SOME OBSERVATIONS ON CALCIUM-INDUCED MITOGENESIS IN RAPIDLY DIVIDING TISSUES OF THE RAT.

A Thesis submitted for the Degree

of

Doctor of Philosophy

by

Grenville Robert Smith.

Department of Biological Sciences,
University of Aston in Birmingham,
May 1976.

SUMMARY

Magnesium ions stimulate mitosis in rat lymphopoietic and haematopoietic tissue in males and females. This is achieved by a different mechanism to that by which calcium ions promote division, since although calcium is able to exert its full mitogenic potential in the male, this action is impeded in the intact female. Replacement of sex steroids in ovariectomised rats revealed that calcium injections enhanced mitosis if progesterone was given, but failed to be effective in the presence of oestrogen. This inhibitory action of oestrogen on the response to calcium injections is not a consequence of some interference with the calcium homoestatic hormones, since a similar regime of injections did not provoke mitosis in the thyroparathyroidectomised female. A direct action of oestrogen in preventing calcium influx into the cell is inferred, which is supported by *in vitro* observations. Even though oestrogen impedes calcium-induced mitogenesis in the female, the mitotic rhythm throughout the oestrous cycle showing maximal activity at oestrus and minimal at pro-oestrus correlated well with the plasma total calcium concentration. These observations are discussed in the light of the slower proliferative responses that occur in these tissues of the female.

Another rapidly renewing tissue, the mucosa of the small intestine, was studied to establish another possible tissue influenced by physiological fluctuations in extracellular calcium ion concentration in the male. However, cell renewal in this tissue was unaffected by such changes. Even though a derangement of calcium metabolism exists in diabetic animals, no evidence was obtained to substantiate a role for calcium in the pathogenesis of intestinal hyperplasia of such animals. Nevertheless, polar metabolites of vitamin D_3, which are intimately linked with the intestinal handling of calcium, enhance division in normal animals, and an abnormal metabolite of vitamin D_3 could well account for the cellular changes observed in the diabetic intestine.

INDEX

Contents

GENERAL INTRODUCTION.

"He who increases knowledge
increases sorrow....."

Ecclesiastes, ch. 1, v.18

General Introduction.

The calcium ion has for a long time been implicated in many important control systems of the body, such as its involvement in nerve conduction (Shanes, 1958); muscle contraction (Shanes, 1963); blood clotting (Ferguson, 1936); and the secretion from certain endocrine glands (Douglas & Rubin, 1963, Douglas & Poisner, 1964). An equally important facet of this cation is its mitogenic action in the rapidly dividing cell populations of lymphopoietic and haematopoietic tissues of the male rat (Perris, Whitfield & Tolg, 1968).

This thesis is concerned with three rapidly dividing tissues, namely the bone marrow, the thymus gland and the epithelial lining of the duodenum. The first part of the study is devoted to cationic (Ca and Mg) initiation of mitosis in the lymphopoietic and haematopoietic tissues. Particular attention is focused on the interaction of the oestrogen molecule with these events and the physiological consequences thereof.

The later part examines the possible role that calcium may play in govening physiological rate of cell proliferation in the small intestine of healthy animals. This was extended to see if there was any correlation between the calcium status in the body and the gastrointestinal hypertrophy in diabetic animals.

INTRODUCTION.

1.1. Classification of tissue types.

In the developing mammal overall body growth is rapid and every tissue contributes to this process. Tissue growth itself is in part the manifestation of cellular hypertrophy and in part of cellular hyperplasia which obviously requires cell proliferation. Cellular growth is in fact intimately related to cell division since a certain amount of cellular growth is a prerequisite for division and may itself be the ultimate trigger for division (Fantes, Grant, Pritchard, Sudbery & Wheals, 1975).

At maturity growth essentially abates, but division still continues in some tissues. For example, the periodic breakdown of the uterine and vaginal epithelia during the oestrus cycle requires constant replacement by division from the germinal layers (Long & Evans, 1922); similarly in the male spermatogenesis keeps pace with the loss of spermatozoa by resorption or ejaculation (Goss, 1967); also loss of blood-borne cells of limited life span is balanced by continual generation and release of new cells into the circulation (Goss, 1967).

With these facts in mind, Bizzozero in 1894 proposed a classification of tissues and organs based on mitotic potentialities. Along similar lines Messier & Leblond (1960) have designated tissue types as:-

(i) Static - these tissues lack the capacity for mitosis altogether. Such tissues are formed when all cells of a given kind become so fully differentiated that they can no longer divide. The cells of static tissues have the potentiality for living as long as the organism survives. Examples of static tissues are various neurones in the central and peripheral nervous system, cardiac muscle of adult mammals, and striated muscle fibres.

(ii) Expanding - these tissues never lose the capacity for mitosis; they resort to mitosis only to augment a population of permanent

cells. Expanding tissues are able to perform a dual role in the body. In organs such as liver, kidney and many of the endocrine and exocrine glands, cell division normally enables the organ to add to its mass in keeping pace with somatic growth. Secondly, these tissues possess the ability to divide in order to replace accidentally lost cells, and may also grow in response to increased functional demand or load (Gibadulin, 1962).

(iii) Renewing - these tissues undergo cell division to replenish cells lost during the normal process of cellular atrition. Such tissues are unique in that they retain a subpopulation of immature undifferentiated stem cells; it is from these germinal cells that those of the differentiated compartment are recruited.

For example, in the male the germinal layer of cells of the seminiferous tubules is represented by the spermatogonia. These divide mitotically to produce primary spermatocytes. Meiotic division then results in secondary spermatocytes, which divide further producing spermatids which mature into spermatozoa (Nelson, 1971). The vaginal epithelium of the female is continually being replenished from cells of the germinal layers; the moribund cells of the cornified layer are shed into the lumen at the end of each four-day cycle (Long & Evens, 1922). Circulating blood cells have a finite life span and are replenished from pluripotential stem cells in the marrow after a series of stages involving cell division and maturation (Lajtha, 1970). Similarly circulating lymphocytes of variable life span are replenished via division of appropriate blast cells (Gowans & McGregor, 1964). The circulating cells of both haemopoietic and lymphopoietic tissue may arise from a common stem cell population. The columnar epithelium cells lining the entire gastrointestinal tract are replenished approximately every two days by menas of proliferation of a stem cell compartment in the crypts of Lieberkuhn (Loehry, Croft, Singh & Creamer, 1969). The differentiated cells of these renewing tissues all have relatively short life spans, thus requiring a constant flow of new cells into the mature population.

1.2 The Cell Cycle.

In the adult mammal division in cell populations is almost entirely restricted to the renewing tissues. The chain of events through which a cell passes after its genesis by means of mitosis until two new daughter cells are produced at the next division is termed the cell cycle (Mazia, 1974). Howard and Pelc (1953) have partitioned the cell cycle into four separate time periods:-

i) G_1 - during this phase RNA and proteins necessary for DNA replication are synthesised.

