J. Physiol. (1977), **264**, *pp.* 125–139
With 4 text-figures
Printed in Great Britain

CALCIUM ABSORPTION IN DIPHOSPHONATE-TREATED RATS: EFFECT OF PARATHYROID FUNCTION, DIETARY CALCIUM AND PHOSPHORUS

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(*Received 3 May 1976*)

SUMMARY

1. The role of parathyroid hormone (PTH) and 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) in modulation of intestinal Ca absorption was studied in rats, using disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP), which is known to reduce 1,25-$(OH)_2D_3$ formation.

2. EHDP decreased intestinal Ca absorption. This effect could be abolished by small amounts of 1,25-$(OH)_2D_3$, whereas even large doses of PTH were ineffective. EHDP also decreased Ca absorption in thyroparathyroidectomized (TPTX) rats. Therefore the effect of EHDP on 1,25-$(OH)_2D_3$ production is unlikely to be mediated through PTH.

3. The correction by PTH of the decreased Ca absorption in TPTX rats was inhibited by EHDP. Since EHDP inhibits formation of 1,25-$(OH)_2D_3$ the effect of PTH on Ca absorption is likely to be mediated through this vitamin D_3 metabolite.

4. In normal rats both a low Ca and a low P diet stimulated Ca absorption. In EHDP-treated intact rats low Ca still stimulated Ca absorption, whereas the effect of low P was abolished. This indicates that low Ca and low P diets affect Ca absorption through different mechanisms.

5. Intestinal adaptation to a low Ca diet was still observed in EHDP-treated TPTX rats. Thus, in the rat, intestinal adaptation to low Ca diet can occur without PTH.

INTRODUCTION

It is well known that the rate of intestinal Ca absorption varies in response to the needs of the organism. In other words, according to the dietary supply and the capacity of the bone to retain Ca, the intestine adapts its capacity of absorption. A few years ago it was suggested (Morgan, Bonjour, Gasser, O'Brien & Fleisch, 1971) that diphosphonates, because

of their effect on the bone Ca retention could be used as tools for investigating the regulation of Ca absorption. Since then several studies have been conducted using the diphosphonate, disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP). When EHDP is given in large doses (10 mg P/kg day s.c.) to rats one observes an inhibition of both bone mineralization (Schenk, Merz, Mühlbauer, Russell & Fleisch, 1973) and intestinal Ca absorption (Gasser, Morgan, Fleisch & Richelle, 1972; Bonjour, Russell, Morgan & Fleisch, 1973*b*). The mechanism responsible for the blockage of bone mineralization is thought to be due to a physicochemical effect on crystal formation. It is now well established that the EHDP-induced inhibition of intestinal Ca absorption is due to decreased formation of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$). Indeed, an impaired synthesis of 1,25-$(OH)_2D_3$ has been observed in both rats (Hill, Lumb, Mawer & Stanbury, 1973) and chicks (Baxter, DeLuca, Bonjour & Fleisch, 1974) treated with large doses of EHDP. Furthermore in rats, the administration of 1,25-$(OH)_2D_3$ can prevent or correct the EHDP-induced depression of intestinal Ca absorption, whereas the precursors vitamin D_3 or 25-hydroxycholecalciferol, at 100 times the dose of 1,25-$(OH)_2D_3$ have no effect (Bonjour, DeLuca, Fleisch, Trechsel, Matejowec & Omdahl, 1973*a*; Bonjour, Trechsel, Tröhler, Fleisch, Baxter & DeLuca, 1974; Bonjour, Trechsel, Fleisch, Schenk, DeLuca & Baxter, 1975). How large doses of EHDP bring about a decreased formation of 1,25-$(OH)_2D_3$ is still unknown. In the rat, production of this active metabolite of vitamin D_3 is influenced by parathyroid hormone (Garabedian, Holick, DeLuca & Boyle, 1972), the amount of dietary Ca (Boyle, Gray & DeLuca, 1971) and inorganic phosphate (P_i) (Tanaka & DeLuca, 1973). It is therefore possible that the effect of EHDP on 1,25-$(OH)_2D_3$ production would depend upon one of these factors. If so, the removal or the administration of parathyroid hormone (PTH), and the variation in the dietary content of Ca or P_i should modify the intestinal response to EHDP treatment.

The first part of this paper discusses the effect of EHDP (10 mg P/kg day s.c. for 7 days) on Ca absorption, which was studied in intact and thyroparathyroidectomized rats whether or not they were substituted with PTH. The effect of PTH substitution was compared to that following the administration of 1,25-$(OH)_2D_3$. In the second part, the interaction between the amount of dietary Ca or P_i and EHDP treatment on the capacity of the intestine to absorb Ca is reported. The results show that parathyroid hormone is probably not involved in the intestinal response to EHDP. Furthermore they indicate that the stimulation of Ca absorption capacity in response to a low Ca diet does occur in EHDP-treated rats and is independent of parathyroid hormone.

