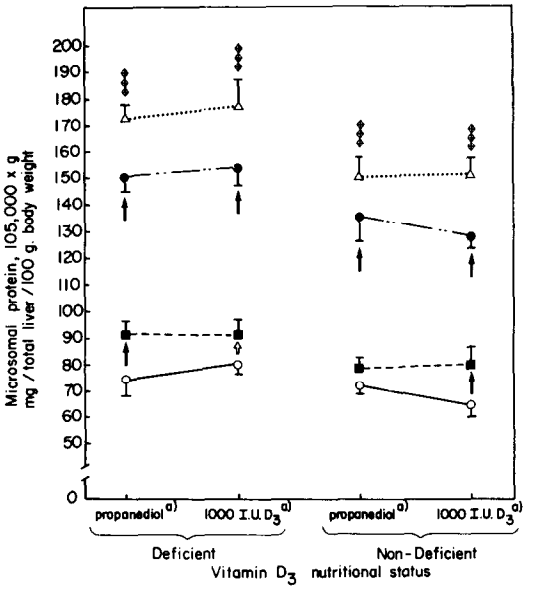

Fig. 3. Influence of a 3-week drug pretreatment and vitamin D_3 nutritional status on the amount of microsomal proteins. Key: (○——○) control, (■- - -■) DPH, (●- - - -●) PB, (△·····△) PB–DPH, and (a) given *per os*, 48 hr before death. Each value represents the mean ± S. E. M.; n = five animals per group: Main effect of drug pretreatment: (‡) $P < 0.001$; and single effect of drug pretreatment: (†) $P < 0.01$, and (†) $P < 0.05$.

liver weight, microsomal proteins, cytochrome P-450 and the enzymatic parameters in control and ACV drug-treated animals that were not supplemented with D_3 before death. Vitamin D deprivation contributed to significantly lower body weight in all but the PB–DPH-treated group. Compared to their D+ counterpart, aniline hydroxylase activity was reduced in all ACV drug-treated D− animals and aminopyrine *N*-demethylase activity in control, DPH and PB-treated D− rats. The deficiency had, on the contrary, a stimulatory effect on cytochrome P-450 in all groups; microsomal proteins were also increased slightly in DPH and PB–DPH D− animals. Liver

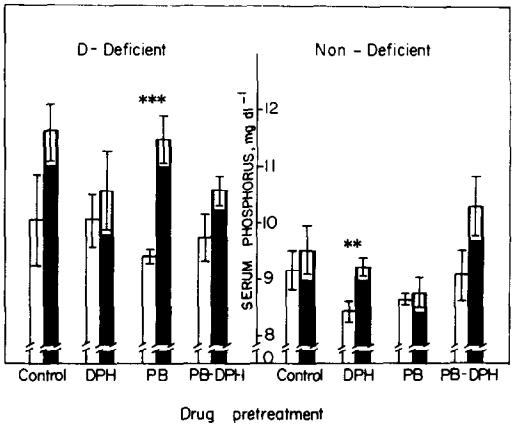

Fig. 2. Influence of a 3-week drug pretreatment and vitamin D_3 nutritional status on the concentration of serum phosphorus. Key (■) 1000 I.U. D_3 *per os*, 48 hr before death; and (□) 1,2-propanediol *per os*, 48 hr before death. Each value represents the mean ± S. E. M.; n = five animals per group. Statistically significant from animals receiving 1,2-propanediol only: (**) $P < 0.01$, and (***) $P < 0.001$.

