Hormonal modulation of mineral metabolism in reproduction

By C. J. Robinson, Judith Hall and S. O. Beshir, *Department of Physiological Sciences, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Pregnancy and lactation present a considerable stress to calcium homoeostasis in mammals. This paper attempts to outline those mechanisms whereby the mother effects the appropriate alterations in Ca fluxes to respond to the increased Ca demands imposed by pregnancy and lactation, and to identify the factors, hormonal and otherwise, that contribute to the adaptative processes of pregnancy and lactation.

As there are considerable variations in the degree of Ca stress induced by such conditions and in the patterns of hormones that affect Ca metabolism in the various species, it is impossible to present a truly global review. The following discussion is, therefore, devoted largely to studies in the rat, with comparable observations on events occurring in man and ruminants included where appropriate. For a more complete review of mineral metabolism in ruminants, the recent Symposium of the Nutrition Society (Field, 1981; Gueguen & Perez, 1981; Scott & McLean, 1981) should be consulted.

Extent of the alterations in Ca metabolism

In humans, where the skeletal content of Ca is approximately 25 mol, the normal Ca turnover is approximately 10 mmol/d. The amount of Ca acquired by the foetus from its mother at term is of the order of 750 mmol, the great majority of which is acquired over the last trimester (Pitkin, 1975); the subsequent lactational drain is of the order of 4–7·5 mmol/d.

In the rat, whose total Ca content is approximately 60 mmol, approximately 4 mmol Ca are transferred to the litter by the time of parturition, the great majority in the last 7 d of pregnancy (Simkiss, 1967). However, up to 25 mmol Ca (equivalent to over 40% of the total maternal content of Ca) may be transferred to the litter during the 3-week-period of lactation. Thus, whilst pregnancy poses a challenge to the Ca homoeostatic mechanism in these species, the challenge is considerably greater during lactation. Similar considerations apply for sheep (Braithwaite *et al.* 1969, 1970) cows (Simkiss, 1967) and pigs (Gueguen & Perez, 1981).

Provided the dietary supply of Ca is adequate, there is little evidence of a reduction in mineral content of the maternal skeleton during pregnancy. Indeed, in both rats (Warnock & Duckworth, 1944; Spray, 1950) and humans (Hummel *et al.* 1936) there appears to be deposition of a 'Ca reserve' (Simkiss, 1967). This indicates that the adaptations more than compensate for the mineral requirement

of the maturing foetal skeleton, although evidence of a mineral deficit has been reported in the sheep (Braithwaite *et al.* 1970). Modest hypocalcaemia during pregnancy has been reported in humans, but when corrections are made for the hypoalbuminaemia associated with volume expansion, there is actually no significant alteration in the physiologically-important ionized Ca concentration (Pitkin, 1975).

Lactation presents a different picture. Here the extent of the alterations varies considerably from species to species. The Ca content of milk and the volume of milk produced (Table 1), are important determinants of the Ca demand. They in turn are affected by the number of young to be suckled (from the human and cow who normally produce only one, to pigs and rats who frequently have litters in excess of ten) and the duration of lactation.

As the Ca requirement of lactation is much greater than that of pregnancy, it is not too surprising that modest hypocalcaemia and/or a loss of skeletal mineral are observed in a number of species including the rat (Warnock & Duckworth, 1944), sheep (Braithwaite *et al.* 1969) and man (Atkinson & West, 1970). There is also the acute post-partum hypocalcaemia of cows (Payne, 1964).

Adaptative changes during pregnancy

Ca metabolism may be altered primarily by:
    (1) Deposition and resorption of Ca in the skeleton.
    (2) The reabsorption of Ca by the kidney.
    (3) The absorption of Ca from the gastrointestinal tract.

As indicated earlier, apart from instances of Ca deprivation, there is little evidence to suggest any diminution in the Ca content of the skeleton. Indeed, the evidence from rats and man would appear to indicate an increase rather than a decrease in skeletal Ca content during pregnancy.

A reduction in urinary Ca loss during pregnancy has been observed in humans (Goss, 1962), particularly during the last month of pregnancy (Heaney & Skillman, 1971), whilst the plasma Ca concentration remains relatively constant. This implies some hormonal modulation of Ca retention, rather than a consequence of alterations in the filtered load of Ca (Nordin & Peacock, 1969). However, the relatively small excretion of Ca in humans (a total of only 5 mmol/d) would

Table 1. *Volume of milk produced and calcium content in various species**

Species	Ca content of milk (mmol/l)	Vol. of milk produced (l/d)	Total milk Ca (mmol/d)
Rat	80	0·006–0·012	0·48–0·96
Human	8·25	0·35–0·8	2·9–6·6
Sheep	40	0·3–1·4	12–56
Pig	55	5·0–7·0	275–385
Cow	30	7·0–24·0	210–720

*Modified from Simkiss, 1967; Braithwaite *et al.* 1969; Baksi & Anderson, 1971; Hart *et al.* 1978.

preclude any marked contribution of the kidney to the Ca requirements of pregnancy, and even less to those of lactation.