METHODS

All experiments were carried out on male or female Wistar rats fed from weaning on a commercial diet containing 1·1–1·3% Ca, 1·0% P and 2800 i.u./kg vitamin D_3. At a weight of 150 ± 10 g they were used for one of the following protocols.

Protocol I: comparative study of the effect of 1,25-$(OH)_2D_3$ and PTH on the EHDP-induced reduction of Ca absorption in intact rats

Rats were pair-fed throughout the experimental period the commercial diet they received from weaning time. The animals received either 10 mg P (160 μmole)/kg s.c. of EHDP given in a volume of 2 ml./kg body wt. or 2 ml./kg body wt. of 0·15 M-NaCl s.c. (non-treated group). EHDP and NaCl injections were given at 7.30 a.m. every morning for 7 days. After the seventh injection animals were fasted overnight. On the 8th day Ca absorption was measured as described below. In addition, half the animals of the EHDP-treated group were given either 1,25-$(OH)_2D_3$ or PTH. 1,25-dihydroxycholecalciferol, prepared according to the procedure described by Boyle, Miravet, Gray, Holick & DeLuca (1972), was generously supplied by Dr H. F. DeLuca. 1,25-$(OH)_2D_3$ was dissolved in 20 μl. ethanol 95% and 13·5 pmole given I.P. daily during the 7 days of EHDP treatment. PTH (TCA extract 250 i.u./mg, Wilson Laboratories) was dissolved in 0·2 ml. of a solution containing 0·15 g% bovine albumin, 0·38 g% NaCl, 0·85 g% glucose and 0·2 g% glutathione. 40 i.u. PTH was given s.c. at 7.30 a.m., 3.30 p.m. and 11.30 p.m. during the last 2 days of EHDP treatment. A last injection of PTH (40 i.u.) was given the next morning at 7.30 a.m., about 3 hr before the measurement of Ca absorption. Preliminary experiments had shown that PTH given at a dose of 3 × 15 i.u./day for 2 days had no influence on the EHDP-induced reduction in Ca absorption.

Protocol II: influence of EHDP on Ca absorption in thyroparathyroidectomized rats

Rats were pair-fed throughout the experimental period the commercial diet they received from weaning time. They were thyroparathyroidectomized surgically under ether anesthesia. 48 hr later a blood sample (0·2–0·3 ml.) was taken from the vein of the foot of a hind limb for plasma Ca determination. Only animals having a plasma Ca concentration below 8 mg/100 ml. were kept for further experimentation. Then the animals received either EHDP (10 mg P/kg day s.c. for 7 days) or NaCl (0·15 M s.c.) in a volume of 2 ml./kg body wt. After 7 days of treatment, animals were fasted overnight. The next morning a blood sample was taken for determining the concentration of plasma Ca ($[Ca]_{pl}$) and calcium absorption was determined as described below. The concentration of plasma inorganic phosphate ($[Pi]_{pl}$) was also determined in a group of thyroparathyroidectomized rats which underwent the same protocol.

Protocol III: influence of EHDP in operated and sham-operated rats supplemented with PTH

Rats were pair-fed throughout the experiment the commercial diet they received from weaning time. Animals were either sham-operated or thyroparathyroidectomized. 48 hr later a blood sample was taken as described above. In the experimental group, only animals having a $[Ca]_{pl}$ below 8 mg/100 ml. were kept for further experimentation. 72 hr after the surgical procedure, all rats were given s.c. PTH 3 × 40 i.u./day at 7.30 a.m., 3.30 p.m. and 11.30 p.m. as well as for the following 6 days. Half the animals of the sham-operated and the experimental group received EHDP at 10 mg P/kg day s.c. The other half received 0·15 M-NaCl at 2 ml./kg day s.c. EHDP and NaCl injections were given at 7.30 a.m. every morning for 7 days.

On the seventh day of treatment all animals were fasted overnight. The next morning, animals received the last PTH injection around 7.30 a.m. 60 min later a second blood sample was taken. Finally, Ca absorption was measured (around 10.30 a.m.) as described below.

Protocol IV: intestinal response to low Ca or P diet in EHDP-treated intact rats

From the beginning of the experimental period, rats were pair-fed a diet containing either 1·1% Ca and 1·0% P, 0·1% Ca and 1·0% P, or 1·1% Ca and 0·2% P. The three diets were prepared from a commercial chow (Altromin C1730) containing 0·04% Ca and 0·16% P, to which Ca gluconate or/and a mixture of K_2HPO_4/KH_2PO_4 (P ratio 7/3) was added. Since the Altromin C1730 diet is poor in vitamin D a supply of D_3 was given by gastric intubation at a dose of 25 i.u. dissolved in 0·2 ml. vegetable oil 3 times a week. After 1 week a first blood sample was taken, the animals being non-fasted. Then half the rats of each dietary group received every morning 10 mg P/kg day s.c. of EHDP in a volume of 2 ml./kg body wt. The other half was given 0·15 M-NaCl s.c. at 2 ml./kg. After the seventh injection, animals were fasted overnight. The next morning, a second blood sample was taken and Ca absorption was determined as described below.