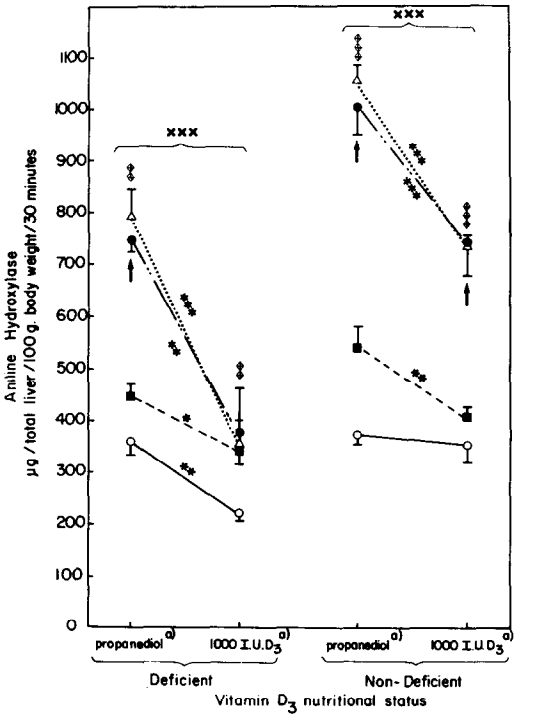

Fig. 4. Influence of a 3-week drug pretreatment and vitamin D_3 nutritional status on aniline hydroxylase activity. Key: (○——○) control, (■- - -■) DPH, (●- - - -●) PB, (△·····△) PB–DPH, and (a) given *per os*, 48 hr before death. Each value represents the mean ± S. E. M.; n = 5 animals per group. Main effect of drug pretreatment: (‡) $P < 0.001$, and (‡) $P < 0.01$; and single effect of drug pretreatment: (†) $P < 0.01$. Main effect of 1000 I.U. of D_3. (× × ×) $P < 0.001$; and single effect of 1000 I.U. of D_3: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$.

weight/100 g body weight was not affected by the deficiency. Figures 1 and 2 present serum concentrations for calcium and phosphorus with and without vitamin D supplementation in D− and D+ animals. Serum calcium concentrations were reduced significantly ($P < 0.001$) by vitamin D deprivation with mean concentrations ranging between 5.8 and 6.6 mg dl^{-1}. All D− animals responded by a marked increase ($P < 0.001$) toward normal values after D_3 supplementation. D+ animals showed a non-significant increase in serum calcium concentrations after D_3 supplementation except for those treated with the combination of the two drugs ($P < 0.01$). Concentrations of serum phosphorus were significantly higher in D− animals ($P < 0.001$) and the administration of 1000 I.U. of D_3 contributed both in D− and D+ groups to increase serum phosphorus levels. No significant interaction between drug treatment and cholecalciferol administration was observed on serum calcium and phosphorus concentrations.

The influence of ACV drugs and vitamin D_3 supplementation on the hepatic parameters studied in D+ and D− animals are shown in Figs. 3–6. Microsomal proteins (Fig. 3) were not affected by vitamin D_3 supplementation but were stimulated by drug treatment ($P < 0.001$). Aniline hydroxylase activity (Fig. 4) was also stimulated by ACV drugs, in both D+ and D− animals. D_3 supplementation contributed to lower the enzymatic activity ($P < 0.001$) accounting for a highly significant interaction ($P < 0.001$) between drug treatment and cholecalciferol supplementation in both D+ and D− rats. In D deficient animals, as much as 49.2 per cent ($P < 0.01$) and 57 per cent ($P < 0.001$) of the enzymatic activity was lost after administration of 1000 I.U. of D_3 during PB and PB–DPH treatment; control and DPH-treated animals showed a loss of 39.4 per cent ($P < 0.05$) and 23.3 per cent ($P < 0.01$) in enzymatic activity respectively. In D+ animals, D_3 supplementation decreased aniline hydroxylase activity by 5.9 per cent in control (NS), 25 per cent in DPH ($P < 0.01$), 26.1 per cent in PB ($P < 0.001$) and 30.8 per cent in PB–DPH treated animals ($P < 0.001$).