Undoubtedly, the major site of adaptation is the gastrointestinal tract, where Ca absorption is frequently doubled (Heaney & Skillman, 1971). This locus of Ca transport has the capacity to provide all the Ca transported to the foetus via the placenta. That the increase in Ca absorption occurs is without question, what is not clear is the mechanism by which it is effected. In vitro studies (Schachter *et al.* 1960; Halloran & DeLuca, 1980) indicate that the active transport of Ca is greatly increased in pregnant rats. Exactly which hormone or hormones contribute to this response will be discussed in the subsequent section. However, the possibility cannot be discounted that at least part of the increased absorption reflects intestinal hypertrophy which is associated with both pregnancy and lactation in rats (Craft, 1970) and mice (Harding & Cairnie, 1975), as is the increase in mucosal surface area (Boyne *et al.* 1966). Whether these latter responses are directly hormonally mediated, or reflect induced hyperphagia, remains to be defined, as do their occurrence in ruminants and man.

Possible hormonal mediators of the alterations in pregnancy

Until 15 years ago, the only hormone believed to be involved in Ca homoeostasis in pregnancy was parathyroid hormone (PTH). However, a number of other hormones, including the lactogenic hormones, have now been implicated in the response.

Parathyroid hormone. PTH is elevated in late pregnancy in man (Cushard *et al.* 1972; Samaan *et al.* 1974; Retallack *et al.* 1977). Additionally, parathyroid hypertrophy has been observed in pregnant rats (Toverud *et al.* 1978) and cows (Capen *et al.* 1965). This latter observation may explain the chronic elevation of PTH levels in the presence of near-normal plasma Ca values.

PTH has three major actions (Robinson, 1974). It increases the tubular reabsorption of Ca in the kidney, it increases the resorption of Ca from the skeleton and it stimulates Ca absorption from the intestine. It would therefore follow that PTH is probably responsible for the reduction in urinary Ca (Goss, 1962; Heaney & Skillman, 1971), although both prolactin and placental lactogen may also be involved (James *et al.* 1978; Makeen, 1980).

The action on the skeleton appears to be of little significance since most species, with the possible exception of sheep (Braithwaite *et al.* 1970), show an increase in skeletal Ca content in pregnancy, probably as a result of the increased calcitonin levels. However, in animals on a limited Ca ration, with relative hypocalcaemia, there is evidence of PTH-induced bone resorption (Bodansky & Duff, 1941).

Since PTH stimulates intestinal Ca absorption (MacIntyre & Robinson, 1969), probably by an effect on the synthesis of 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) (Fraser & Kodicek, 1973), the elevated levels of PTH seen in pregnancy undoubtedly contribute to the increased Ca absorption. However, studies in our laboratory have indicated that the elevation of Ca absorption in late pregnancy is

little influenced by the absence of the parathyroid glands, suggesting that they are not the major influence.

1,25(OH)$_2$D$_3$. This is the major active metabolite of vitamin D and, like PTH, it has three actions, on bone, kidney and the intestine. The resorption of bone is only seen with very high levels of 1,25(OH)$_2$D$_3$ and the effects on the renal handling of Ca are small and controversial (Pushett *et al.* 1974; Bonjour *et al.* 1978).

1,25(OH)$_2$D$_3$ levels are significantly elevated at the end of pregnancy (Table 2, Halloran & DeLuca, 1980). How much of the elevated 1,25(OH)$_2$D$_3$ is of renal origin and how much is synthesized in the placenta (Weisman *et al.* 1979) remains to be established. However, 1,25(OH)$_2$D$_3$ is probably responsible for much of the increase in Ca absorption seen at this time as it is an extremely potent stimulator of Ca transport (Norman, 1978) and is probably the common pathway through which a number of hormones exert their effect upon Ca absorption. Experiments are in progress in our laboratory to attempt to determine in vivo that proportion of the increase in Ca absorption due to 1,25(OH)$_2$D$_3$ and that due to the increase in surface area of the intestine (Craft, 1970) during pregnancy and lactation.