Protocol V: influence of EHDP on Ca absorption and plasma Ca concentration in sham-operated and thyroparathyroidectomized rats fed a high or low Ca diet

Rats were either sham-operated or thyroparathyroidectomized surgically under ether anesthesia. 48 hr later a blood sample (0·2–0·3 ml.) was taken for determination of plasma calcium. Among the experimental group, only animals having a plasma Ca concentration below 8 mg/100 ml. were kept for further experimentation. From 72 hr after the surgical procedure rats were pair-fed diet containing either 0·1% Ca and 1·0% P or 1·1% Ca and 1·0% P. Both low and high Ca diets were prepared from a commercial chow (no. 195 OPC Nafag) containing 0·1% Ca and 0·2% P to which was added a mixture of K_2HPO_4/KH_2PO_4 (P ratio 7/3) and either glucose or Ca gluconate to yield the above mentioned Ca and P content. Sham-operated and experimental rats were given one of the two diets and received either EHDP (10 mg P/kg day s.c.) or NaCl (0·15 M s.c.) in a volume of 2 ml./kg body wt. After 7 days of treatment, animals were fasted overnight. The next morning a blood sample was taken and Ca absorption was determined as described below.

Measurement of Ca absorption

Ca absorption was estimated by an already described modification (Bonjour *et al.* 1973*a*) of the *in situ* ligated loop method (Wasserman, 1963; Morrissey & Wasserman, 1971). The animals were anaesthetized with an I.P. injection of 30–40 mg pentobarbitone/kg (Nembutal Abbott®). A first incision of the small intestine was made at the level of the bile duct aperture. A 1 cm long glass cannula was threaded through this first incision and tied in place. A second incision was made 8–12 cm distal to the glass cannula and the segment located between the two incisions (referred to as the duodenum) was rinsed with 5 ml. 0·15 M-NaCl. The lumen was emptied by a stream of air. The segment was then ligated and filled with 0·5 ml. of a solution containing 0·15 M-NaCl and either 4×10^{-5} M-$CaCl_2$ (protocol I, II and V), or 4×10^{-4} M-$CaCl_2$ (protocol III and IV), or 4×10^{-3} M-$CaCl_2$ (protocol IV). All solutions contained 0·1 μc/0·5 ml. of ^{45}Ca (specific activity 30 mc/mg). After a 5 or 15 min incubation the ligated loops were excised, the incubated solution cleaned from mucosal and cellular debris by centrifugation and radioactivity determined in two 0·1 ml. aliquots of the supernatant added to 10 ml. liquid scintillation solution. Ca absorption capacity was estimated by calculating

the % ^{45}Ca which disappeared from the incubation solution after 5–15 min. The radioactivity in the intestinal walls of the incubated segments was also determined as described previously (Bonjour *et al.* 1973*a*). The percent of the incubated dose of ^{45}Ca found in the intestinal wall of the duodenal segment varied inversely with the concentration of cold Ca in the solution. It was found to be (mean ± S.E. of mean) 5·48% ± 0·56 (n = 15); 8·87% ± 0·51 (n = 20); 12·99% ± 1·38 (n = 8) at concentrations of 4×10^{-3} M, 4×10^{-4} M and 4×10^{-5} M $CaCl_2$ respectively in control rats fed a high Ca (1·1%) and high P diet (1·0%). At the concentration tested, EHDP treatment, 1,25-$(OH)_2D_3$, PTH, thyroparathyroidectomy, low Ca or low P diet did not significantly modify the amount of ^{45}Ca found in the intestinal wall. Therefore, when comparing the groups, the difference in ^{45}Ca which disappeared from the incubation solution corresponds to the difference in the % Ca actually absorbed in the body.

Chemical determinations

Ca in plasma was measured by atomic absorption spectroscopy (Perkin Elmer Model 290 B) after diluting the samples with 0·5% $LaCl_2$.

Inorganic phosphate in plasma was measured according to the method of Chen, Toribara & Warner (1956).

Statistical analysis: all experimental results are expressed as mean ± S.E. of mean. Significance of difference between groups was evaluated by Student's t test.