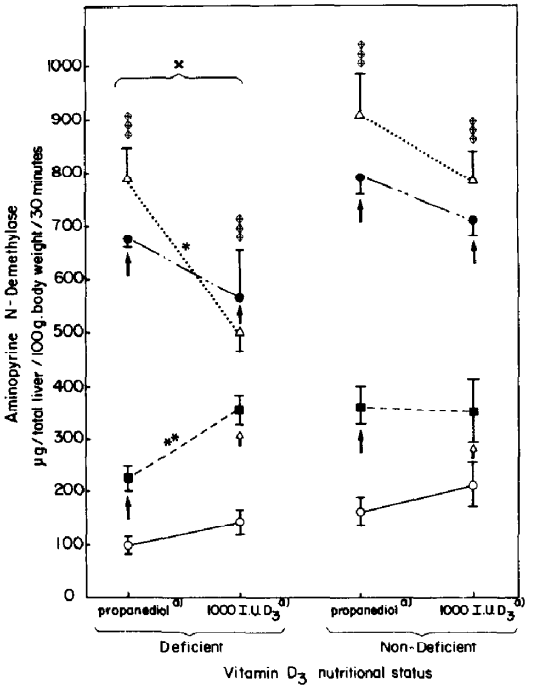

Fig. 5. Influence of a 3-week drug pretreatment and vitamin D_3 nutritional status on aminopyrine *N*-demethylase activity. Key: (○— ○) control, (■– – –■) DPH, (●———●) PB, (△·····△) PB–DPH, and (a) given *per os*, 48 hr before death. Each value represents the mean ± S. E. M.; n = five animals per group. Main effect of drug pretreatment: (‡) $P < 0.001$; and single effect of drug pretreatment: (†) $P < 0.01$, and (♀) $P < 0.05$. Main effect of 1000 I.U. of D_3 (×) $P < 0.10$; and single effect of 1000 I.U. of D_3: (*) $P < 0.05$, and (**) $P < 0.01$.

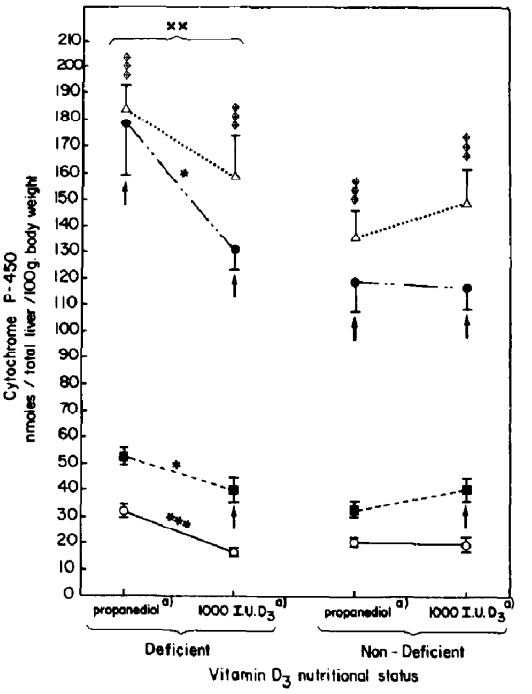

Fig. 6. Influence of a 3-week drug pretreatment and vitamin D_3 nutritional status on cytochrome P-450 content. Key: (○— —○) control, (■– – –■) DPH, (●———●) PB, (△·····△) PB–DPH, and (a) given *per os*, 48 hr before death. Each value represents the mean ± S. E. M.; n = five animals per group. Main effect of drug pretreatment: (‡) $P < 0.001$; and single effect of drug pretreatment: (†) $P < 0.01$. Main effect of 1000 I.U. of D_3 (××) $P < 0.01$; and single effect of 1000 I.U. of D_3: (*) $P < 0.05$, and (***) $P < 0.001$.

Aminopyrine *N*-demethylase activity is presented in Fig. 5. The factorial analysis shows again an overall significant effect of ACV drugs ($P < 0.001$) and a non-significant effect of cholecalciferol supplementation in D+ animals. In D− animals, cholecalciferol supplementation had an inhibitory effect on the enzymatic activity of 36.5 per cent for PB–DPH ($P < 0.01$) and 16 per cent for PB-treated animals (NS), but on the contrary, had a stimulatory effect of 42.2 per cent in control (NS) and 57.4 per cent in DPH-treated animals ($P < 0.01$). A significant interaction ($P < 0.01$) was observed between drug treatment and vitamin D_3 supplementation in D− rats only; this observation suggests a highly variable response of aminopyrine *N*-demethylase activity to drug treatment depending on whether or not supplementation is given in this group.

