Comparative Studies on the 25-Hydroxylations of Cholecalciferol and 1 α -Hydroxycholecalciferol in Perfused Rat Liver

By MASAFUMI FUKUSHIMA,* YASUHO NISHII,* MICHICO SUZUKI† and TATSUO SUDA††

*Research Laboratories of Chugai Pharmaceutical Co., Takada, Toshima-ku, Tokyo, Japan 171, and

†Department of Biochemistry, School of Dentistry, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, Japan 113

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The 25-hydroxylations of [^3H]cholecalciferol and 1 α -hydroxy[^3H]cholecalciferol in perfused rat liver were compared. Results showed that about twice as much 1 α (OH) D_3 (1 α -hydroxycholecalciferol) was incorporated into the liver as cholecalciferol. 25-Hydroxy[^3H]cholecalciferol and 1 α -25-dihydroxy[^3H]cholecalciferol were not incorporated significantly. Livers isolated from vitamin D-deficient rats formed the 25-hydroxy derivatives of cholecalciferol and 1 α (OH) D_3 respectively linearly with time for at least 120 min. The rate of 1 α ,25(OH) $_2\text{D}_3$ (1 α ,25-dihydroxycholecalciferol) production increased exactly 10-fold on successive 10-fold increases in the dose of 1 α (OH) D_3 , suggesting that hepatic 25-hydroxylation of 1 α (OH) D_3 is not under metabolic control. On the other hand, the rate of conversion of cholecalciferol into 25(OH) D_3 (25-hydroxycholecalciferol) did not increase linearly with increase in the amount of cholecalciferol in the perfusate. The 25-hydroxylation of cholecalciferol seemed to proceed at a similar rate to that of 1 α (OH) D_3 at doses of less than 1 nmol, but with doses of more than 2.5 nmol, the conversion of cholecalciferol into 25(OH) D_3 became much less efficient, though the linear relation between the amounts of substrate and product was maintained. A reciprocal plot of data on the 25-hydroxylation of cholecalciferol gave two K_m values of about 5.6 nM and 1.0 μM , whereas that for the 25-hydroxylation of 1 α (OH) D_3 gave a single K_m value of about 2.0 μM . These results suggest that there are two modes of 25-hydroxylation of cholecalciferol in the liver, which seem to be closely related to the mechanism of control of 25(OH) D_3 production by the liver.

It is well known that the 25-hydroxylation of cholecalciferol, at least in mammals, takes place mainly in the liver (Horsting & DeLuca, 1969; Ponchon *et al.*, 1969). In normal human subjects 25(OH) D_3 usually circulates at approx. 20–30 ng/ml (Haddad & Chyu, 1971; Ohara *et al.*, 1976), but when 30 000–50 000 i.u. of cholecalciferol is administered daily to patients with chronic renal failure, their plasma concentrations of 25(OH) D_3 rise to more than 100 ng/ml (Ohara *et al.*, 1976). These data suggest that the hepatic 25-hydroxylation of cholecalciferol is regulated at least in a physiological dose range.

Bhattacharyya & DeLuca (1973a) first reported the existence of a mechanism for regulating liver 25-hydroxylase activity. Animals pretreated with chole-

calciferol showed decreased liver 25-hydroxylase activity, but prior administration of large amounts of 25(OH) D_3 did not affect the conversion of cholecalciferol into 25(OH) D_3 (Bhattacharyya & DeLuca, 1973a; Rojanasathit & Haddad, 1976). Thus liver 25-hydroxylase activity for cholecalciferol is not regulated by the circulating concentration of 25(OH) D_3 or its further metabolite(s).

We reported previously that 1 α (OH) D_3 is rapidly converted into 1 α ,25(OH) $_2\text{D}_3$ in isolated perfused rat liver (Fukushima *et al.*, 1975, 1976). In the present work, we have compared the 25-hydroxylations of cholecalciferol and 1 α (OH) D_3 in perfused liver. Results showed that the 25-hydroxylation of 1 α (OH) D_3 was not under metabolic control, whereas that of cholecalciferol was. The latter seemed to be due to two modes of 25-hydroxylation of cholecalciferol in the liver.