RESULTS

(1) *Comparative study of the effect of 1,25-$(OH)_2D_3$ and PTH on the EHDP-induced reduction of Ca absorption*

Previous experiments in intact rats have shown that 1,25-$(OH)_2D_3$ given in a single dose as small as 32·5 p-mole I.V. can correct within 6 hr the low Ca absorption induced by EHDP given at the daily dose of 10 mg P/kg S.C. for 7 days (Bonjour *et al.* 1974). The results shown in Fig. 1 confirm that 1,25-$(OH)_2D_3$ can also prevent the reduction in Ca absorption induced by EHDP. In contrast administration of PTH (3 × 40 i.u./day), given during the last 2 days of the EHDP treatment, did not alter the effect of the diphosphonate on Ca absorption in intact rats. These results suggested that PTH was not involved in the EHDP-induced reduction in Ca absorption.

(2) *Influence of EHDP on Ca absorption in thyroparathyroidectomized rats*

To further assess the role of PTH on the intestinal response to EHDP treatment, the effect of the diphosphonate was studied in surgically treated rats. In those animals, EHDP increases significantly the concentration of plasma Ca (Table 1). This elevation in plasma Ca is associated with a decrease in the concentration of plasma inorganic phosphate ($[P_i]_{Pl}$). Measured in the same conditions $[P_i]_{Pl}$ was found to be 10·0 ± 0·43 mg% (n = 7) in EHDP-treated surgical rats as compared to 15·5 ± 0·5 mg% (n = 6) in non-treated TPTX animals. Even after the thyroparathyroid

glands were removed EHDP still inhibited intestinal Ca absorption (Table 1). Furthermore PTH (3 × 40 i.u/day) administered from the onset of the EHDP treatment did not prevent the fall of Ca absorption in both surgically operated and sham-operated animals (Fig. 2). In these rats

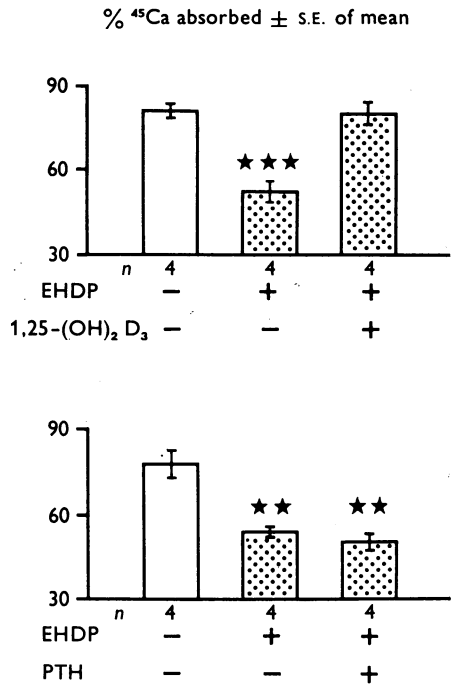

Fig. 1. Comparative study of the effect of 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$) and parathyroid hormone (PTH) on the EHDP-induced reduction of Ca absorption in intact rats. EHDP was given at a dose of 10 mg P/kg day s.c. for 7 days. $1,25\text{-}(OH)_2D_3$ was given during the 7 days of EHDP treatment at a daily dose of 13·5 p-mole I.P. 40 i.u. PTH was given during the last 2 days of EHDP treatment at 7.30 a.m., 3.30 p.m. and 11.30 p.m. and 3 hr before the measurement of Ca absorption. Ca absorption was measured in duodenal segments as described in the text. Ca concentration in the incubation solution was 4×10^{-5} M. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$ as compared with the non-treated group (open bar). n = number of animals.

supplemented with PTH, $[Ca]_{Pl}$ (mg %) measured 2 hr before the tied-loop experiment in the surgically treated rats was (mean ± S.E. of mean): non-treated $10{\cdot}0 \pm 0{\cdot}36$, $n = 4$; EHDP-treated $11{\cdot}4 \pm 0{\cdot}27$, $n = 4$; $P < 0{\cdot}05$. In the sham-operated rats it was: non-treated $10{\cdot}0 + 0{\cdot}21$, $n = 6$; EHDP-treated $10{\cdot}7 \pm 0{\cdot}10$, $n = 6$; $P < 0{\cdot}05$.

3. *Intestinal response to low Ca or P diet in non-treated and EHDP-treated intact rats*

In the non-treated rats reducing either Ca or P in the diet stimulated the amount of Ca absorbed (Fig. 3). This response was more apparent when Ca absorption was measured at the higher intraluminal Ca concentration (4×10^{-3} M).