ACV drugs significantly increased cytochrome

Table 2. Hexobarbital sleeping time*

Drug treatment†	Sleeping time (min) Vitamin D deficient‡	Non-deficient‡
Control	36.5 ± 2.8	24.9 ± 4.0§
Diphenylhydantoin	24.8 ± 1.9	16.9 ± 1.5‖
Phenobarbital	11.0 ± 2.5	5.2 ± 0.9§
Diphenylhydantoin + phenobarbital	6.2 ± 1.8	7.5 ± 1.0

*Each value represents the mean ± S. E. M.; n = six to eight animals per group. Sodium hexobarbital was administered i.p. at a dose of 120 mg kg^{-1}.

†All drugs were administered daily at a dose of 50 mg kg^{-1} each for a period of 3 weeks.

‡Effect of the drug treatment on the sleeping time: vitamin D deficient: $P < 0.001$, non-deficient: $P < 0.01$.

§Statistically different from animals of the same treatment but of different vitamin D nutritional status, $P < 0.05$.

‖Statistically different from animals of the same treatment but of different vitamin D nutritional status, $P < 0.01$.

P-450 content in both D+ and D− animals (Fig. 6). Vitamin D_3 supplementation contributed to significantly reduce microsomal cytochrome P-450 content in D− animals ($P < 0.01$). Control animals suffered the greatest proportional decrease in content with a loss of 49.2 per cent ($P < 0.001$) followed by PB-treated animals with a loss of 27.6 per cent ($P < 0.05$), DPH-treated animals with a loss of 24.0 per cent ($P < 0.05$) and PB–DPH-treated animals with a loss of 13.5 per cent (NS). There were no statistically significant changes in cytochrome P-450 content in D+ animals after cholecalciferol supplementation. No interaction between drug treatment and cholecalciferol administration was observed.

Hexobarbital sleeping time (Table 2) was significantly shorter in ACV drug-treated D+ ($P < 0.01$) and D− ($P < 0.001$) animals when compared to their respective controls. When animals of the same treatment but of different nutritional status were compared, hexobarbital sleeping time was significantly longer in D− animals with the exception of PB–DPH treatment where no significant difference was observed between the two groups.

DISCUSSION

Anabolic hydroxylations in liver and kidney increase vitamin D mediated calcium mobilization from intestine and bone. The highly significant rise in serum calcium both in control and ACV drug-treated D− animals, even in the presence of a 16-hr fast before death clearly indicates that a sub-chronic ACV drug treatment does not affect the capacity of the animals to respond to a single pharmacological dose of cholecalciferol. This observation would suggest that the metabolic capacity of the liver and kidney to transform the vitamin into more active hydroxylated metabolites, as well as end organ responsiveness, are not lost during sub-chronic ACV drug treatment, confirming clinical observations that epileptic patients suffering from "ACV osteomalacia" respond to pharmacological doses of vitamin D [32, 33]. *In vivo*, plasma concentrations of 25(OH)D [18, 34, 35] as well as $1,25(OH)_2D$ [36, 37] have, in fact, already been reported to be stimulated by ACV drugs although *in vitro* loss of end organ responsiveness has been suggested [38, 39]. The present experiment unequivocally shows the ability of D− ACV drug-treated animals to mobilize calcium and phosphorus ions. The relative lack of response of serum calcium and phosphorus to vitamin D_3 supplementation in D+ animals also indicates normal regulatory mechanisms of calcium and phosphorus metabolism after D_3 administration during ACV drug treatment. These data on the *in vivo* response of serum calcium and phosphorus to a single pharmacological dose of D_3, however, do not reveal intrinsic and fine variations, mediated by the drug treatment, on vitamin D metabolism. Such conclusions could only be reached by analysis of more direct parameters of vitamin D metabolism.