Materials and Methods

Cholecalciferol compounds

[1 α ,2 α - ^3H]Cholecalciferol (12.3 Ci/mmol) and

Abbreviations used: 25(OH) D_3 , 25-hydroxycholecalciferol; 1 α (OH) D_3 , 1 α -hydroxycholecalciferol; 1 α ,25(OH) $_2\text{D}_3$, 1 α ,25-dihydroxycholecalciferol.

† Present address: Department of Biochemistry, School of Dentistry, Showa University, Hatanodai, Shinagawa-ku, Tokyo, Japan 142.

25(OH)[26(27)- ^3H] D_3 (7.3 Ci/mmol) were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). $1\alpha(\text{OH})[2\text{-}^3\text{H}]\text{D}_3$ (4.2 Ci/mmol) was synthesized chemically in our laboratory (Tohira *et al.*, 1977). $1\alpha,25(\text{OH})_2[26(27)\text{-}^3\text{H}]\text{D}_3$ (7.3 Ci/mmol) was synthesized biologically from 25(OH)[26(27)- ^3H] D_3 by using a chick-kidney incubation system *in vitro* (Boyle *et al.*, 1972) and purified by Celite column chromatography (Haussler & Rasmussen, 1972). Crystalline cholecalciferol and 25(OH) D_3 were purchased from Philips-Duphar (Apollolaan, Amsterdam, The Netherlands). $1\alpha(\text{OH})\text{D}_3$ and $1\alpha,25(\text{OH})_2\text{D}_3$ were synthesized chemically by Dr. I. Matsunaga in our laboratory. All radioactive compounds obtained commercially were purified by Sephadex LH-20 column chromatography before use.

Animals

Weanling male rats (Sprague-Dawley) were maintained in individual stainless-steel cages, and given a modified (Suda *et al.*, 1970) vitamin D-deficient diet (Steenbock & Black, 1925) (Ca, 0.45%; P₁, 0.3%) and deionized water *ad libitum*. Rats weighing 190–240 g, after 6 weeks on this diet, were used for experiments. Their average serum calcium concentration was about 5.0 mg/100 ml.

Liver perfusion

Liver was perfused by a slight modification of the method of Miller *et al.* (1951) as follows. Animals were starved overnight and then anaesthetized with Nembutal (5 mg/100 g body wt.). Their peritoneal cavity was opened. Connective tissue around the liver was carefully removed and the common bile duct was cannulated. Then the portal vein and the inferior vena cava were isolated, and 0.3 ml of sodium heparin solution (1000 i.u./ml) was injected into the inferior vena cava. After 3 min a glass cannula was rapidly inserted into the portal vein, and the thorax was opened for cannulation of the superior vena cava. After about 20 ml of warm Krebs-Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), had been passed from the portal vein into the superior vena cava, the inferior vena cava was rapidly ligated. Livers with attached diaphragms were quickly dissected out and placed on a Petri dish in a perfusion apparatus controlled at 37–38°C (Metaloglass, Boston, MA, U.S.A.). The livers were then perfused from the portal vein to the superior vena cava. The perfusate was a mixture of 30 ml of heparinized arterial blood from vitamin D-deficient rats and 45 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, equilibrated with O_2/CO_2 (19:1).

When the flow of perfusate became constant (30–40 ml/min), graded doses of [^3H]cholecalciferol or $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ dissolved in 95% ethenol were added to the perfusate. After perfusion was termi-

nated, the livers were homogenized with 3 vol. of water.

In a separate set of perfusions, viability of the livers used in these experiments was checked for 150 min by monitoring the synthesis of urea from NH_4Cl (700 μmol) and ornithine (175 μmol). The rate of urea formation was almost identical 60 min later (360 $\mu\text{mol}/20\text{ min}$) with that 120 min later (363 $\mu\text{mol}/20\text{ min}$).

Experiments performed *in vivo*

Graded doses of either [^3H]cholecalciferol or $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ were injected into the jugular vein of vitamin D-deficient rats, and 2 h later the blood was collected by heart puncture under light diethyl ether anaesthesia.