Prolactin. This is primarily considered to be a lactogenic hormone although it is now known that it has many other actions. Not least among these is that it exerts an effect on Ca metabolism in terms of Ca retention by the kidney (Makeen, 1980) and the stimulation of Ca transport both in vitro (Mainoya, 1975) and in vivo (James *et al.* 1977). The latter effect is mediated largely by an action on the synthesis of 1,25(OH)$_2$D$_3$ (Spanos *et al.* 1976). Prolactin also increases feeding drive (C. J. Robinson and D. Stephenson, unpublished results) and fluid intake in the rat (Kaufman, 1981) and thus may increase the amount of Ca available for absorption.

Table 2. *Effects of bromocriptine* (0·5 *mg twice daily for* 3 *d*) *in rats*

(Mean values with their standard errors)

	n	Weight (g) Mean	SEM	Number of foetuses Mean	SEM	Plasma Ca (mmol/l) Mean	SEM	Ca absorption (% dose absorbed) Mean	SEM	Plasma 1,25-dihydroxy vitamin D$_3$ (pmol/l) Mean	SEM
Non-pregnant + vehicle	6	208	7	—	—	2·54	0·07	52·7	5·1	48	14
Pregnant + vehicle	3	245*	19	7·7	1·3	2·55	0·07	86·2*	4·7	264*	53
Pregnant + bromocriptine	4	274*	7	9·5	0·6	2·30	0·13	77·4*	7·7	264*	43

*Significantly different (*P*<0·05) from non-pregnant group.

However, since plasma prolactin levels do not rise in the rat (Amenomori *et al.* 1970), goat, sheep and cow (Cowie *et al.* 1980) until just before parturition, it is unlikely that prolactin plays an important role in Ca homoeostasis at this stage of reproduction in those species. This is born out by a study (Table 2) in which bromocriptine treatment was given for 3 d to block the pre-parturient rise in plasma prolactin levels. The elevations in Ca absorption and plasma $1,25(OH)_2D_3$ were almost identical in control and bromocriptine-treated pregnant groups, indicating that the presence of prolactin was not necessary for the adaptive response to occur. However, since prolactin levels rise progressively from the beginning of the third trimester in humans (Del-Pozo *et al.* 1977) the possibility that prolactin contributes to the response in human pregnancy cannot be excluded.

Calcitonin. Although one report (Swaminathan *et al.* 1974) indicates that chronic calcitonin treatment may depress Ca absorption in the pig, the majority of evidence (Krawitt, 1967; Robinson *et al.* 1969) indicates that calcitonin has no direct effect upon Ca transport by the gut. However, the possibility of an indirect effect, by inducing secretion of PTH in animals with intact parathyroid glands, cannot be discounted.

Again, the effects of calcitonin on the kidney are limited and controversial. The hormone has only one major action in Ca metabolism—that of blocking bone resorption, including that induced by PTH and $1,25(OH)_2D_3$.

Surprisingly, in spite of a tendency to hypocalcaemia in the later stages of pregnancy, calcitonin levels are actually significantly elevated just before parturition (Samaan *et al.* 1974). Whilst the physiological advantage of blocking any bone resorption that might otherwise be induced by the elevated PTH concentration is apparent, the reason why the calcitonin levels are elevated remains to be determined. One suggestion is that since gastrointestinal hormones such as gastrin, cholecystokinin–pancreozymin and enteroglucagon, released post-prandially, are potent calcitonin secretagogues, and since the food intake before parturition is nearly double the normal intake (Lichtenberger & Trier, 1979) it seems reasonable to presume that the hyperphagia contributes to the elevated calcitonin levels.

Placental lactogen. Heaney & Skillman (1971) first suggested that placental lactogen, in addition to its other actions in pregnancy, may play a role in the control of Ca metabolism. It is structurally similar to growth hormone (GH) and prolactin, both of which exert effects upon Ca metabolism by stimulation of the production of $1,25(OH)_2D_3$ (James *et al.* 1978). Placental lactogen is ideally suited to exert such a role because its levels rise progressively from about mid-term and reflect placental development and, therefore, the increasing demands of the foetus. Moreover, it is present in the plasma in far higher quantities than any other hormone, reaching concentrations measured in micrograms per ml in humans (Josimovich, 1977) and goats (Cowie *et al.* 1980).

Human placental lactogen injected at a dose predicted to produce plasma concentrations approximately equal to those seen just before parturition, virtually doubled Ca absorption in rats (James *et al.* 1978).

Subsequent work (Spanos, 1979) has shown that placental lactogen, like prolactin and GH, increases the renal production of $1,25(OH)_2D_3$ and indicated that this is probably the mechanism whereby placental lactogen increases Ca absorption.