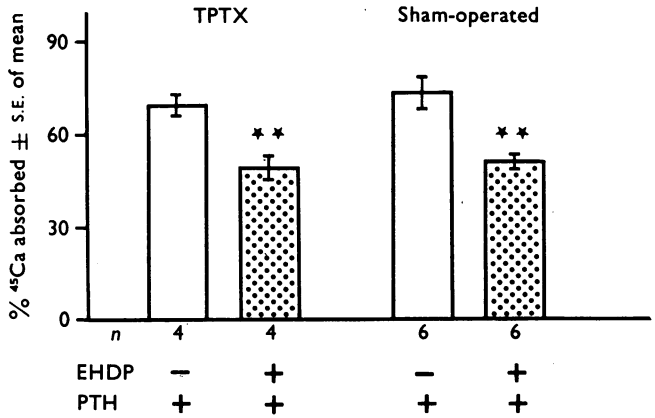

Fig. 2. Maintenance of the effect of EHDP on Ca absorption in thyroparathyroidectomized (TPTX) and sham-operated animals supplemented with parathyroid hormone (PTH). EHDP was given at a dose of 10 mg P/kg s.c. for 7 days. 40 i.u. of PTH was given during the 7 days of EHDP treatment at 7.30 a.m., 3.30 p.m. and 11.30 p.m. and 3 hr before the measurement of Ca absorption. Ca absorption was measured in duodenal segments as described in the text. Ca concentration in the incubation solution was 4×10^{-4} M. $^{**}P < 0{\cdot}01$ as compared with the corresponding group which did not receive EHDP. n = number of animals.

In the EHDP-treated rats the response to a low Ca diet appeared the same as in the non-treated animals. However EHDP treatment inhibited the rise in the Ca absorption capacity elicited by a low P diet. The difference in the response to a low Ca as compared to a low P intake was particularly apparent when Ca absorption was assessed at an intraluminal Ca concentration of 4×10^{-4} M. Under these experimental conditions, the inhibitory effect of EHDP on Ca absorption was nearly abolished in rats fed a low Ca diet, whereas it seemed to be more pronounced with the low P diet when compared to the non-treated rats fed the same diet. Indeed the EHDP-treated/non-treated ratios for Ca absorption measured at an intraluminal Ca concentration of 4×10^{-4} M were (mean ± S.E. of mean): high Ca–high P diet $0{\cdot}65 \pm 0{\cdot}04$ (n = 8); low Ca-high P diet $0{\cdot}93 \pm 0{\cdot}04$ (n = 4); high Ca-low P diet $0{\cdot}54 \pm 0{\cdot}04$ (n = 4).

In Table 2 are presented the results concerning the determination of

plasma Ca and phosphate. A small elevation of plasma Ca is observed under EHDP treatment. A statistically significant difference is observed only in the rats fed the low Ca–high P diet. As in surgically operated rats EHDP also brings about a reduction in plasma phosphate in intact rats when fed a Ca 1·1/P 1·0 g% diet. This effect is very likely due to a change

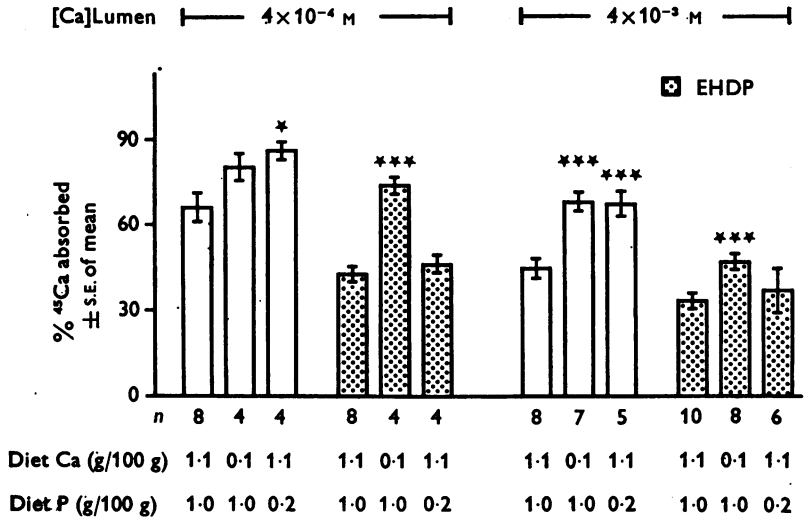

Fig. 3. Intestinal response to low Ca or P diet in non-treated and EHDP-treated intact rats. Rats were fed the indicated diet for 14 days. EHDP was given at a dose of 10 mg P/kg day s.c. for the last 7 days. Ca absorption was measured in duodenal segments as described in the text. As shown, Ca concentration in the incubation solution was either 4×10^{-4} or 4×10^{-3} M. Open bars = non-treated rats; dotted bars = EHDP-treated rats. * $P < 0{\cdot}05$; *** $P < 0{\cdot}001$ as compared with the corresponding group fed Ca 1·1 and P 1·0% diet. n = number of animals.

in the renal handling of P_i (Troehler, Mühlbauer, Hugi, Fleisch & Bonjour, 1976). Finally treatment with EHDP does not prevent the elevation of plasma P_i which takes place when animals fed a low P_i diet are fasted.