Measurement of synthesis of 25-hydroxy derivatives

Liver homogenates, perfusate and blood plasma were separately extracted by the method of Bligh & Dyer (1959). The chloroform extracts were concentrated in a rotary evaporator and applied to Sephadex LH-20 columns (Holick & DeLuca, 1971). For analysis of [^3H]cholecalciferol metabolism, columns (1.5 cm $\times$ 40 cm) containing 23 g of the Sephadex were used with 50% (v/v) chloroform in hexane as solvent, and fractions (3 ml) were collected. For analysis of $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ metabolism, the chloroform extracts were chromatographed on columns (1.5 cm $\times$ 30 cm) containing 17 g of the Sephadex with 65% (v/v) chloroform in hexane as solvent, and fractions (7 ml) were collected. The flow rate on each column was adjusted to 40 drops/min. The solvents were evaporated from the eluates under a stream of air and the resulting residues were dissolved in 10 ml of toluene scintillator [4 g of 2,5-diphenyloxazole and 400 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene/litre of toluene]. For determination of the radioactivity in bile, 0.1 ml of bile was dissolved in 10 ml of Bray's (1960) solution. Radioactivity was measured in a Nuclear-Chicago mark 1 liquid-scintillation spectrometer. Before applying extracts to the columns, 200 μg of either authentic 25(OH) D_3 or $1\alpha,25(\text{OH})_2\text{D}_3$ was added. The A_{265} of each fraction was determined.

K_m -like values were calculated by reciprocal plots of the amounts of the substrates added and those of their 25-hydroxy derivatives produced for 120 min with the liver perfusion. The values are referred to simply as K_m hereafter in the text.

Results

Distribution of total radioactivity during liver perfusion

Fig. 1 shows the distribution of total radioactivity between the perfusate, liver and bile at intervals during 120 min of perfusion after addition of 0.1 μg of [^3H]cholecalciferol (Fig. 1a) or $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ (Fig. 1b) to the perfusate. In each case, the total

Fig. 1. Changes in total distribution of total radioactivity between the perfusate, liver and bile during 120 min of perfusion after the addition of 0.1 μg of $[^3H]$ cholecalciferol (a) or $1\alpha(OH)[^3H]D_3$ (b) to the perfusate
The total amount of radioactivity recovered in the perfusate (○), liver (●) and bile (△) was always more than 90% of that added initially. Each point represents a single perfusion experiment.

Table 1. Distribution of metabolites between the perfusate, liver and bile 120 min after the addition of 0.1 μg of $[^3H]$ cholecalciferol, $1\alpha(OH)[^3H]D_3$, $25(OH)[^3H]D_3$ or $1\alpha,25(OH)_2[^3H]D_3$ to the perfusate
Values are percentages of the total radioactivity recovered in the perfusate, liver and bile. In each case, the total recovery was more than 90% of the initial amount.

Metabolites	Compound added ...	Metabolite distribution (%)			
		Cholecalciferol	1 α (OH)D ₃	25(OH)D ₃	1 α ,25(OH) ₂ D ₃
Perfusate					
Cholecalciferol		58.3	—	—	—
1 α (OH)D ₃		—	20.0	—	—
25(OH)D ₃		17.1	—	89.4	—
1 α ,25(OH) ₂ D ₃		—	23.5	—	81.7
Others		1.8	4.1	3.0	1.5
Liver					
Cholecalciferol		14.0	—	—	—
1 α (OH)D ₃		—	38.7	—	—
25(OH)D ₃		2.1	—	1.9	—
1 α ,25(OH) ₂ D ₃		—	3.4	—	6.1
Others		3.2	3.8	1.3	2.8
Bile					
Unknown		3.5	6.5	4.4	7.9
Total		100.0	100.0	100.0	100.0

radioactivity in the perfusate decreased rapidly in the first 20–40 min and then remained fairly steady. The radioactivity incorporated into the liver reached saturation in 60 min and then gradually decreased. The saturation point with $1\alpha(OH)[^3H]D_3$ was about

twice that with $[^3H]$ cholecalciferol. With both compounds, the radioactivity in the bile gradually increased, but it was never more than 5% of the total. Thus only the extracts of the perfusate and liver were used for further analyses of the metabolism of chole-

calciferol and $1\alpha(\text{OH})\text{D}_3$. It is noteworthy that radioactive 25-hydroxy derivatives, such as $25(\text{OH})[^3\text{H}]\text{D}_3$ or $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$, when added to the perfusate, were not incorporated into the liver to any great extent (Table 1).