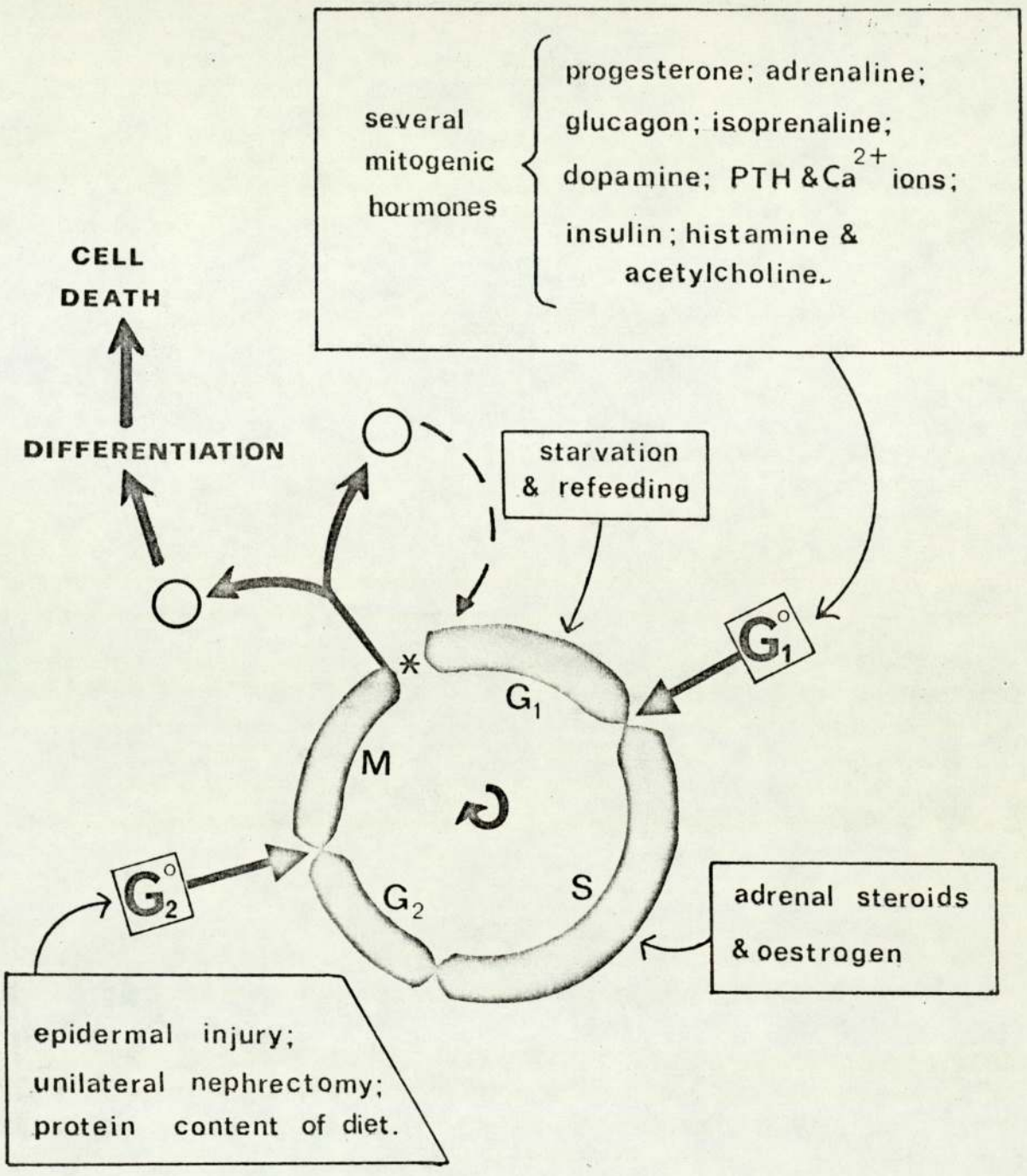
ii) S - during which DNA is synthesised.

iii) G_2 - during which further protein and RNA are synthesised in preparation for mitosis.

iv) M - cell division takes place, resulting in the production of two new cells. One of these cells normally leaves the actively dividing population, to differentiate and replace lost cells, whilst the other usually remains in the cycle and re-enters G_1 to keep the stem cell pool constant.

Tc is designated as the total cell cycle time (See Fig. 1).

The cell cycle is similar in virtually all dividing cells except for the duration of the various phases (Howard & Pelc, 1953; Herrmann, Marchok & Boril, 1967; Baserga, 1968; Cameron, 1971). Although exceptions do occur, the duration of the S, G_2 and M periods are relatively constant in mammalian cell populations in vivo. It is in the G_1 period that most of the time variations in the overall cell cycle of different tissues occurs (Baserga, 1965; Cameron, 1971). A comparison of cell cycle parameters in normal and abnormal tissues in various species is shown in Table 1. Not only is the duration of the G_1 phase variable for the different cell types, but it is also the most variable within any one particular cell



***BULLOUGH'S 'DICOPHASE' – MAJOR SITE OF ACTION OF CHALONES.**

Fig. 1: Generalised representation of the Cell Cycle indicating sites of action of various modulating agents. Effects shown at G_1^o and G_2^o are only applicable to specific tissues exhibiting these quiescent cell populations - see text.

TABLE 1.

Duration of the phases of the cell cycle in some normal and malignant tissues, demonstrating the variability of G_1.

Species	Tissue	(time in hours)					Reference
		G_1	S	G_2	M	T_c	
Rat (newborn)	small intestine crypt.	5.6	5.6	1.7	0.6	13.5	Maurer, Pilgrim, Wegener, Hollweg & Lennartz, 1965.
	Cartilage.	30.7	7.3	1.8	0.7	40.5	ibid.
	Epidermis.	11.0	5.5	1.7	0.7	18.9	ibid.
Rat (adult)	Small intestine crypt.	1.5	6.5	1.0	1.0	10.0	Cairnie, Lamerton, & Steel, 1965.
Cow	Lymphocytes.	0.8	5	0.5	0.7	7	Vincent, Borner, Chanana, Cronkite, Greenberg, Joel, Shiffer & Stryckmans 1969.
Hamster	Pouch epithelium. (Normal)	128	10.2	1.6	2.6	142	Reiskin & Mendelsohn, 1964.
	Pouch epithelium (Malignant)	8.7-11.9	6.1	2.2	0.5	17.5	
Mouse (adult)	Vaginal epithelium (Oestrus)	15.4	7.6	1	1	25	Thrasher, Clark & Clarke, 1967.
	Vaginal epithelium (dioestrus)	62.5	7.5	1	1	72	

population. Variations in evironmental conditions such as nutrition, pH, and cell population density result in marked changes in the duration of G_1 (Cameron, 1971).

With the observation that not all cells with mitotic potential are continually cycling, the model of the cell cycle propounded by Howard & Pelc has been further modified to incorporate both the cells that are dividing all the time, and those cells that are quiescent yet can be induced to enter DNA synthesis by appropriate stimuli. It is pertinent at this stage to discuss the nature and possible significance of such quiescent cell populations. At least two types of resting periods within the cell cycle have been observed. These are the G^o (Patt & Quastler, 1963) and the $G_2{}^o$ (Gelfant, 1962; Epifanova & Terskikh, 1969). For convenience the G^o and $G_2{}^o$ populations will be considered separately, since some differences are apparent. Both types of quiescent cell population remain in interphase for prolonged periods. The G^o population enters the cell cycle at the G_1/S boundary and the cells in the $G_2{}^o$ population enter the cycle at the G_2/M boundary.

A large number of tissues contain cells in a G^o phase; the majority of these are renewing tissues, while a few are expanding tissues (see section 1.1). Under the appropriate stimulus the quiescent G^o cells in the tissues listed below enter mitosis between 12-24 hours later, in accordance with the average S, G_2 duration. Examples of G^o recriutment are to be found in the regenerating liver following partial hepatectomy (Grisham, 1962; Bucher, 1963, 1967a,b ; Church & McCarthy, 1967a, b); oestrogen-stimulated uterus (Epifanova, 1966; Hamilton, 1968); erythropoietin on the spleen (Hodgson, 1967); and phytohemagglutinin-stimulated lymphocytes in culture (Cooper, Barkhan & Hale, 1963). More detailed biochemical discussion concerning stimulated cell populations may be found

in the reviews by Baserga, 1968 and Cooper, 1971.

The triggering mechanisms governing the progression of cells through the cycle seem to be located in the G_1 since induction of division is most frequently preceeded by recruitment of cells into DNA synthesis. However in adult mouse ear epidermis, where the normal cell cycle phases have been reported as G_1 (22 days); S (30 hr); G_2 (6.5 hr); M (3.8 hr) by Sherman, Quastler & Wimber, 1961, cutting provokes a burst of mitosis within 4 hours (Gelfant, 1959). Clearly insufficient time would have elapsed for such cells to complete a normal S + G_2 phase before dividing. Since the cells did not incorporate tritiated thymidine when it was available continuously after wounding before they divided, they must have been arrested in a quasi G_2 phase (G_2°) awaiting the inducement of wounding before entering mitosis directly (Gelfant, 1962). These so called G_2° cells differ from the G° in a number of ways:-

(i) they are blocked in G_2 indifinitely and some of these cells can be mobilized into division immediately upon stimulation. The time course of the response is thus far shorter than with a G° population.