Placental lactogen is present in quantities far in excess of any of the other hormones that affect Ca absorption, and its concentration increases with foetal size. It thus follows that it may well be the hormone which is responsible for the increased Ca absorption in pregnancy, and thus provides a mechanism whereby intestinal absorption of Ca can be increased to reflect the increased mineral demand.

It has yet to be determined whether placental lactogen acts directly on the kidney and whether it is responsible for some or all of the extra-renal production of $1,25(OH)_2D_3$ by the placenta. Notwithstanding, the evidence indicates that placental lactogen deserves to be considered, possibly in conjunction with PTH, as one of the major primary hormonal modulators of Ca absorption in pregnancy, and suggests a new and important role for the hormone.

Parturient hypocalcaemia. Before proceeding to review the control of Ca metabolism in lactation it seems pertinent to make reference to this disorder, which largely affects ruminants, usually beginning approximately 24 h prior to parturition. It is associated with elevated $1,25(OH)_2D_3$ and PTH levels (Horst *et al.* 1978) and a reduced calcitonin content in the blood (Mayer *et al.* 1975). The aetiology is not certain. One proposal is that the rapid withdrawal of Ca from the circulation, to be secreted in the colostrum (which in cows has a higher concentration of Ca than milk), is responsible and that it reflects the failure of the homoeostatic mechanism (Simkiss, 1967).

A simplistic explanation, complementary to that of Simkiss, is as follows. It is recognized that the bovine lactational requirement of Ca is extremely high (Table 1) and that this is primarily satisfied by increased absorption of Ca. However, just prior to parturition, in the majority of species, there is an acute reduction of food intake. This results in a reduced secretion of gastrointestinal hormones, which will tend to cause lowered plasma calcitonin levels. The hypocalcaemia resulting from a cessation of Ca transport from the gut will also lower plasma calcitonin levels, but tend to produce the elevated $1,25(OH)_2D_3$ and PTH levels. These obviously do not act upon the gut since there is little Ca in the gastrointestinal tract. Moreover, the time lag of the skeletal response to these agents is inadequate to satisfy acutely the large demand of the mammary tissue. Accordingly, hypocalcaemia results until such time as the food drive is increased and the hormones exert their effects again on the gastrointestinal tract and, in the short-term, the skeleton.

Adaptive changes during lactation

The Ca demand of lactation is greater than that of pregnancy (Table 1) and, moreover, increases progressively until weaning (Baksi & Anderson, 1971). This is paralleled by alterations in the adaptative processes, similar to those observed in pregnancy but larger in magnitude:

(1) A reduction in urinary Ca excretion (Retallack *et al.* 1977).
(2) An increase in food intake (Lichtenberger & Trier, 1979).
(3) An increase in intestinal absorption of Ca (Fournier & Susbielle, 1952).

In spite of these, skeletal demineralization has been reported in lactating rats (Warnock & Duckworth, 1944), sheep (Braithwaite, 1978) and humans (Atkinson & West, 1970), indicating a failure to match the demand of lactation. This may be a matter of some concern in repeated pregnancies or prolonged lactations.

The reduction in urinary Ca excretion is partly due to the reduced filtered load, consequent upon the modest lowering of plasma Ca frequently seen in this situation, and also the conservative effect of the resultant increased PTH level (Samaan *et al.* 1974; Retallack *et al.* 1977) upon the kidney. However, the amount of Ca conserved is so small in comparison with the Ca secreted in the milk that, with the possible exception of humans (Table 1, Retallack *et al.* 1977), it contributes probably less than 5% of milk Ca.

The increase in food intake is well recognized, with up to a quadrupling of food intake being recorded in rats (Lichtenberger & Trier, 1979). This has two advantages to the animal, it increases the amount of Ca available for absorption and by its bulk contributes to the intestinal hypertrophy that facilitates increased absorption.

The mechanism of the increase in food intake remains uncertain. Our studies (C. J. Robinson and D. Stephenson, unpublished results) indicate that prolactin injected twice daily can induce a modest 20% increase in food intake in virgin female rats, but nothing approaching the 300% increase observed in the lactating female rat. Admittedly, in our studies the animals employed were virgin rats so that the possibility of interactions with other hormones could not be excluded, nor could the effect of suckling itself (Cotes & Cross, 1954) which may be important in generating such a drive.