Metabolism of $[^3\text{H}]\text{cholecalciferol}$ and $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ in perfused rat liver

The chromatographic profile of extracts of the perfusate after perfusion for 120 min with $0.1\text{ }\mu\text{g}$ of $[^3\text{H}]\text{cholecalciferol}$ has been shown in the previous report (Suda *et al.*, 1977). $[^3\text{H}]\text{cholecalciferol}$ was converted into one less-polar and one more-polar metabolite, and the more-polar metabolite migrated in exactly the same position as synthetic $25(\text{OH})\text{D}_3$ added to the extract.

The chromatographic profile on a Sephadex LH-20 column of extracts of the perfusate after perfusion for 120 min with $0.1\text{ }\mu\text{g}$ of $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ is shown in Fig. 2. The results showed that $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ was converted into one less-polar and one more-polar metabolite than $1\alpha(\text{OH})\text{D}_3$ itself. The more-polar metabolite migrated in exactly the same position on a Sephadex LH-20 column as authentic $1\alpha,25(\text{OH})_2\text{D}_3$ added to the extract. Final proof that this radioactive metabolite was $1\alpha,25(\text{OH})_2\text{D}_3$ was obtained by g.l.c.-mass spectrometry (Fukushima *et al.*, 1975).

Fig. 3 shows the time course of change in the amounts of unchanged cholecalciferol (Fig. 3a) and $1\alpha(\text{OH})\text{D}_3$ (Fig. 3b) in the perfusate after $0.1\text{ }\mu\text{g}$ of

either $[^3\text{H}]\text{cholecalciferol}$ or $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ had been added. It was noteworthy that after 120 min as much as 60% of the added $[^3\text{H}]\text{cholecalciferol}$ and only

Fig. 2. Chromatographic profile on a Sephadex LH-20 column of extracts of perfusate after 120 min of perfusion with $0.1\text{ }\mu\text{g}$ of $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$.

Before applying extracts to the column, $200\text{ }\mu\text{g}$ of synthetic $1\alpha,25(\text{OH})_2\text{D}_3$ was added. ●, Radioactivity; ○, A_{265} .

Fig. 3. Time course of change in the amounts of unchanged $[^3\text{H}]\text{cholecalciferol}$ (a) and $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$ (b) in the perfusate. Livers were perfused with $0.1\text{ }\mu\text{g}$ of $[^3\text{H}]\text{cholecalciferol}$ or $1\alpha(\text{OH})[^3\text{H}]\text{D}_3$. Each point represents a single perfusion experiment.

20% of the added 1 α (OH)[³H]D₃ were recovered unchanged. In each case, the radioactivity in the liver was recovered mainly as unchanged [³H]cholecalciferol or 1 α (OH)[³H]D₃ (Table 1).

The radioactive 25-hydroxy derivatives were found in the perfusate within 20 min of addition of

their precursors, and their concentrations increased linearly with time for at least 120 min (Fig. 4). The concentration of 25(OH)D₃ in the liver seemed to attain saturation in 20–40 min and did not change significantly thereafter, whereas the amount of 1 α ,25(OH)₂D₃ in the liver appeared to increase

Fig. 4. Time course of change in the amounts of 25(OH)[³H]D₃ (a) and 1 α ,25(OH)₂[³H]D₃ (b) after adding 0.1 μ g of [³H]cholecalciferol and 1 α (OH)[³H]D₃ respectively

—, Sum of the amounts of 25-hydroxy metabolites recovered in extracts of the perfusate (●) and liver (○). Each point represents a single perfusion experiment.

Fig. 5. Relation between the amounts of cholecalciferol and 1 α (OH)D₃ added and the amounts of their 25-hydroxy metabolites produced by perfused rat liver in 120 min

The amounts of 1 α ,25(OH)₂D₃ (●) and 25(OH)D₃ (○) indicate the sum of the amounts of these metabolites recovered in extracts of the perfusate and liver. The relationship between the amounts of substrates and their respective products in a physiological range is shown in the insert. Each point represents a single perfusion experiment.

gradually. In each case, about 80–90% of the synthesized 25-hydroxy derivatives were found in the perfusate after 120 min of perfusion.