(ii) G_2° cell populations have been found in the ear epidermis (Gelfant, 1962); lens epithelium (Harding, Feldherr & Srinivasan, 1961); urinary bladder epithelium (Walker, 1959); kidney tubular epithelium (Pederson & Gelfant, 1970) and duodenal crypt epithelium (Pederson & Gelfant, 1970). There does not appear to be any relation between the normal rates of proliferation in these tissues and the possession of a G_2° reserve population. The mitotic activity of unstimulated mouse renal, skin, and intestinal epithelia exists in ratios of 1:100:1000, yet they all contain non cycling G_2° cells.

(iii) The kind of stimulus needed to initiate G_2° recruitment so far studied are:-

a) injury to epidermis (Gelfant, 1962)

b) unilateral nephrectomy (Pederson & Gelfant, 1970)

c) changing from low to high protein diet (Pederson & Gelfant, 1970).

It would appear that although both G^{o} and G_2^{o} stimulation result in cell proliferation that the former is the result of physiological chal-lenge, whereas the latter has a more pathological basis. The essential feature of the cell life cycle is the alternation of resting periods and periods of active proliferation. The completion of mitosis does not imply that the daughter cell will undergo differentiation or immediately re-enter the cell cycle; in the absence of adequate stimulus it may pass again into a resting period (Epifanova & Terskikh, 1969).

1.3 Endogenous factors influencing the cell cycle.

The cell cycle time is characteristic for a given tissue type and apart from the fluctuations that occur with age, notably in the G_1 phase (Herrmann, Marchok & Boril, 1967; Buetow, 1971), each phase in the cell cycle exhibits remarkable consistancy.

However, there are a multitude of factors which can potentially alter the dynamics of the cell cycle. One may anticipate that the factors which control cell proliferation operate at some point prior to the onset of the DNA synthetic phase. Indeed, mitosis is believed to be initiated during the G_1 period, since both physiologically and experimentally induced cell proliferation first results in DNA synthesis (Cronkite, Bond, Fliedner & Rubini, 1959; Quastler & Sherman, 1959; Leblond, Messier & Kopriwa, 1959; Mendelsohn, Dohan Jr. & Moore, 1960), except the G_2^{o} recruitment already described. Any agent having an influence on the cycle could be expected either to:-

(i) Cause a recruitment of quiescent cells from a resting period into the actively dividing cell population, so in a given time the mitotic index of that tissue is raised (e.g. progesterone).

(ii) affect the proportion of cells in G_1, in some way, prior to entering the S phase of the cycle (e.g. starvation).

(iii) in some way cause an alteration in the duration of one of the sub-divisions of the cycle, so the overall cycle time is respectively altered (e.g. adrenal steroids, oestrogens). Several physical and humoral factors are thus able to alter the proportion of cells in a population that are dividing. A more detailed analysis of these agents follows.

To ensure basal mitotic activity is maintained, all the nutrients required for the various synthetic events occurring throughout the cell cycle and vital for its completion must be present. It has been found that there is a general suppression of mitotic activity during periods of starvation (Blumenthal, 1950). It is believed that in the duodenal mucosa, that starvation holds cells in the G_1 part of the cycle. Analysis of this tissue shows that refeeding initiates cell division by stimulating cells to emerge from G_1 into S, (Cameron & Cleffmann 1964).

Experimental animals are frequently exposed to stressful situations such as handling, isolation and injections. Such conditions must at least temporarily alter the normal functioning of the animals autonomic and endocrine systems (Selye, 1971). Just be confining mice to small cylindrical holes, Cameron (1968), was able to show that this resulted in a lowered mitotic activity. Stress intimately involves the steroids secreted from the adrenal cortex; the dominating steroid in man is cortisol, whereas in rat it is corticosterone (Starling & Lovatt Evans, 1968). Laguchev & Aventisyan, 1972, showed that cortisol caused a lengthening of the S phase of the cycle of duodenal epithelial cells. Peckham, Ladinsky & Kiekhofer 1963; Epifanova, 1966; Galand, Rodesch, Leroy & Chretien, 1967 observed a reduction in the duration of the S-phase in various tissues of oestrogen-treated animals.

It is convenient at this point now to look more closely at the role that hormones in general play in this complex scheme of events. The main attribute of hormones in the mammalian body is the maintainence of homeostasis, and in fact they do not initiate new reactions within cells but only influence existing ones. They exert a wide variety of actions upon cellular metabolism (Needham, 1964), and indeed several hormones have been shown to be mitogenic (Table 2). Swann 1958 has suggested that hormonal regulation of cell proliferation is possible only in tissues with high mitotic activity, where there are always a large number of cells ready to enter mitosis. Mitogenic hormones could potentially affect any stage of the cycle where a reaction may be a rate-limiting step. Alternatively by influencing a rate-limiting reaction governing movement of quiescent cells into the cell cycle, they could also act as mitogens, e.g. progesterone recruiting G^{o} cells (Bresciani, 1968a). Nearly all of the available data on the effects of hormones on the various phases of the cell cycle have been obtained mainly with the oestrogens (Epifanova, 1971). It would appear that most mitogenic hormones affect some rate-limiting step at or near the G_1/S houndary (Peckham, Ladinsky & Kiekhofer, 1963; Epifanova, 1966; Galand, Rodesch, Leroy & Chretien, 1967; Bresciani, 1968a; Morgan & Perris, 1974; Perris & Morgan, 1975).

The classification of hormones as mitotic stimulators or inhibitors is to a certain degree arbitary since the response of a tissue largely depends on the initial state of that tissue. It is well known that only a few injections of a mitogenic hormone gives rise to cell proliferation in target tissues, while continual treatment results in a fall of mitotic activity and the tissue becomes insensitive to further stimulation. This is true for both oestrogens (Bullough, 1965; Galand, Rodesch, Leroy & Chretien, 1967; Finn, Martin & Carter, 1969) and androgens (Sheppard, Tsien, Mayer & Howie, 1965; Coffey, Shimazak & Williams-Ashman, 1968; Tuohimaa & Niemi, 1968). Although these hormones exert an influence on cell division this biphasic action might be explained if one assumes the

TABLE 2.

Humoral influences upon cellular proliferation in rapidly dividing tissues.

Tissue	Humoral Factor	Reference
i) EPIDERMIS	Oestrogens	Allen 1955; 1956 Bullough 1950
	Adrenaline	Bullough 1952
	Adrenaline	Bullough & Laurence 1966
	Glucocorticoids	Bullough & Laurence 1968
	Androgens	Bullough & Van Oordt 1950
	Testosterone/ Thyroxine	Eartly, Grad & Leblond 1951
	Oestrogens	Ebling 1954
	(Review article)	Ebling 1974
	(Review article)	Pochi & Strauss 1974
ii) LIVER (regenerating)	Identity of humural substance not characterised.	Adibi, Paschkis & Cantarow 1959 Alston & Thompson 1963 Becker 1973 Bucher 1963; 1967a, b Cater, Halmer & Mee, 1957 Leduc 1964 Moolten & Bucher 1967 Paschkis, Goddard, Cantarow & Adibi 1959 Sakai 1970 Virolainen 1967 Wrba & Rabes 1967
iii) LYMPHOID TISSUE	Thymosin	Klein, Goldstein & White 1965
	Histamine, Glucagon, Insulin	Perris & Morgan 1975
	Parathyroid gland	Perris, Weiss & Whitfield, 1970
	Oestrogen	Smith, Gurson, Riddell & Perris 1975
	Cortisol	Whitfield, MacManus & Rixon 1970
	Growth hormone, neuro-hormones, PTH, prolactin	Whitfield, Perris & Youdale 1969
iv) HAEMOPOIETIC TISSUE	Testosterone	Diamond, Jacobsohn & Sidman 1967
	Oestrogens (review)	Fried, Tichler & Dennenberg 1974 Gordon 1957
	Erythropoietin	Kuna, Gordon, Morse, Lane & Charipper, 1959
	Erythropoietin	Matoth & Kaufmann 1962
	Erythropoietin	Peschle, Rappaport & Sasso 1973
	Erythropoietin	Reissman & Samorapoompichit 1970
	Testosterone	Rencricca, Solomon, Fimian, Howard, Rizzoli & Stohlman 1969.

Table 2 cont.