The two *in vitro* enzymatic parameters studied, as well as cytochrome P-450 content, were found to be stimulated by ACV drug treatment regardless of vitamin D nutritional status. In all groups, under our present experimental conditions, the combined PB–DPH treatment produced only additive effects on the parameters studied. The *in vivo* hexobarbital sleeping time was, in a parallel fashion, depressed by ACV drug treatment regardless of vitamin D nutritional status, in good agreement with the *in vitro* observations. These findings stress the capacity of the animals, in a state of vitamin D deficiency, to respond to liver enzyme inducers such as PB and DPH. Although vitamin D deficiency did not affect enzymatic activity qualitatively, it tended to lower the latter after ACV drug administration, despite the presence of higher cytochrome P-450 content. This depression in enzymatic activity could be attributed to many factors such as a different molecular composition, in the D− state, of the microsomal membrane which contains the enzymes involved in aniline hydroxylation and aminopyrine *N*-demethylation. It has been shown, for example, that microsomal phospholipids are necessary for the catalytic activity of the MFO [40]. In connection with this, it is interesting to note that vitamin D, at least in the intestinal mucosa, stimulates phospholipid incorporation [41]. The MFO could also be influenced by abnormal extra- and intracellular calcium and phosphorus concentrations as well as the high levels of parathyroid hormone which accompany the deficiency. Correction of these factors by vitamin D_3 repletion could explain, in part, the lowering toward normal values of cytochrome P-450 in D− animals and its lack of effect in the D+

animals. Correction of serum calcium could not explain, however, the important inhibition of aniline hydroxylase activity since it was observed in both D− and D+ animals. The liver has a high uptake capacity for D_3 [42] and the latter is subsequently metabolized at the level of the microsomes as well as the mitochondria [22, 23]. PB, on the other hand, selectively stimulates the association of the vitamin with the smooth endoplasmic reticulum and seems to favor the microsomal production of compounds more polar than 25(OH)D [43, 44]. It is tempting, therefore, to postulate that vitamin D_3 or a product of its metabolism may be competing with aniline for enzymatic hydroxylation in liver microsomes. The effects of D_3 supplementation on aminopyrine *N*-demethylation are more intricate; D_3 does not modify significantly the activity in the D+ animals and seems to exert different influences on the effect of the various drug treatments in the D- state. Numerous dietary factors, such as cholesterol and fatty acids [45], are known to influence the catalytic activity of the mono-oxygenase complex. It now appears that, under certain circumstances, vitamin D_3 and/or extracellular factors associated with calcium homeostasis could play such a role.

Contrary to the 1-hydroxylation in kidney mitochondria [46, 47], the involvement of cytochrome P-450 in vitamin D metabolism has not yet been clearly characterized in liver microsomes although both vitamin D_2 and 25(OH)D_3, as well as the synthetic analogue dihydrotachysterol, have been shown to give rise to type I substrate when bound to hepatic microsomal proteins [48]. Based on the extremely low K_s of 25(OH)D_3 for cytochrome P-450, Cinti *et al.* [48] propose that this hemoprotein plays a role in the degradation of vitamin D and its metabolites, a phenomenon which could be accentuated by ACV drug treatment. Ghazarian *et al.* [49] also reported the production of inactive compounds by liver and kidney microsomes, while conflicting results of induction and inhibition of the D_3 25-hydroxylase by PB treatment have been published [23, 50]. It is now well accepted that multiple forms of cytochrome P-450 exist [51] with different substrate specificity [52–54] and the form which anabolically transforms vitamin D may not be induced by PB and DPH, contrary to what is observed in this study with aniline hydroxylase and aminopyrine *N*-demethylase activities. Direct measurement of the enzyme(s) involved in the metabolism of D_3 will have to be made in the presence of inducers of different forms of cytochrome P-450. A careful control of factors such as extracellular calcium, phosphorus, magnesium, parathyroid hormone and calcitonin will also have to be monitored.

The statistical analysis of the present study demonstrates no interaction in D+ animals and a weak interaction in D− animals, after D_3 supplementation, on aminopyrine *N*-demethylase activity. On the contrary, a strong interaction on the hydroxylation of aniline, both in D− and D+ animals, is present. These data suggest a certain involvement of the MFO in vitamin D metabolism as well as the specificity for the pathway involved although the nature of the substrate(s) reacting with the mono-oxygenase complex is not apparent in the present study. This inhibitory effect of D_3 supplementation on the MFO, including cytochrome P-450 could explain, in part, the reduced capacity reported by Bhattacharyya and DeLuca [55] of the D repleted animals to hydroxylate the vitamin in position 25.