The increase in Ca absorption is the most important factor responsible for satisfying the majority of the lactational drain of Ca. Recent evidence (Halloran & DeLuca, 1980) would suggest that the response can be divided into two parts, that attributable to intestinal hypertrophy, which may be of mechanical or hormonal origin, and that attributable to facultative alterations in the concentration of $1,25(OH)_2D_3$ in the blood. At present the proportional contribution of these two remains to be defined, but it is now quite clear that $1,25(OH)_2D_3$ approximates to the so-called 'endogenous factor' (Nicolaysen, 1943) which alters Ca absorption to match body demands. As the production of $1,25(OH)_2D_3$ by the kidneys is sensitive to alterations in a number of putative controlling hormones, there has been some doubt as to which agent, or agents, was actually responsible for the elevated levels in lactation. The subsequent section deals with the possible candidates of this role and others that contribute to the maintenance of Ca homoeostasis in lactation.

Alteration of Ca-regulating hormones during lactation

Calcitonin. The levels of calcitonin are elevated during lactation (Toverud *et al.*

1978). Again, the cause for this is uncertain in view of the normocalcaemic or even slightly hypocalcaemic state. However, the hyperphagic-stimulated release of calcitonin secretagogues from the gastrointestinal tract, in concert with the transient post-prandial hypercalcaemia resulting from the increased absorption of Ca, may well contribute significantly to this response. Studies in animals given limited rations will allow definition of this putative axis.

If calcitonin is present in increased concentrations in the blood, is it fulfilling any physiological role? Our experimental studies (Table 3) show that animals deprived of their thyroids but with auto-transplanted parathyroid glands (calcitonin deficient) had significantly less Ca in their skeleton, and their Ca absorption was reduced, compared with intact controls in whom the Ca requirement for lactation was obtained from the diet rather than the maternal skeleton (Lewis *et al.* 1971). Thus, calcitonin has indeed an important physiological role—that of protecting the skeleton from the demineralizing actions of PTH, whilst permitting its other actions on Ca transport, including that in the gut, to go unhindered.

Parathyroid hormone. Certainly PTH levels are elevated during early lactation (Samaan *et al.* 1974; Retallack *et al.* 1977). However, there is some controversy concerning later lactation where one report (Cushard *et al.* 1972) indicates that PTH falls towards normal levels whilst lactation is still progressing, thus casting doubts on the contribution of PTH to this adaptive response. Undoubtedly, the elevated PTH levels are responsible for the reduction in urinary Ca in lactation, and may also contribute to skeletal demineralization, since the skeletons of parathyroidectomized animals are heavier at the end of lactation than those of comparable controls (Bodansky & Duff, 1941). PTH does stimulate the production of $1,25(OH)_2D_3$ by the kidney (Fraser & Kodicek, 1973) and may well contribute to the elevated levels observed in lactation. However, in the light of the observation that PTH levels can fall whilst lactation is still progressing and recent experimental

Table 3. *Effect of calcitonin deficiency on bone constituents and calcium absorption in lactating rats*

(Mean values with their standard errors)

| | | Femur (mg) | | | | | Ca absorption (% dose absorbed) | | | |
| | | Weight | | Ca | | Phosphorus | | Duodenum | | Jejunum | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	7	570·7	16·6	101·8	3·7	53·6	3·0	98·1	0·4	40·1	3·2
Calcitonin-deficient	9	518·9	5·5	91·3	1·3	43·7	1·0	96·6	0·6	29·7	1·7
Difference		51·8	15·2	10·5	3·4	9·9	2·8	1·5	0·7	10·4	3·4
Significance of difference (P)		<0·005		<0·005		<0·001		<0·05		<0·005	

Table 4. *Effect of bromocriptine and prolactin (0·5 mg twice daily for 3 d) in lactating rats**

(Mean values with their standard errors)

	Plasma Ca (mmol/l)			Ca absorption (% dose absorbed)			1,25-dihydroxy vitamin D_3** (pmol/l)			Parathyroid hormone (µg eq/l)		
	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Non-lactating controls	9	2·53	0·02	9	17·4	1·5	3	62·5	4·8	10	0·205	0·032
Lactating controls	8	2·42[a]	0·03	8	25·3[a]	2·8	4	95·7[a]	8	9	0·383[a]	0·053
Lactating + bromocriptine	7	2·58	0·03	7	16·2[b]	2·9	4	60·8[b]	15·4	9	0·374	0·063
Lactating + bromocriptine + prolactin	7	2·62[b]	0·03	8	25·7	5·6	4	70·45	15·4	10	0·484	0·073

*The results are not comparable to those in Table 2 as the animals were fed on a diet of different Ca and vitamin D contents.
**Pooled values from two to three animals.
Significantly different from [a]non-lactating controls ($P<0·05$), [b]lactating controls ($P<0·05$).

results on the role of prolactin in Ca absorption (see p. 178), it seems unlikely that PTH is the sole controlling hormone regulating Ca absorption in lactation.