	Tissues	Humoral Factor	Reference
v)	INTESTINAL EPITHELIUM	Adrenocortical hormones	Baker & Bridgman 1954
		Vitamin D	Birge & Alpers 1973
		Prolactin	Cairnie & Bentley 1967
		Prolactin	Campbell & Fell 1964
		Thyroid/testicular	Carriere 1965
		Adrenaline	Epifanova & Tchoumak 1963
		Insulin (alloxan-diabetic)	Jervis & Levin 1966
		Cortisol	Laguchev & Aventisyan 1972
		Growth hormone/Thyroxine	Leblond & Carriere 1955
		Insulin	Mackay, Callaway & Barnes 1940
		Insulin (alloxan-diabetic)	Nakabou, Okita, Takano & Hagihara 1974
		Secretin/pentagastrin	Pansu, Berard, Dechelette & Lambert 1974
		ACTH stress hormones	Rasanen 1963
		Vitamin D	Spielvogel, Farley & Norman 1972
		Serotonin	Tutton 1974
		Adrenaline	Tutton & Helme 1974
		Vitamin D	Urban & Schedl 1969
		Thyroid hormone	Wall, Middleton, Pearse & Booth 1970
		Testosterone	Wright, Morley & Appleton 1972
vi)	ACCESSORY SEX ORGANS (♀)		
	a) Mammary	Female Sex Hormones	Folley 1955 Jacobsohn 1958 Smith 1955
	b) Uterine	Female Sex Hormones	Epifanova 1966 Lee, Rogers & Trinder 1974 Martin, Das & Finn, 1973 Tachi, Tachi & Lindner 1972
	c) Vagina	Female Sex Hormones	Lee & Rogers 1972 Martin & Claringbold 1960 Thrasher, Clark & Clarke 1967
vii)	ACCESSORY SEX ORGANS (♂)	Male sex hormones	Allen 1958 Burkhardt 1940 Tuohimaa & Niemi 1968

existence of a negative feedback control mechanism within these, and possibly other tissues, providing some endogenous autoregulation of growth.

In multicellular adult organisms there is a dynamic equilibrium between cell gain and cell loss. To maintain a constancy of the cell population, this process mustbe controlled by some precise homeostatic mechanism. Several models have been postulated to explain the control of cell proliferation in the body. Many of these models are based on the assumption that each specific cell population produces a substance which regulates its own proliferative activity. Any model must account for the balance between cell production, cell differentiation, and eventually cell loss in the tissues. It must also explain the fact that increased wear and tear (e.g. in the epidermis) brings about hyperplasia of the germinal cells (Iverson & Elgjo 1967). Thirdly it must account for the specificity of compensatory organ regeneration. For example, partial hepatectomy results in the remaining portion of the liver undergoing hypertrophy and hyperplasia, while at the same time other organs and tissues remain unchanged (but note Paschkis, Goddard, Cantarow & Adibi 1959). The above evidence points to the control of cell division residing in the tissue itself, as opposed to a general alteration in some hormone concentration throughout the entire body.

The concept of autonomous regulation of cell proliferation has been developed by the model of Weiss & Kavanau 1957. A negative feedback control mechanism within a tissue providing autoregulation of growth has since been suggested by Glinos 1967; Iversen & Elgjo 1967; and Weiss 1968. The model in essence consists of a generative compartment and a differentiating compartment; the differentiated compartment produces a specific inhibitor substance which controls cell proliferation in the generative compartment. Bullough & Laurence (1964 a,b) have proposed that such a tissue-specific mitotic inhibitor should be called a chalone.

Bullough 1967 defines a chalone as 'an internal secretion produced by a tissue for the purpose of controlling by inhibition the mitotic activity of that some tissue.' It is reasonable to assume that the rapidly dividing tissues that respond to mitogenic stimuli would be antagonised by an endogenous factor that would be inhibitory in nature. The exception to this would be the antimitotic adrenal steroids, the compensatory actions to which may be controlled by some other mechanism.

Much has been written regarding chalones: a complete discussion of the role of these 'factors' in controlling cell proliferation is beyond the scope of this review and only a brief consideration is presented here. For detailed reports see Bullough & Laurence 1967 and Rytomaa 1973, and Bullough 1975b. The most important biological properties of chalones are:

i) they inhibit cell proliferation in vivo;

ii) they are produced by the same tissue on which they act;

iii) their action is reversible;

iv) their action is cell line specific.

The chalone systems that have been reported are:

Tissue	Reference
i) Epidermis	Bullough & Laurence 1964a Elgjo 1974 Frankfurt 1971 Inversen & Elgjo 1967 Marks 1971 Marrs & Vorhees 1971 Voaden 1968.
ii) Granulocyte	Benestad, Rytomaa & Kiviniemi 1973 Paukovits 1971 Rytomaa & Kiviniemi 1968
iii) Erythrocyte	Bateman 1974 Kivilaakso & Rytomaa 1971
iv) Lymphocyte	Bullough & Laurence 1970a Chung 1973 Garcia-Giralt, Lasalvia, Florentin & Mathe 1970 Houck, Irasquin & Leikin 1971 Moorhead, Paraskova-Tchernozemska, Pirrie & Hayes 1969

Tissue	Reference
v) Sebaceous gland	Bullough & Laurence 1970b
vi) Melanocyte	Bullough & Laurence 1968 Dewey 1973
vii) Kidney	Saetren 1956
viii) Liver	Saetren 1956 Scaife 1970 Verley, Deschamps, Pushpathadam & Desrosiers 1971
ix) Lung	Simmett, Fisher & Heppleston 1969
x) Fibroblast	Houck, Cheng & Sharma 1973
xi) Endometrial tissue	Volm, Wayss & Hinderer 1969
xii) Thryoid gland	Garry & Hall 1970
xiii) Intestinal epithelia	Tutton 1973
xiv Testis	Clermont & Mauger 1974.

Most of the chalones have been studied only very superficially - only the epidermal, granulocytic and lymphocytic chalones have been studied in depth. The concept of internal regulation of tissue specific cell proliferation by the chalone is a satisfying concept, since the theory has obvious implications for cancer therepy by employing a natural agent formed by the body. Proof that their concentration within a tissue varies inversely with mitotic activity in vivo is as yet lacking. Such a finding is essential for the establishment of a true physiological role for these compounds. There is some evidence for mitotic activity depression by chalones in vitro (Wiley, Williams & McDonald 1973), and also dose response depression effects of chalones in vitro (Boldingh & Laurence 1968). In recent years, additional factors such as the 'anti-chalones' (Rytomaa & Kiviniemi 1968) and the mesenchymal factor (Bullough 1975a), both of which are stimulatory (and may be identical), have been introduced, to explain experimental results. Despite the vast amount of literature on this

subject, the authenticity of chalones depends on a high degree of purification and eventual chemical analysis of the chalone and the subsequent analysis of their in vivo actions. Such sophistication is still awaited.

A diurnal variation in mitotic activity of tissues has been reported by many workers. Most have concluded that cell proliferation is lowest during the waking hours and maximal during sleep (Vasama & Vasama 1958; Bullough & Laurence 1961). Bullough 1965 believes that the diurnal rhythm is related to the adrenaline concentration in the blood, which is high during periods of wakefulness, and low during periods of sleep. Adrenaline itself is not a mitotic inhibitor, but acts as a co-factor forming a chalone-adrenaline complex (Bullough 1965; 1967). Bullough believes this complex is the most potent epidermal mitotic inhibitor known. Other tissues exhibiting diurnal mitotic activity may owe this fluctuation to a similar chalone-adrenaline interaction.

Although exogenous administration of the hormones listed in Table 2 influence division, it has not been possible with any certainty to ascribe to these hormones a physiological role in the control of division, such that mitotic activity varies in accordance with the hormone concentration and the demand for new cell production. However, since the demonstration of the mitogenic action of calcium in haematopoietic and lymphopoietic tissue of the rat (Perris & Whitfield 1967) several situations have been found whereby the variation of plasma calcium concentration is the determining factor for the fluctuation in mitotic activity in these tissues. There have been shown to be calcium-dependent mitotic changes observed in the growth of the rat (Perris, Whitfield & Tolg 1968); following haemorrhage (Perris, MacManus, Whitfield & Weiss 1971); accompanying circadian rhythm (Hunt & Perris 1974); accompanying erthropoietin induced mitosis (Hunt & Perris 1973), and following immunological

challenge (Edwards & Perris 1975, unpublished results). In these situations where calcium has been shown to fluctuate physiologically, then there might be an interaction with some endogenous factor such as the chalones.