4. *Intestinal adaptation to low Ca diet in sham-operated and thyroparathyroidectomized rats treated with HHDP*

As shown in Fig. 4, the low Ca diet did not seem to stimulate the absorption of Ca in the non-treated sham-operated group. This apparent lack of stimulation of the calcium transport system is probably related to the low Ca concentration (4×10^{-5} M) of the incubation solution used in this experiment. It could be due to an experimental artifact resulting from the high percent of Ca absorbed after an incubation of 15 min. However, this cannot explain the similar absorption observed after 5 min

in both the rats fed high and low Ca diets. Therefore it seems valid to conclude that the alteration in the Ca transport system resulting from Ca deprivation is revealed only above a critical concentration of substrate in the intestinal lumen. Thus the threshold for the expression of the Ca

TABLE 1. Influence of EHDP on plasma Ca ($[Ca]_{pl}$) and Ca absorption in thyroparathyroidectomized rats

	Non-treated	EHDP-treated
n ...	10	10
$[Ca]_{pl}$ (mg/100 mg) (1)	7·4 ± 0·2	7·6 ± 0·2
(2)	6·5 ± 0·1	8·6 ± 0·2***
Ca absorption (%)	71·3 ± 2·8	58·5 ± 2·9**

Rats were fed a commercial diet containing Ca 1·1% and P 1·0%. EHDP was given at the dose of 10 mg P/kg day S.C. for 7 days.

(1) determined in plasma samples taken two days after thyroparathyroidectomy.

(2) determined in plasma samples taken after an overnight fast, just before the measurement of Ca absorption.

Ca concentration in the incubation solution was 4×10^{-5} M. All values are mean ± S.E. of mean. n = number of animals.

**$P < 0.01$; ***$P < 0.001$ as compared to the non-treated group.

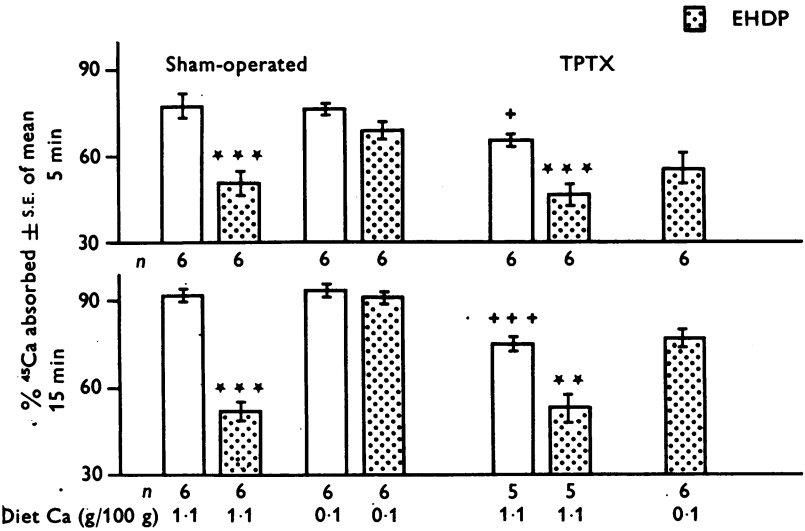

Fig. 4. Intestinal adaptation to low Ca diet in sham-operated and thyroparathyroidectomized (TPTX) rats treated with EHDP. Rats were fed the indicated diets for 7 days. EHDP was given at a dose of 10 mg P/kg S.C. for 7 days. Ca absorption was measured in duodenal segments as described in the text. Ca concentration in the incubation solution was 4×10^{-5} M. Open bars = non-treated rats; dotted bars = EHDP-treated rats. ** $P < 0.01$; *** $P < 0.001$ as compared with the corresponding non-treated group; + $P < 0.05$; + + + $P < 0.001$ as compared with the corresponding sham-operated group receiving the 1·1% Ca diet. n = number of animals.

TABLE 2. Influence of EHDP on the plasma concentration of Ca ($[Ca]_{pl}$) and inorganic phosphate ($[P_i]_{pl}$) in intact rats fed high or low Ca or P diets

Ca-P in diet (g/100 g) ...	Ca 1·1/P 1·0		Ca 0·1/P 1·0		Ca 1·0/ P 0·2	
Blood I: before EHDP-treatment (rats non-fasted)						
n	21		23		14	
$[Ca]_{pl}$ (mg %)	10·1 ± 0·11		9·8 ± 0·09		11·2 ± 0·24	
$[P_i]_{pl}$ (mg %)	7·3 ± 0·11		7·4 ± 0·21		4·7 ± 0·22	
Blood II: after EHDP-treatment (rats fasted overnight)	Non-treated	EHDP-treated	Non-treated	EHDP-treated	Non-treated	EHDP-treated
n	10	11	11	12	7	7
$[Ca]_{pl}$ (mg %)	10·1 ± 0·16	10·4 ± 0·19	10·1 ± 0·09	10·5 ± 0·09*	10·0 ± 0·17	10·2 ± 0·27
$[P_i]_{pl}$ (mg %)	8·8 ± 0·56	6·7 ± 0·24**	7·9 ± 0·26	7·5 ± 0·23	8·9 ± 0·41	10·2 ± 0·70