As well as raising the question of the role of vitamin D on drug metabolism and its own metabolism, this paper offers some indirect evidence that pharmacological doses of cholecalciferol, through the unchanged vitamin and/or its metabolites, might act as a modifier of drug metabolism *in vitro*. It is clear that additional studies on different forms of cytochrome P-450 are needed to evaluate the enzymatic machinery involved in vitamin D metabolism, the role of each component of the MFO on its anabolism and catabolism, as well as the role played by vitamin D in drug disposition.

Acknowledgements—The authors are grateful to Mrs. Sanae Yamaguchi, Mr. Normand Dansereau and Mr. Michel Crowley for their technical assistance, Mrs. Elizabeth Pérès-Nault for the preparation of the graphs, Mr. Jacques Bernier for the photography and Miss Carole Dufort for the preparation of the manuscript.

REFERENCES

1. F. Schmid *Fortschr. Med.* **85**, 381 (1967).
2. R. Kruse, *Mschr. Kinderheilk.* **116**, 378 (1968).
3. A. D. Borgstedt, M. F. Bryson, L. W. Young and G. B. Forbes *J. Pediat.* **81**, 9 (1972).
4. C. Christiansen, M. Kristensen and P. Rødbro, *Br. med. J.* **3**, 738 (1972).
5. E. A. Sotaniemi, H. K. Kakarainen, J. A. Puranem and R. O. Lahti, *Ann. intern Med.* **77**, 389 (1972).
6. T. J. Hahn, S. J. Birge, S. C. Scharp, and L. V. Avioli, *J. clin. Invest.* **51**, 741 (1972).
7. T. C. B. Stamp, J. M. Round, D. J. F. Rowe and J. G. Haddad, *Br. med. J.* **4**, 9 (1972).
8. F. Lifshitz, and N. K. MacLaren, *J. Pediat.* **83**, 612 (1973).
9. C. Christiansen, P. Rødbro, and M. Lund, *Br. med. J.* **2**, 208 (1973).
10. J. Silver, T. J. Davies, E. Kupersmitt, M. Orme A. Petrie, and F. Vajda, *Arch Dis. Childn.* **49**, 344 (1974).
11. R. B. Shafer and F. Q. Nuttall, *J. clin. Endocr. Metab.* **41**, 1125 (1975).
12. T. J. Hahn, B. A. Hendin, C. R. Scharp, V. C. Boisseau and J. G. Haddad, *New Engl. J. Med.* **292**, 550 (1975).
13. R. Bouillon, J. Reynaert, J. H. Claes, W. Lissens and P. DeMorr, *J. clin. Endocr. Metab.* **41**, 1130 (1975).
14. T. J. Hahn, B. A. Hendin, C. R. Scharp and J. G. Haddad, Jr., *New Engl. J. Med.* **287**, 900 (1972).
15. H. U. Koch, D. Kraft, D. von Herrath and K. Schaefer, *Epilepsia* **13**, 829 (1972).
16. J. Silver, G. Neale and G. R. Thompson, *Clin. Sci.* **46**, 433 (1974).
17. M. Villareale, L. V. Gould, R. H. Wasserman, A. Barr R. T. Chiroff and W. H. Bergstrom, *Science N.Y.* **183**, 671 (1974).
18. M. Gascon-Barré, M. G. Côté, F. H. Glorieux and E. Delvin. *Pharmacologist* **18**, 160 (1976).
19. J. G. Haddad, Jr. and J. C. Chyu *J. clin. Endocr.* **33**, 992 (1971).
20. S. B. Arnaud, M. Matthusen, J. B. Gilkinson and R. S. Goldsmith, *Am. J. clin. Nutr.* **30**, 1082 (1977).
21. M. R. Haussler, D. J. Baylink, M. R. Hughes, P. F. Brumbaugh, J. E., Wergedal, F. H. Shen, R. L. Nielson, S. J. Counts, K. M. Bursac and T. A. McCain, *Clin. Endocr.* **5**, 1515 (1976).