Comparison of the 25-hydroxylations of cholecalciferol and $1\alpha(\text{OH})\text{D}_3$ in vivo and in perfused liver

Fig. 5 shows the relation between the amounts of cholecalciferol or $1\alpha(\text{OH})\text{D}_3$ added to perfusates and the amounts of their 25-hydroxy derivatives produced in 120 min. The 25-hydroxylation of $1\alpha(\text{OH})\text{D}_3$ proceeded much more rapidly than that of cholecalciferol. After addition of 39 nmol of cholecalciferol or $1\alpha(\text{OH})\text{D}_3$ to the perfusate, as much as 7.5 nmol of $1\alpha,25(\text{OH})_2\text{D}_3$ was produced from $1\alpha(\text{OH})\text{D}_3$, whereas only 0.5 nmol of $25(\text{OH})\text{D}_3$ was formed

from cholecalciferol. The amount of $1\alpha,25(\text{OH})_2\text{D}_3$ produced increased exactly 10-fold for each 10-fold increase in the amount of $1\alpha(\text{OH})\text{D}_3$.

On the other hand, the amount of $25(\text{OH})\text{D}_3$ produced did not increase linearly with increase in the amount of cholecalciferol added to the perfusate. When less than 1 nmol of cholecalciferol was added to the perfusate, 25-hydroxylation of cholecalciferol seemed to proceed at a similar rate to that of $1\alpha(\text{OH})\text{D}_3$; the amount of $25(\text{OH})\text{D}_3$ synthesized was fairly constant with between 1.0 and 2.5 nmol of substrate, but with more than 2.5 nmol the conversion of cholecalciferol into $25(\text{OH})\text{D}_3$ was much less efficient, though the amount of substrate was still linearly related to the amount of product (Fig. 5). A similar relationship was observed *in vivo* when graded amounts of [^3H]cholecalciferol were administered to vitamin D-deficient rats and the $25(\text{OH})[^3\text{H}]\text{D}_3$ in their blood plasma was measured 2 h later. The 25-hydroxylation of cholecalciferol was saturated with a minute amount of substrate, and the plasma concentration of $25(\text{OH})\text{D}_3$ remained fairly constant when less than 1.3 nmol of cholecalciferol was administered (Fig. 6). However, with doses of more than 1.3 nmol, the amount of $25(\text{OH})\text{D}_3$ in the plasma increased linearly. These results are similar to the observations obtained independently by E. B. Mawer and M. B. Clark (personal communications).

The dependence of the rates of 25-hydroxylation of cholecalciferol and $1\alpha(\text{OH})\text{D}_3$ in the liver on their substrate concentrations in the perfusate is shown in Figs. 7 and 8 respectively. These reciprocal plots of the data obtained with the liver-perfusion system gave two K_m values of about 5.6 nM and 1.0 μM for the 25-hydroxylation of cholecalciferol (Fig. 7) and a single K_m value of about 2.0 μM for that of $1\alpha(\text{OH})\text{D}_3$ (Fig. 8).

Fig. 6. Effect of the dose of cholecalciferol injected intravenously into vitamin D-deficient rats on the amount of $25(\text{OH})\text{D}_3$ in their blood plasma 2 h later. Each point represents a single experiment *in vivo*.

Fig. 7. Effect of the concentration of cholecalciferol in the perfusate on its 25-hydroxylation. Values were calculated from those of the liver-perfusion experiment shown in Fig. 5.

Fig. 8. Effect of the concentration of $1\alpha(\text{OH})\text{D}_3$ in the perfusate on its 25-hydroxylation. Values were calculated from those of the liver-perfusion experiment shown in Fig. 5.

Discussion

It is well known that the 25-hydroxylation of cholecalciferol in the liver is under at least partial control (Bhattacharyya & DeLuca, 1973*a,b*; Rojanasathit & Haddad, 1976). This control, which appears to be less sensitive than that of 1 α -hydroxylation of 25(OH)D₃ in the kidney, may nevertheless decrease the risk of hypercalcaemia.