Rats were fed the indicated diets for 14 days. $[Ca]_{pl}$ and $[P_i]_{pl}$ was measured one week after starting the experimental diets, animals being non-fasted (blood I). Then EHDP was given at the dose of 10 mg P/kg day S.C. over the last 7 days. The non-treated animals received equivalent volumes of 0·15 M-NaCl vehicle. After the seventh injection animals were fasted overnight. The next morning a second blood sample was taken (blood II) before measuring the intestinal absorption of Ca (Fig. 3). All values are mean ± S.E. of mean. n = number of rats. *$P < 0.05$; **$P < 0.01$ as compared with the corresponding untreated group.

adaptation phenomenon, in the rat duodenum under our experimental conditions, seems to be set between 4×10^{-5} and 4×10^{-4} M (see Figs. 3 and 4).

In the sham-operated EHDP-treated group intestinal adaptation in response to a low Ca diet does take place, confirming results presented in Fig. 3. Furthermore this experiment also shows that Ca absorption, when assessed at low intraluminal Ca concentration, does not seem to be affected by EHDP treatment in rats fed a low Ca diet.

In the thyroparathyroidectomized non-treated rats, it was not possible to evaluate the effect of lowering the dietary supply of Ca in presence of a high P intake. Indeed, as mentioned below, most of the animals of this group died, probably of hypocalcaemia (see Table 2) before Ca absorption could be measured.

TABLE 3. Influence of EHDP on plasma Ca concentration (mg/100 mg) in sham-operated and thyroparathyroidectomized (TPTX) rats fed a high or low Ca diet

Ca-P in diet (g/100 g) ...	Ca 1·1/P 1·0		Ca 0·1/P 1·0	
	Non-treated	EHDP-treated	Non-treated	EHDP-treated
Sham-operated	10·3 ± 0·1 (12)	10·5 ± 0·2 (12)	10·2 ± 0·1 (12)	10·9 ± 0·1*** (12)
TPTX	6·2 ± 0·6 (11)	9·2 ± 0·3*** (10)	3·5 ± 0·1 (7)	6·8 ± 0·5** (12)

EHDP was given at the dose of 10 mg P/kg day S.C. for 7 days. After an overnight fast blood was taken for plasma Ca determination just before the measurement of calcium absorption the data of which are presented in Fig. 4. All values are mean ± S.E. of mean. Number in parentheses = number of rats. $^{**}P < 0.01$; $^{***}P < 0.001$ as compared to the corresponding non-treated group.

In the thyroparathyroidectomized EHDP-treated rats, the dietary Ca intake markedly influenced the percent of Ca absorption. In these treated rats, fed the low Ca and high P diet, Ca absorption was significantly ($P < 0.01$) higher than that observed in the treated rats fed the high Ca and high P diet. Therefore the results of these experiments showed that in both sham-operated and thyroparathyroidectomized rats EHDP treatment did not abolish the intestinal response to low Ca diet. Finally, the known inhibitory effect of thyroparathyroidectomy on Ca absorption was clearly shown in the untreated rats fed the high Ca–high P diet (Fig. 4). However in the EHDP-treated rats thyroparathyroidectomy influenced Ca absorption only when the animals were fed a low Ca–high P diet. Indeed low rate of Ca absorption achieved by EHDP in animals fed a high Ca diet was not further depressed by removal of the thyroparathyroid glands (Fig. 4).

In this experiment calcaemia measured in rats (fasted overnight) 8 days after starting the EHDP treatment, i.e. 24 hr after the last injection

of EHDP, is presented (Table 3). In the sham-opearated group, EHDP-treated animals displayed a slightly higher plasma calcium than the non-treated group. As in Table 2 statistical significant difference was obtained only in the rats fed the low Ca diet. In the operated animals fed the high Ca diet, EHDP markedly raised the plasma Ca level to a value close to that observed in the sham-operated animals. In the operated animals fed a low Ca diet, rats of the non-treated group which survived the overnight fast had (Table 3) a very low $[Ca]_{Pl}$ ($3{\cdot}5 \pm 0{\cdot}13$ mg %). Very interestingly, under these experimental conditions, EHDP prevented a fall in the calcaemia to levels incompatible with survival.