PTH does not seem to affect the transfer of Ca in the mammary gland in the goat (Hove & Tollman, 1981). Moreover, rats deprived of their parathyroid glands have been shown to be able to reproduce and suckle their young almost normally (Fry *et al.* 1979), the only impairment of pup growth appearing between days 6 and 12 of lactation. It thus follows that whilst PTH should be present for optimal reproductive capacity and litter growth, its presence may not be essential for lactation to proceed and for an adequate supply of Ca to be provided to the mother and foetus.

Prolactin. This appears to be the most likely candidate for the role of primary controller of Ca absorption mediated by $1,25(OH)_2D_3$ during lactation for the following reasons:

(1) Prolactin has a direct stimulatory action on renal $1,\alpha$-hydroxylase (MacIntyre *et al.* 1979) and it causes rapid elevation of plasma $1,25(OH)_2D_3$ levels in intact animals (Spanos *et al.* 1976).

(2) Prolactin has been shown to stimulate Ca absorption in the rat both in vitro (Mainoya, 1975) and in vivo (James *et al.* 1977).

(3) Prolactin is ideally suited to regulate the supply of Ca during lactation as it is the only lactogenic hormone, with the exception of GH in ruminants (Hart, 1973), whose levels are regulated by suckling (Cowie & Tindal, 1971) and lactational demand.

The following studies (Robinson *et al.* 1982), using the agent bromocriptine which suppresses prolactin secretion, provide evidence which supports this suggestion. Lactating rats were divided into two groups, one of which received bromocriptine treatment for 3 d. Appropriate control groups were also included. At the end of the treatment Ca absorption and levels of plasma Ca, 1,25(OH)$_2$D$_3$ and PTH were measured. The results (Table 4) show that Ca absorption increased during lactation, confirming previous observations and, moreover, that the levels of both 1,25(OH)$_2$D$_3$ and PTH were elevated. However, treatment with bromocriptine lowered Ca absorption and 1,25(OH)$_2$D$_3$ levels to normal, whilst PTH levels remained elevated.

The results, therefore, indicate that the increased Ca absorption seen in the lactating rat is caused by the elevated levels of prolactin. Further evidence of this is provided by the fact that exogenously administered prolactin prevented the fall in Ca absorption and 1,25(OH)$_2$D$_3$ levels in rats treated with bromocriptine. Thus these results strongly suggest that prolactin, rather than PTH, is the major hormone controlling Ca absorption in the lactating rat.

Hypothesis

Pregnancy and lactation present considerable Ca stresses on maternal homoeostatic mechanisms, the stress due to lactation being considerably greater in the majority of mammals. The Ca demands are largely satisfied in four ways:

(1) By a facultative reduction in urinary Ca excretion.
(2) By increased food intake.
(3) By intestinal hypertrophy and increase of mucosal area.
(4) By a facultative increase in the intestinal absorption of Ca, consequent upon elevated levels of 1,25(OH)$_2$D$_3$.
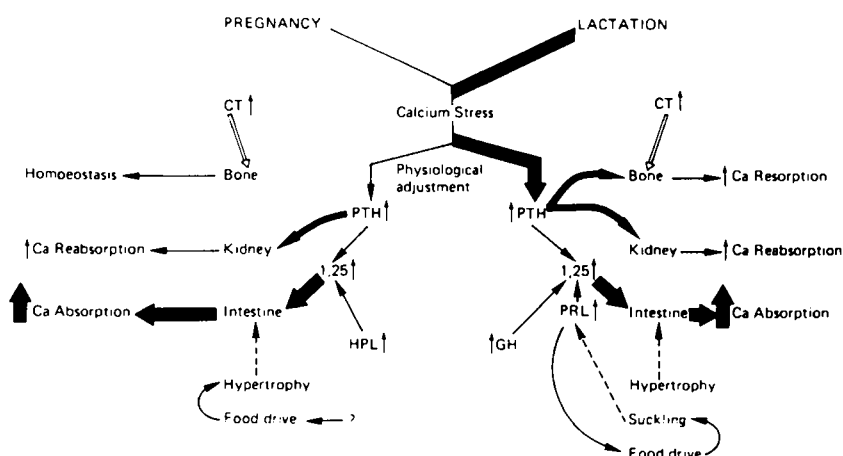

Fig. 1. The proposed hormonal control of calcium metabolism in pregnancy and lactation. CT, calcitonin; PTH, parathyroid hormone; HPL, human placental lactogen; GH, growth hormone; PRL, prolactin; 1,25, 1,25-dihydroxy vitamin D$_3$.