It is the major purpose of this thesis to investigate the role of calcium, magnesium and certain hormones on mitosis in three renewal tissues of the rat; i.e. the thymus, the bone marrow and the small intestine *in vivo*. A detailed consideration of the function and cell turnover in these tissues is given in Section 2, and considerations of calcium and magnesium homeastasis and the possible involvement of these ions in the control of division in Section 3.

2.1 Lymphoid Tissue and Lymphopoiesis.

Present in the blood of mammals are between $2 \times 10^8 - 5 \times 10^8$ nucleated non-dividing cells called lymphocytes (Yoffey 1960). An equal or higher number are present in the lymph; the number in lymph is difficult to estimate as lymph volume is not known, and the concentration of lymph highly variable.

Attention must be focussed on the sources of lymphocytes within the body and their functional differences. Lymphoid tissue can be classified as Primary lymphoid tissue (Thymus gland and Bursa of Fabricius) and Secondary lymphoid tissue (spleen, lymph nodes, Peyers patches and tonsils).

At birth, when the spleen and lymph nodes are poorly developed, the thymus is already a prominent lymphoid organ. When considered in isolation the thymus is an organ actively engaged in the production of large numbers of small lymphocytes with the consequent deficit of large lymphocytes (Metcalf 1966). Large lymphocytes (diameter > 11u) transform into medium sized (diameter 7-11u) and these in turn divide to produce the small lymphocyte (< 7u). The large and medium cells undergo 3-4 division cycles daily being actively engaged in lymphopoiesis. It has long been appreciated since the work of Kindred (1940), Andreasen & Christensen (1949) and Andreasen & Ottesen (1945) that the mitotic activity of the thymic cortex is 7-10 times that of the peripheral lymph node tissue. At each level of division the more primitive cells continually reproduce themselves as well as maintaining the process of lymphopoiesis (Sainte-Marie & Leblond 1965). Rapid lymphopoiesis is observed in the thymus in accordance with the short life span of 3-4 days (Metcalf 1964a). Although division rate is extremely rapid, the majority of these dividing cells die *in situ*. With increasing age, there is thymic involution, and the rate of lymphopoiesis declines (Metcalf 1964a).

Ford (1966) demonstrated that the primitive cells of the thymus are slowly replaced by cells that migrated from the bone marrow. He showed that there were two kinds of lymphocytes found in the body, both originating from a common stem cell in the bone marrow. The so-called T-dependent lymphocytes emigrate from the bone marrow and are dependent upon the thymic environment for their future development. These cells reside both in the thymus itself, and the thymus-dependent (T-dependent) areas of the secondary lymphoid tissue (i.e. the paracortical areas of the lymph nodes, and the periarteriolar lymphocytic sheaths of the splenic white pulp).

Another group of cells have the propensity to emanate from the bone marrow and settle in lymphoid tissue other than the thymus (Ford 1966). These B-lymphocytes directly repopulate the B-dependent areas of the secondary lymphoid organs (lymphoid follicles and germinal centres of the lymph nodes and much of the white pulp of the spleen), and for most part are sessile in these tissues. In the chick the Bursa of Fabricius is the processing organ of these migrating cells (Cooper, Peterson, South & Good, 1966); the equivalent of the Bursa in man has not been identified, although gut associated lymphoid tissue, such as tonsils, appendix, Peyer's patches, lymphoid follicles themselves, and the haematopoietic tissue have been nominated as possible condidates. It follows from this property of the bone marrow to function as a stem cell population, that its division rate will be high.

A separate and discrete population of lymphocytes was brought to light by Gowans & McGregor (1964), who showed that small lymphocytes formed by lymphopoiesis in the T-dependent areas of the lymph nodes and spleen, comprise a pool of lymphocytes that are long lived (Little, Brecher, Bradley & Rose 1962; Craddock, Naka, Fukuta & Vanslager 1964; Norman, Sasak, Ottoman & Fingerhut 1965), with the qualities of being able to survive repeated recirculation through the blood and lymph, of migrating

into and through most of the body's tissues (but not the thymus) and returning to the lymph nodes. In the rat these long lived recirculating lymphocytes have life spans of a year or more.

It is a co-operation between the various groups of lymphocytes that consitutes the immune response. Whilst most of the thymic lymphocytes die *in situ*, and are themselves mitotically unresponsive to antigen (Craddock, Winkelstein, Matsuyuki & Laurence 1967), some are destined to become T-lymphocytes. Under the appropriate antigenic challenge, either the T- or the B-lymphocytes respond. Thus when a cell-mediated response is elicited such as painting picryl chloride onto the skin to induce contact hypersensitivity, there is a marked proliferation in the T-dependent areas with the consequent production of lymphoblasts. On the other hand, the pneumococcus antigen leads to proliferation in the B-dependent areas and subsequently production of humonal antibody. The cellular co-operation between the T-lymphocytes, the B-lymphocytes, and the macrophages in the immune response is shown schematically in Fig. 2. Claman, Chaperon & Triplett (1966) have shown a co-operation between the T- and the B-lymphocytes in actual synthesis of antibody. The T-lymphocytes, without themselves producing antibody, form an association such that they enable antigenic stimulation of the B-lymphocyte to be more effective (Mitchison, Taylor & Rajewsky 1970). Phagocytic macrophages, emanating from the bone marrow (Volkman & Gowans 1965a,b) are involved in the immune response such that their movement away from their site of action is prevented by the release of the lymphokine MIF (macrophage migration inhibitory factor) (David, 1971) by the T-lymphocyte (Rocklin, Meyers, & David 1970) in cell mediated reactions. In antigenic reactions with subsequent antibody production, the macrophage also plays a role by processing the invading antigen in some way (Feldman & Globerson 1972), forming a close association with the B-lymphocyte and ultimately releasing humoral antibody from the