DISCUSSION

The present work confirms that in rats the inhibitory action of large doses of EHDP on intestinal Ca absorption can be prevented by physiological doses of 1,25-$(OH)_2D_3$ (Bonjour *et al.* 1973*a*, 1974, 1975). The data indicate that, contrary to 1,25-$(OH)_2D_3$, PTH is probably not involved in the intestinal response to EHDP. Indeed in intact rats, administration of PTH at doses which normalize the calcaemia of thyroparathyroidectomized rats cannot reverse the EHDP-induced reduction in Ca absorption. Furthermore the inhibitory action of the diphosphonate is still present in the operated rats whether or not they were supplemented with PTH. Thus the EHDP-induced inhibition of Ca absorption does not appear to be mediated by a reduced secretion of PTH. Such a mediation could have been suspected since the action of EHDP on the intestine is accompanied by an increase in plasma Ca (Gasser *et al.* 1972; Bonjour *et al.* 1973*a*). Furthermore, in the non-treated animals, thyroparathyroidectomy decreases Ca absorption (Fig. 4) and this effect can be corrected by PTH substitution (Fig. 2), confirming several studies on this subject (Rasmussen, 1959; Kimberg, Schachter & Schenker, 1961; Cramer, 1963; Toverud, 1964; Shah & Draper, 1966; Sammon, Stacey & Bronner, 1970). Our data show that EHDP antagonizes the action of PTH on Ca absorption (Fig. 2). These results strongly support the hypothesis (Garabedian *et al.* 1972) that the PTH effect on intestinal Ca absorption in normal animals is mediated through 1,25-$(OH)_2D_3$. The diphosphonate also antagonizes the stimulatory effect of P deprivation on Ca absorption. Whether these effects of EHDP are taking place at the renal level on the production of 1,25-$(OH)_2D_3$ remains to be established.

In contrast, the diphosphonate does not impair the enhancement of Ca absorption capacity in response to Ca deprivation. Furthermore our results also indicate that the intestinal response to EHDP treatment seems to be modulated by the calcium intake. This finding would corroborate our

original hypothesis (Morgan *et al.* 1971; Bonjour *et al.* 1973*a*,*b*) which considered the decreased Ca absorption as an adaptive phenomenon in response to the inability of the bone to incorporate dietary Ca in presence of EHDP. The modulation of the effect of EHDP on the intestine by the Ca intake could well be mediated by various degrees of inhibition in the production of 1,25-$(OH)_2D_3$.

Recent studies on this subject in the chick have produced contradictory results. Baxter *et al.* (1974) have shown very little influence of dietary Ca on the inhibitory effect of EHDP (given at 5 mg P/kg day s.c. for 2 weeks) on the 1-hydroxylase. In contrast Taylor, Mawer & Reeve (1975) have found no inhibition of the renal 1-hydroxylation in birds maintained on a low Ca diet and receiving EHDP at 10 mg P/kg day s.c. From our results on Ca absorption capacity in the rat we would predict a partial inhibition of the production of 1,25-$(OH)_2D_3$.

The intestinal adaptation to a low Ca diet in the EHDP-treated animals probably does not involve PTH. Indeed this adaptation by the Ca intake is observed in thyroparathyroidectomized rats. This latter effect is especially interesting with respect to the regulation of Ca absorption. Indeed the influence of lowering dietary Ca without decreasing the P intake is difficult to study in surgically operated animals. Usually, and as in one of our experiments, the animals died. To our knowledge only one study has been reported concerning the effect of lowering dietary Ca, without decreasing the P intake, on the Ca absorption of thyroparathyroidectomized rats (Kimberg *et al.* 1961). In this investigation, five out of nine animals which survived this treatment in spite of a very low calcaemia (2·8–4·2 mg/100 ml.) exhibited a greater Ca transport (studied *in vitro* using duodenal gut sacs) than did the operated rats fed a high Ca diet. This observation suggests that Ca adaptation to a low Ca diet could occur in the operated rats in absence of a concomitant phosphate deprivation. Our experiments show that large doses of EHDP elevate the level of plasma Ca through an unknown mechanism. This probably allows operated animals to survive on a low Ca–high P diet in satisfactory condition. In these circumstances a duodenal adaptation to a reduction in the dietary Ca supply is observed in the operated rats in spite of a high P intake. This represents the first definite evidence that the capacity of the intestine to transport Ca can be modulated by Ca alone according to homoeostatic requirement in absence of the thyroparathyroid glands. The possible involvement of 1,25-$(OH)_2D_3$ for promoting this intestinal response remains to be investigated.

This work has been supported by the U.S. National Institute of Health (grant AM 07266), the Swiss National Foundation for Scientific Research (grant 3.121.73) and the Procter and Gamble Company, U.S.A.

We thank Mrs B. Fosco, Mrs I. Moret, Miss U. Zwicky and Mrs I. Tschudi for their excellent technical help, Mrs C. Stieger for assistance in illustration and Mrs B. Gyger for the preparation of the manuscript.

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