22. M. H. Bhattacharyya and H. F. DeLuca, *Archs Biochem. Biophys.* **160**, 58 (1974).
23. I. Björkhem and I. Holmberg, *J. biol. Chem.* **253**, 842 (1978).
24. G. Guroff, H. F. DeLuca and H. Steenbock, *Am. J. Physiol.* **204**, 833 (1963).
25. P. Mazel, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. LaDu, H. G. Mandel and E. L. Way), Chap. 27, p. 546. Williams & Wilkins, Baltimore (1971).
26. Y. Imai, A. Ito and R. Sato, *J. Biochem. Tokyo* **60**, 417 (1966).
27. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
28. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
29. E. M. Gindler and J. D. King, *Am. J. clin. Path.* **58**, 376 (1972).
30. S. Natelson, *Techniques of Clinical Chemistry*, p. 576. Charles C. Thomas, Springfield, 911 (1971).
31. L. Lison, *Statistique appliquée à la biologie expérimentale*, Chap. VI, p. 155, Gauthier-Villars, Paris (1968).
32. N. McLaren and F. Lifshitz, *Pediat. Res.* **7**, 914 (1973).
33. T. J. Hahn and L. V. Avioli, *Archs intern. Med.* **135**, 997 (1975).
34. A. W. Norman, J. D. Bayless and H. C. Tsai, *Biochem. Pharmac.* **25**, 163 (1976).
35. R. T. Matheson, J. J. Herbst, W. Jubiz, J. W. Freston and K. G. Tolman, *J. clin. Pharmac.* **16**, 426 (1976).
36. W. Jubiz, M. R. Haussler, T. A. McCain and K. G. Tolman, *J. clin. Endocr. Metabol.* **44**, 617 (1977).
37. J. C. Levison, G. N. Kent, G. K. Worth and L. W. Retallack, *Endocrinology* **101**, 1898 (1977).
38. M. W. Jenkins, M. Harris and M. R. Willis, *Calc Tissue Res.* **16**, 163 (1974).
39. R. A. Corradino, *Biochem. Pharmac.* **25**, 863 (1976).
40. H. W. Strobel, A. Y. H. Lu, J. Heidema and M. J. Coon, *J. biol. Chem.* **245**, 4851 (1970).
41. V. W. Thompson and H. F. DeLuca, *J. biol. Chem.* **239**, 984 (1964).
42. S. Rojanasathit and J. G. Haddad, *Biochim. biophys. Acta* **421**, 12 (1976).
43. T. J. Hahn, C. R. Scharp and L. V. Avioli, *Endocrinology* **94**, 1489 (1974).
44. G. Neale, *Front Gastrointest. Res.* **2**, 32 (1976).
45. M. Lang, *Gen. Pharmac.* **7**, 415 (1976).
46. J. G. Ghazarian and H. F. DeLuca, *Archs Biochem. Biophys.* **160**, 63 (1974).
47. J. G. Ghazarian, C. R. Jefcoate, J. C. Knutson, W. H. Orme-Johnson and H. F. DeLuca, *J. biol. Chem.* **249**, 3026 (1974).
48. D. L. Cinti, E. E. Golub and F. Bronner, *Biochem. biophys. Res. Commun.* **72**, 546 (1976).
49. J. G. Ghazarian, P.-Y. Hsu and B. L. Peterson, *Archs Biochem. Biophys.* **184**, 596 (1977).
50. S. Sulimovici and M. S. Roginsky, *Life Sci.* **21**, 1317 (1977).
51. P. E. Thomas, A. Y. H. Lu, D. Ryan, S. B. West, J. Kawalek and W. Levin, *J. biol. Chem.* **251**, 1385 (1976).
52. A. Y. H. Lu, R. Kuntzman, B. West and A. H. Conney, *Biochem. biophys. Res. Commun.* **42**, 1200 (1961).
53. A. Y. H. Lu, R. Kuntzman, S. West, M. Jacobson and and A. H. Conney, *J. biol. Chem.* **247**, 1727 (1972).
54. D. W. Nebert, J. K. Hedeman, H. W. Sirobel and M. J. Coon, *J. biol. Chem.* **248**, 7631 (1973).
55. M. H. Bhattacharyya and H. F. DeLuca, *Biochem. biophys. Res. Commun.* **59**, 734 (1974).