In pregnancy the demand (Fig. 1) is satisfied by all four mechanisms. In the case of point (4), the increased synthesis of $1,25(OH)_2D_3$ is mediated by PTH and placental lactogen. This gives the latter hormone, whose levels rise as pregnancy progessses, a new and important physiological role.

In lactation. Again, all mechanisms contribute to satisfy the demand for Ca and to a greater absolute extent than in pregnancy. The elevated concentration of $1,25(OH)_2D_3$ which causes the increased absorption of Ca may in part be controlled by PTH, but the main controlling hormone is prolactin whose plasma levels reflect the lactational demand. It is thus suggested that this represents a major new physiological role for prolactin in lactation.

REFERENCES

Amenomori, Y., Chen, C. L. & Meites, J. (1970). *Endocrinology* **86,** 506.
Atkinson, P. J. & West, R. R. (1970). *J. Obstet. Gynaec., Br. Commonw.* **77,** 555.
Baksi, S. N. & Anderson, R. R. (1971). *Proc. Soc. exp. Biol. Med.* **137,** 215.
Bodansky, M. & Duff, V. B. (1941). *J. Nutr.* **21,** 235.
Bonjour, J. P., Preston, C., Rizzoli, R. & Fleisch, H. (1978). In *Endocrinology of Calcium Metabolism*, p. 172 [D. H. Copp and R. V. Talmage, editors]. Amsterdam: Excerpta Medica.
Boyne, R., Fell, B. F. & Robb, I. (1966). *J. Physiol.* **183,** 570.
Braithwaite, G. D. (1978). *Br. J. Nutr.* **39,** 213.
Braithwaite, G. D., Glascock, R. F. & Riazuddin, Sh. (1969). *Br. J. Nutr.* **23,** 827.
Braithwaite, G. D., Glascock, R. F. & Riazuddin, Sh. (1970). *Br. J. Nutr.* **24,** 661.
Capen, C. C., Koestner, A. & Cole, C. R. (1965). *Lab. Invest.* **14,** 1673.
Cotes, P. M. & Cross, B. A. (1954). *J. Endocr.* **10,** 363.
Cowie, A. T., Forsyth, I. A. & Hart, I. C. (1980). In *Hormonal Control of Lactation*, p. 109, Monographs on Endocrinology. Berlin: Springer Verlag.
Cowie, A. T. & Tindal, J. S. (1971). *The Physiology of Lactation*. Monographs of the Physiological Society, no. 22. London: Edward Arnold.
Craft, I. L. (1970). *Clin. Sci.* **38,** 287.
Cushard, W. G., Creditor, M. A., Canterbury, J. M. & Reiss, E. (1972). *J. clin. Endocr.* **34,** 767.
Del-Pozo, E., Hiba, J., Lancranjan, I. & Kunzig, H. J. (1977). In *Prolactin and Human Reproduction* [P. G. Crosignani and C. Robyn, editors]. New York: Academic Press.
Field, A. C. (1981). *Proc. Nutr. Soc.* **40,** 267.
Fournier, P. & Susbielle, H. (1952). *J. Physiol., Paris* **44,** 123.
Fraser, D. R. & Kodicek, E. (1973). *Nature, New Biol.* **241,** 163.
Fry, J. M., Curnow, D. H., Gutteridge, D. H. & Retallack, R. W. (1979). *J. Endocr.* **82,** 323.
Goss, D. A. (1962). *Obstet. Gynéc.* **20,** 199.
Gueguen, L. & Perez, J. M. (1981). *Proc. Nutr. Soc.* **40,** 273.
Halloran, B. P. & DeLuca, H. F. (1980). *Am. J. Physiol.* **239,** E64.
Harding, J. D. & Cairnie, A. B. (1975). *Cell. Tiss. Kinet.* **8,** 135.
Hart, I. C. (1973). *J. Endocr.* **57,** 179.
Hart, I. C., Bines, J. A. & Morant, S. V. (1978). *J. Dairy Sci.* **62,** 270.
Heaney, R. P. & Skillman, T. G. (1971). *J. Clin. Endocr.* **33,** 661.
Horst, R. L., Jorgensen, N. A. & DeLuca, H. F. (1978). *Am. J. Physiol.* **235,** E634.
Hove, K. & Tollman, R. (1981). *J. Endocr.* **89,** 297.
Hummel, F. C., Sternberger, H. R., Hunscher, H. A. & Macy, I. G. (1936). *J. Nutr.* **11,** 235.
James, M. F., Makeen, A. M., Foley, S., Stevens, J. & Robinson, C. J. (1977). *J. Endocr.* **75,** 53P.
James, M. F., Spanos, E., Robinson, C. J., Makeen, A. M., Snowball, S., Colston, K. W., Hillyard, C. & MacIntyre, I. (1978). *J. Endocr.* **79,** 38P.