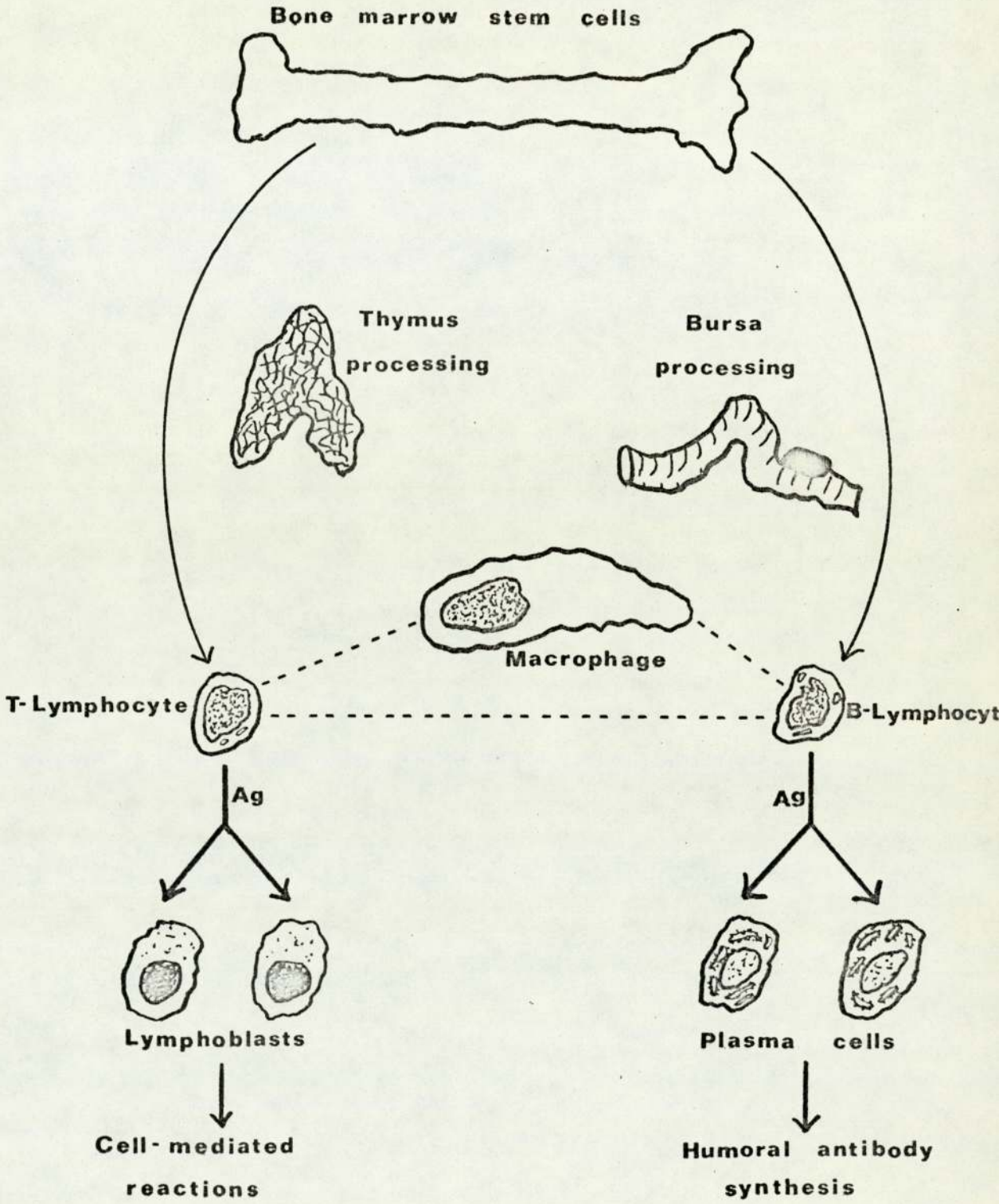


Fig. 2: Pathway whereby stem cells from the bone marrow are processed by thymus and gut-associated lymphoid tissue to become immunocompetent T- and B-lymphocytes respectively. Macrophages form a co-operation with both T- and B-lymphocytes in subsequent antigenic reactions (see text). Proliferation and transformation to cells of the lymphoblast and plasma cell series occurs on exposure to antigen (Ag).

plasma cell. The involvement of the recirculating pool of long lived lymphocytes, the majority of which are T-dependent, has been studied by Rowley, Gowans, Atkins, Ford & Smith (1972).

The life span of the thymic lymphocyte being short necessitates a rapid turnover, and the thymus gland can thus be regarded as a renewing tissue. Unlike the lymph nodes, the thymus does not require the stimulatory action of antigen to induce a proliferative response (Craddock, Winkelstein, Matsuyuki & Lawrence 1967). From studies in germ-free animals, and in animals undergoing antigenic stimulation, it is apparent that cell division in the normal thymus is independent of exogenous antigen. Metcalf has shown convinvingly that the thymus is independent of peripheral feedback inhibitory factors or peripheral stimulatory factors (Metcalf 1964b). This tissue has its own growth stimulatory apparatus and it is believed that the internal milieu of the thymus is influenced by a trophic secretion of the fixed epithelial cells (Metcalf, Sparrow, Nakamura & Ishidate 1961; Pepper 1961; Metcalf 1964b; Dukor, Miller, Hause & Allman 1965; Miller 1965; Matsuyama, Wiadrowski & Metcalf 1966).

The hormone thymosin isolated from thymic tissue (Goldstein, Slater & White 1966), is intimately linked with cell-mediated reactions (Goldstein, Asanuma, Battisto, Hardy, Quint & White 1970). While it is as yet unknown where the natural interaction between thymosin and its target cells occurs it is thought that precursor cells from the bone marrow could undergo changes mediated by thymosin, acting either within or outside the thymic environment, and additional properties acquired by the cells in the peripheral lymphoid tissue are necessary to complete their development to enable reactivity in cell-mediated immune responses (Trainin, Small & Kimhi 1973).

Even though true regulation of division in the thymic population is absent, several hormones such as PTH (Whitfield, Perris & Youdale 1969;

Whitfield, MacManus, Youdale & Franks 1971); growth hormone, neurohormones and prolactin (Whitfield, Perris & Youdale 1969; Whitfield, Rixon & Youdale 1969); cortisol (Whitfield, MacManus & Rixon 1970a; Whitfield, MacManus & Gillan 1973a) and acetylcholine, histamine and insulin (Perris & Morgan 1975) have been shown to promote proliferation of thymic lymphocytes, whereas the ensuing hypocalcaemia following parathyroidectomy depresses proliferation (Perris, Weiss & Whitfield 1970). It has indeed been shown that a major determinant in the physiological control of thymic lymphopoiesis is the plasma calcium ion concentration, in line with the observations that there is a reduced proliferation following parathyroidectomy (Perris, Weiss & Whitfield 1970) and increased proliferation with concommitant calcium elevation (Perris & Whitfield 1967; Perris, Whitfield & Tolg 1968). A more comprehensive account of the calcium involvement in thymic lymphopoiesis is presented in section 3.5.

2.2 Bone Marrow and Erythropoiesis.

Present in the bone marrow are pluripotential stem cells which give rise to a variety of unipotential stem cells (Lajtha 1970). Each unipotential stem cell produces, by multiple divisions, its specific non-dividing mature differentiated cell which passes into the blood and extracellular fluid for the remainder of its finite life span. The numbers of circulating blood cells are subject to control systems which have been delineated to varying degrees. While the factors which maintain granulocyte, platelet and lymphocyte numbers are imperfectly understood, the outlines of the control of circulating red cell mass is better known.

The fundamental stimulus for erythropoiesis is hypoxia (Gordon 1959). Erythropoietin (EPO), a circulating hormone, is now acknowledged to be the prime regulator of erythropoiesis in higher organisms (Gordon 1973). The feedback control mechanisms regulating plasma levels of EPO have also been fully detailed by Gordon (1973). These are shown schematically in Fig. 3.

Briefly, a decrease in red blood cell mass, by lowering blood oxygen content, stimulates the production of renal erythrogenin. This factor then interacts with a substrate in the plasma to yield the functional circulating EPO, which specifically causes differentiation of a haematopoietic precursor cell into the earliest recognizable members of the nucleated erythroid series (Stohlman, Ebbe, Morse, Howard & Donovan 1968; McCulloch 1970). This action of EPO on erythroid precursors is probably a direct one, since EPO will also stimulate red cell production in cultures of bone marrow cells *in vitro* (Kuna, Gordon, Morse, Lane & Charipper 1959; Matoth & Kaufmann 1962; Reissmann & Samorapoompichit 1970). The increased red blood cell mass induced by EPO, through a negative feedback action on the kidney, reduces erythrogenin production. Thus a well controled feedback system regulating red blood mass is accomplished.