It has been shown in rats (Lund & DeLuca, 1966; Bhattacharyya & DeLuca, 1973*a*; Rojanasathit & Haddad, 1976), humans (Stanbury *et al.*, 1973) and chicks (Bhattacharyya & DeLuca, 1974; Fraser, 1975) that, as the amount of a single dose of cholecalciferol is increased, its percentage conversion into 25(OH)D₃ is decreased. This phenomenon was initially explained as being due to feedback inhibition of the hydroxylase enzyme by the 25(OH)D₃ produced (DeLuca, 1971), but Bhattacharyya & DeLuca (1973*a*) subsequently reported that product inhibition could not explain the decrease in enzyme activity observed in response to a dose of cholecalciferol. Rojanasathit & Haddad (1976) clearly demonstrated that the conversion of [³H]-cholecalciferol into 25(OH)[³H]D₃ in vitamin D-deficient rats was not affected by prior administration of a large amount of 25(OH)D₃ (30 times the amount of [³H]cholecalciferol). These findings indicate that the liver 25-hydroxylase activity for cholecalciferol is not regulated by the circulating amount of 25(OH)D₃ or its metabolites.

We are interested in the mechanism of control of the 25-hydroxylation of cholecalciferol in the liver. Since no efficient system *in vitro* for measuring hepatic cholecalciferol 25-hydroxylase activity was available, in this work we used perfused livers isolated from vitamin D-deficient rats to compare the 25-hydroxylations of cholecalciferol and 1 α (OH)D₃.

Bhattacharyya & DeLuca (1973*b*) compared the 25-hydroxylations of cholecalciferol and dihydrotachysterol₃, finding that the rate of appearance of 25(OH)D₃ in the blood only increased about 2-fold with a 100-fold increase in the amount of cholecalciferol, whereas the rate of appearance of 25-hydroxydihydrotachysterol₃ increased 100-fold with 100-fold increase in the amount of dihydrotachysterol₃. The present results indicate that the hepatic 25-hydroxylation of 1 α (OH)D₃ is similar to that of dihydrotachysterol₃. Bhattacharyya & DeLuca (1973*b*) concluded that 25-hydroxylation of dihydrotachysterol₃ is not under metabolic control, and this seems to be true for the 25-hydroxylation of 1 α (OH)D₃ also.

The hepatic 25-hydroxylation of cholecalciferol appeared more complicated. The reciprocal plots (Fig. 7) suggested that, unlike 1 α (OH)D₃, cholecalciferol has two modes of 25-hydroxylation (Table 2). The first mode seems to be responsible for the 25-

Table 2. *Modes of 25-hydroxylation of cholecalciferol and 1 α (OH)D₃ in the liver*

Substrate	Cholecalciferol		1 α (OH)D ₃ Whole range
	<2.5 nmol	>2.5 nmol	
Affinity	High	Low	Low
Capacity	Limited	Large	Large
Velocity	Fast	Slow	Fast

hydroxylation of physiological amounts of cholecalciferol (less than 2.5 nmol/6g of liver), and the K_m value of this was much smaller than that of the second mode of reaction. The amount of enzyme responsible for the first mode appeared limited, because this phase of the reaction was saturated with about 1 nmol of cholecalciferol. The first mode of reaction proceeded at a rate similar to that of the 25-hydroxylation of 1 α (OH)D₃.

The second mode of reaction, on the other hand, appeared responsible for the 25-hydroxylation with larger amounts of substrate; when more than 2.5 nmol of cholecalciferol was added to the perfusate, its conversion into 25(OH)D₃ became much less efficient, though there was still a linear relation between the amounts of substrate and product. Therefore the amount of enzyme available for this mode of reaction appeared rather large. Thus we may consider that the first mode of 25-hydroxylation of cholecalciferol is a 'high-affinity low-capacity reaction', whereas the second mode is a 'low-affinity high-capacity reaction' (Table 2). Further studies are required to discover whether these two modes of reaction are catalysed by a single enzyme or two different enzymes.

Most of the 25(OH)D₃ produced was rapidly released into the perfusate, but a little was found in the liver. Thus it is possible that the latter may exert product inhibition, as previously suggested by Rojanasathit & Haddad (1976). The K_m value obtained by liver perfusion reflects the overall reaction, including transport of substrate in the perfusate, its uptake into the liver, its excretion into the bile and the enzyme reaction. Nevertheless, the values obtained seem to have physiological significance, and the existence of two modes of reaction seems to be related to the mechanism of control of 25-hydroxylation of cholecalciferol in the liver.

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