Josimovich, J. B. (1977). In *Endocrinology of Pregnancy,* p. 191 [F. Fuchs and A. Klopper, editors]. New York: Harper and Row.

Kaufman, S. (1981). *J. Physiol.* **318,** 9.

Krawitt, E. L. (1967). *Proc. Soc. exp. Biol. Med.* **125,** 1084.

Lewis, P., Rafferty, B., Shelley, M. & Robinson, C. J. (1971). *J. Endocr.* **49,** ix.

Lichtenberger, L. M. & Trier, J. S. (1979). *Am. J. Physiol.* **237,** E98.

MacIntyre, I., Brown, D. J. & Spanos, E. (1979). In *Vitamin D Basic Research and its Clinical Application,* p. 523 [A. W. Norman, K. Schaefer, D. von Herrath, H. G. Grigoleit, J. W. Coburn, H. F. DeLuca, E. B. Mawer and T. Suda, editors]. New York: Walter de Gruyter.

MacIntyre, I. & Robinson, C. J. (1969). *Ann. N.Y. Acad. Sci.* **162,** 865.

Mainoya, J. R. (1975). *Endocrinology* **96,** 1165.

Makeen, A. M. (1980). Studies of the effects of hormones upon the metabolism of calcium and other minerals in experimental animals and man. PhD Thesis, University of Newcastle upon Tyne.

Mayer, G. P., Blum, J. W. & Deftos, L. J. (1975). *Endocrinology* **96,** 1478.

Nicolaysen, R. (1943). *Acta. Physiol. Scand.* **5,** 200.

Nordin, B. E. C. & Peacock, M. (1969). *Lancet* **ii,** 1280.

Norman, A. W. (1978). In *Vitamin D,* p. 93 [D. E. M. Lawson, editor]. London and New York: Academic Press.

Payne, J. M. (1964). *Vet. Rec.* **16,** 1275.

Pitkin, R. M. (1975). *Am. J. Obstet. Gynecol.* **121,** 724.

Puschett, J. B., Beck, W. S., Jelonek, A. & Fernandez, P. C. (1974). *J. clin. Invest.* **53,** 756.

Retallack, R. W., Jefferies, M., Kent, G. N., Hitchcock, N. E., Gutteridge, D. H. & Smith, M. (1977). *Calcif. Tiss. Res.* **22,** 142.

Robinson, C. J. (1974). *Clinics Endocr. Metab.* **3,** 389.

Robinson, C. J., Matthews, E. W. & MacIntyre, I. (1969). In *Les Tissus Calcifiés,* Proceedings of the V European Symposium, p. 279 [G. Milhaud, M. Owen and H. J. J. Blackwood, editors]. Paris: S.E.D.E.S.

Robinson, C. J., Spanos, E., James, M. F., Pike, J. W., Haussler, M. R., Makeen, A. M., Hillyard, C. J. & MacIntyre, I. (1982). *J. Endocr.* **94,** 443.

Samaan, N. A., Wigoda, C. & Castillo, S. C. (1974). In *Endocrinology,* 1973, p. 364 [S. Taylor, editor]. London: W. Heinemann.

Schachter, D., Dowdle, E. B. & Schenker, H. (1960). *Am. J. Physiol.* **198,** 263.

Scott, D. & McLean, A. F. (1981). *Proc. Nutr. Soc.* **40,** 257.

Simkiss, K. (1967). In *Calcium in Reproductive Physiology.* London: Chapman and Hall.

Spanos, E. (1979). The regulation of 1,25-dihydroxycholecalciferol production by the kidney. PhD Thesis, University of London.

Spanos, E., Barrett, D. & MacIntyre, I. (1978). *Nature, Lond.* **273,** 246.

Spanos, E., Colston, K. W., Evans, I. M. A., Galante, L. S., MacCauley, S. J. & MacIntyre, I. (1976). *Molec. Cell Endocr.* **5,** 163.

Spray, C. M. (1950). *Br. J. Nutr.* **4,** 354.

Swaminathan, R., Ker, J. & Care, A. D. (1974). *J. Endocr.* **61,** 83.

Toverud, S. U., Cooper, C. W. & Munson, P. L. (1978). *Endocrinology* **103,** 472.

Warnock, G. M. & Duckworth, J. (1944). *Biochem. J.* **38,** 220.

Weisman, Y., Harell, A., Edelstein, S., David, M., Spirer, Z. & Golander, A. (1979). *Nature, Lond.* **281,** 317.

Printed in Great Britain