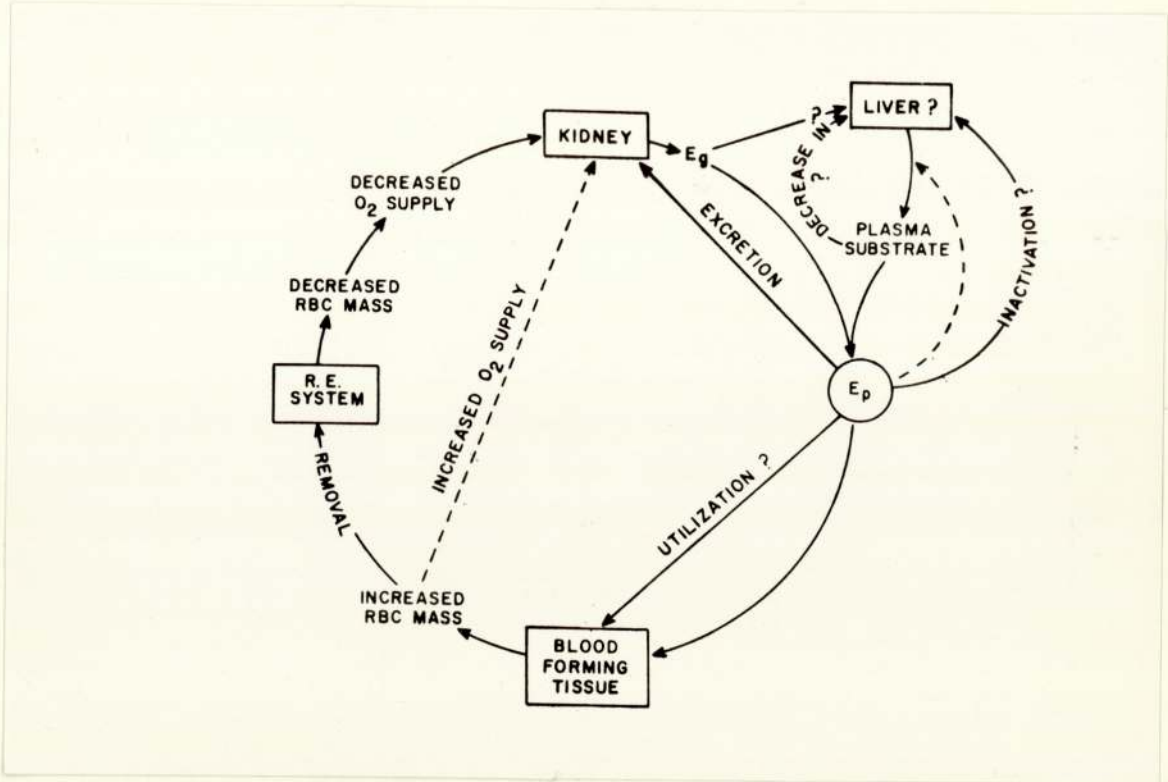


Fig. 3: Scheme of the biogenesis of erythropoietin (Ep) and its feedback regulation. (Solid line indicates stimulation and dotted line inhibition). Destruction of red blood cells (RBC) by decreasing blood oxygen content stimulates production of renal erythrogenin (Eg). Eg (which is probably an enzyme) interacts with a plasma substrate (and may indeed stimulate its production) to yield circulating Ep. Elevation of the circulating Ep inhibits its own production; this appears to be accomplished through reduction in the substrate levels. Consequently, the increased RBC mass induced by Ep reducces Eg production through a negative feedback action on the kidney.

Although other hormones can also influence erythropoiesis, Gordon (1957) has shown that they have a reinforcing role rather than exerting a primary influence upon erythropoiesis. However, even though Gordon has highlighted the role of EPO in erythropoiesis, it has been obvious for some time that certain clinical and experimental observations appear incompatible with the idea that erythropoietin acts as the only control mechanism in erythrocyte regulation (Brecher & Stohlman 1959). Linman & Bethell (1960) have presented evidence which suggests that there appear to be at least two specific plasma erythropoietic factors. The first is true EPO, and the other has been termed erythrocytic antichalone.

An intimate involvement of the calcium homeostatic machinery in erythropoietic events (Perris 1971; Perris, MacManus, Whitfield & Weiss 1971; Perris & Whitfield 1971) has also clearly been shown. Furthermore Hunt & Perris (1973) demonstrated that elevated plasma EPO (as would occur following haemorrhage) resulted in hypercalcaemia. However the exact mechanism whereby EPO interacts with the calcium balance mechanisms is at present not clear. Even though EPO directly stimulates bone marrow erythropoiesis in vitro (Kuna, Gordon, Morse, Lane & Charipper 1959; Matoth & Kaufmann 1962; Reissmann & Samorapoompichit 1970), the calcium-dependent responses are important when rapid restoration of erythrocyte numbers is required, as is the case with the parathyroid-mediated hypercalcaemia following haemorrhage (Perris, MacManus, Whitfield & Weiss 1971).

The bone marrow consists of a heterogenous population of immature nucleated precursor cells of the erythroid, lymphoid and myeloid series. The extracellular calcium ion concentration has also been shown to influence the division in this nucleated cell population. Thus the stimulatory effect of calcium on the nucleated cell population in the bone marrow (Perris, Whitfield & Rixon 1967; Perris & Whitfield 1971; Hunt & Perris 1973) and the reduced division following parathyroidectomy (Perris,

MacManus, Whitfield & Weiss 1971, Perris & Whitfield 1971; Rixon & Whitfield 1972a; Hunt & Perris 1973, 1974) imply a physiological role for calcium in determining the degree of bone marrow cell division. Thus EPO and calcium are well established in the stimulation of erythroid elements in the bone marrow following an erythropoietic stimulus. The calcium ion also plays a major role in the division of other nucleated cell populations in the bone marrow for the hypoplasia which follows parathyroidectomy involves the lymphoid as well as the erythroid elements (Rixon & Whitfield 1972a).

2.3 Small intestinal mucosa and epithelial cell turnover.

The small intestine is an elongated tube which occupies a compacted area within the abdominal cavity. It extends from the pyloric end of the stomach to its junction with the caecum at the ileo-caecal valve. The first few inches of rat small intestine comprise the duodenum; the junction between the duodenum and the jejenum is indicated arbitrarily by the ligament of Treitz. The jejenum and the distal portion of the small intestine, the ileum, constitute roughly equal proportions to the remaining small intestine, with no physical line of demarkation between the two areas.

In man, spiral or circular folds, the valvulae conniventes are readily visible with the naked eye (Bloom & Fawcett 1962), but in the rat these are absent and the interior of the intestines present a comparatively smooth appearance. This uppermost surface is termed the mucosa, which can be divided into three layers. The deepest of these, the muscularis mucosae, consists of a thin sheet of smooth muscle, generally 3-10 cells thick which separates the mucosa from submucosa. The middle layer, the lamina propria, is the subepithelial connective tissue space bounded below by the muscularis mucosae and above by the intestinal epithelium. The lamina propria together with its epithelial covering form the numerous fingerlike villi which project from the surface of the mucosa into the intestinal lumen, as well as the many pitlike indentations, the crypts of Lieberkuhn, located at the base of adjacent villi. The lamina propria contains a heterogenous population of cell types including fibroblasts, macrophages, plasma cells, lymphocytes and mast cells. Blood and lymph vessels, as well as unmyelinated nerve fibres and strands of smooth muscle are also present. It thus provides important structural support for the intestinal epithelium, and also a protective function against invading organisms (Crabbe, Carbonara & Heremans 1965). The third layer of the

intestinal mucosa consists of a continuous sheet of a single layer of columnar epithelium which covers both the villi and the crypts. The major role of the mature epithelial cells is food absorption; various undifferentiated cell types however, occupy the crypt region. Their origin, differentiation and renewal will be the main purpose of this review and will be discussed in detail later.

The size of the intestinal villi decreases gradually from duodenum to ileum (Altmann 1971); these regional differences have been attributed to factors secreted in the intestinal chyme (Altmann & Leblond 1970). The number of villi in the rat small intestine appears to remain remarkably constant with increasing age (Forrester 1972), although the ratio of crypts to villi rises with age (Clarke 1972). As well as the regional size differences, the three area are unique in their specialized functions.

When considering the overall absorption in the intestine both the efficiency of the absorptive process, and the time spent in that region are equally important factors. Thus although the active transport mechanism of calcium in the rat, which is dependent on vitamin D (Dowdle, Schachter, & Schenker 1960), is fastest in the proxinal duodenum, less in ileum, and least in the mid small intestine (Kimberg, Schachter & Schenker 1961), due to the short length of the duodenum and the rapid transit through it, the bulk of the dietary calcium absorption in the rat takes place in the ileum, followed by the jejenum and duodenum (Marcus & Iengemann 1962). This means that the longer residence time of calcium in the ileum compared to the duodenum more than compensates for the greater rate of transit across the duodenal epithelium.

Orally administered vitamin D is primarily absorbed in the jejenum and perhaps to a lesser extent in the ileum (Schachter, Finkelstein & Kowarski 1964). The relative effect of vitamin D on the rates of calcium

transfer across different levels of the intestine parallels the distribution of the active transport mechanism (Schachter, Kimberg & Schenker 1961).

The promotion of vitamin B_{12} absorption by intrinsic factor is maximal in the rat jejenum (Reynell, Spray & Taylor 1957). There is evidence that magnesium is transported more efficiently at the ileal end of the small intestine (Ross 1962; Behar 1974) unlike calcium which is transported more efficiently at the duodenal end (Schachter & Rosen 1959).

Four cell types are found at the base of the crypt of Lieberkuhn (Fig.4) i.e. columnar, entero-endocrine, mucous and paneth cells. All of these cells arise from a common crypt-base columnar cell which represents the functional stem cell population of the small intestine (Cheng & Leblond 1974c).

(i) Columnar epithelial cells.

The gastrointestinal mucosa having been designated a renewing tissue (Messier & Leblond 1960) implies that cell loss is balanced by cell gain. To understand why this classification was made, it is necessary to outline the relevant historical background. In 1894 Bizzozero noted abundant mitotic activity in the crypts and identified this region as the site of cell proliferation. Leblond, Stevens & Bogoroch (1948) demonstrated histological localization of newly formed DNA in the crypt cells, using ^{32}P, confirming earlier ideas of this being the generative zone. In an attempt to elucidate the fate of these numerous mitoses, Leblond & Stevens (1948) concluded that cells were shed from the tip of the villi (the extrusion zone) into the lumen. They envisaged that the entire epithelial lining of the intestine was constantly renewed; immature cells produced in the crypts continually moved up the villus where they differentiated into absorptive cells and were subsequently sloughed off at the extrusion zone.

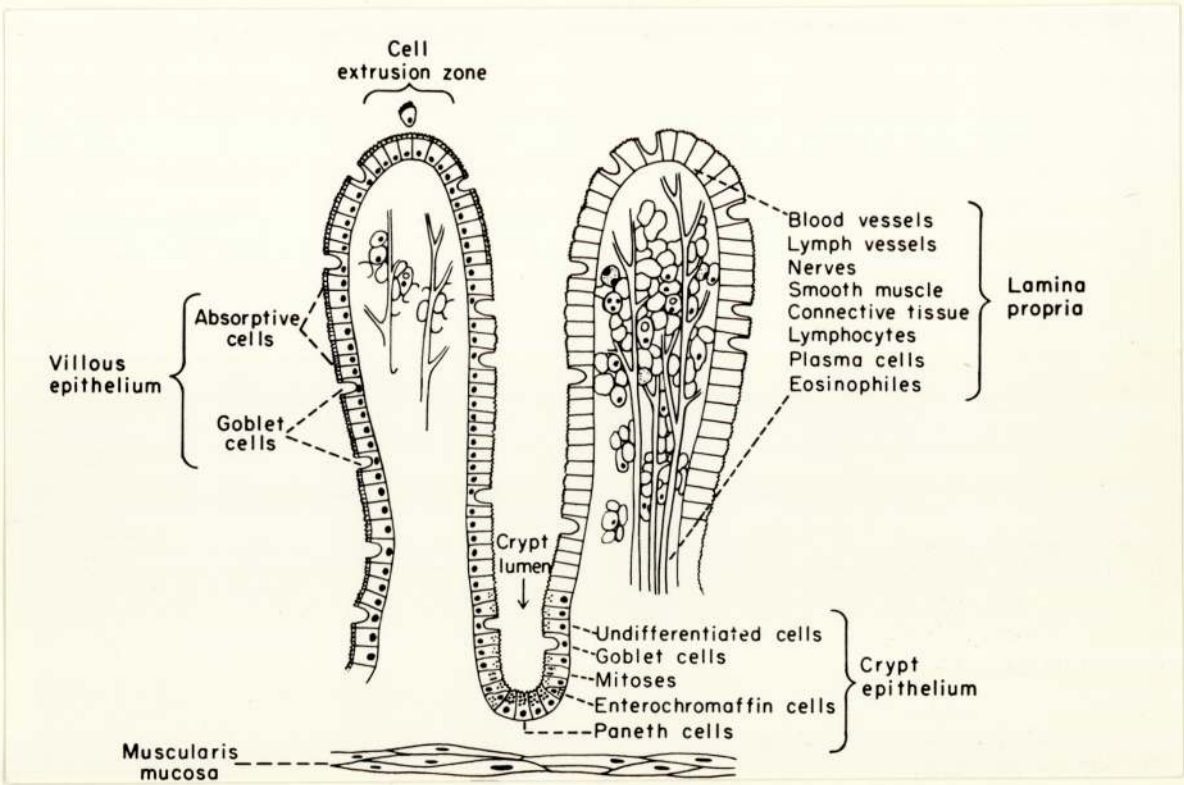


Fig. 4: Architecture and cellular organization of a typical villus. The base of adjacent villi form the crypt of Lieberkuhn; epithelia surrounding this crypt can be distinguished into four types - i) columnar (undifferentiated) cells, ii) entero-endocrine (enterochromaffin) cells, iii) mucous (goblet) cells and paneth cells, all emanating from a common stem cell at the crypt base. Migration of the columnar cells onto the villous epithelium results in maturation and differentiation into absorptive cells. Subsequently (about 40hrs later) these cells that arose originally from the stem cell population are exfoliated at the extrusion zone.

This plausible hypothesis of cell replacement was confirmed by the autoradiographic techniques of Leblond & Messier (1958).

Under steady state conditions, therefore, there is a dynamic equilibrium between cell production and cell loss (Cheng & Leblond 1974a). However under abnormal conditions the steady state situation is lost, and the size of the cell population can be altered either by proliferation or extrusion predominating. Thus in the transplanted fragment of intestine embedded under the kidney capsule, villi were shown to be absent but proliferation in the crypt still comtinued (Rowinski & Kaminski 1973). The functional integrity of the villous cells has been shown (Galjaard, van der Meer-Fienggen & Giessen 1972; Rijke, van der Meer-Fienggen & Galjaard 1974) since these cells exhibit a feedback control on cell proliferation in the crypt. On the other hand starvation arrests proliferating cells in G_1 and although division rate is diminished, extrusion still continues (Cameron & Cleffman 1964).

The time taken for a newly produced crypt cell to achieve full maturity and subsequent loss of function at the extrusion zone has been termed the transit time, and in rat small intestine is of the order of 40 hours (Loehry, Croft, Singh & Creamer 1969). The transit time varies throughout the digestive tract, but the shortest transit times are found in the small intestine; duodenum 1.6 days, jejenum 1.3 days and ileum 1.4 days (Bertalanffy 1960; Bertalanffy & Lau 1962a). In accordance with its designation as a renewal tissue, the small intestine represents one of the most rapidly dividing tissues of the rat (see below).

Turnover time of various cell populations of the rat.

Tissue	Turnover time (days)	Reference
Bronchus	126.0	Blenkinsopp 1967.
Ureter	49.0	Bertalanffy & Lau 1962a
Ear epidermis	34.0	Bertalanffy 1957

Turnover time of various cell populations of the rat (cont...)

Tissue	Turnover time (days)	Reference
Corneal epithelium	6.9	Bertalanffy & Lau 1962b
Cervix	5.5	Bertalanffy & Lau 1963
Vagina	3.9	Bertalanffy & Lau 1963
Tongue epithelium	3.5	Cutright & Bauer 1967
Thymic small lymphocytes	(7.0)	Everett & Tyler 1967
Bone marrow small lymphocytes	(4.0)	Everett & Tyler 1967
Small intestine: duodenum	(1.6)	Bertalanffy & Lau 1962a
Small intestine: ileum	1.4	Bertalanffy & Lau 1962a
Small intestine: jejenum	1.3	Bertalanffy & Lau 1962a

( ) Tissues being studied in this thesis.

(ii) Entero-endocrine cells.

Formerly called enterochromaffin or argentaffin cells, these cells are not limited to the small intestine, but are distributed throughout the gastrointestinal epithelia (Macklin & Macklin 1932). They are abundant in the intestinal epithelium of man, but quite uncommon in rat (Patzelt 1936). The entero-endocrine cell population has been shown to divide slowly, and is renewed in about 4 days in mouse via villous extrusion (Cheng & Leblond 1974b). The majority of these cells are located in the crypt region, and progressively decrease in number towards the villous tip (Benditt & Wong 1957).

Grutzner & Menzel in 1879 first described histological identification of these cells by virtue of their ability to take up silver. The functional role of the entero-endocrine cell is unknown. It has been well established that these cells contain significant quantities of 5-hydroxytryptamine (5-HT) (Erspamer & Asero 1952; Benditt & Wong 1957). Since pharmacological doses of 5-HT are an effective smooth muscle stimulant (Page 1954) it has been postulated that these cells may function in the regulation of

gastrointestinal motility, but this view is still a speculative one.

(iii) Mucous Cells.

Mucous cells may be classified into oligomucous or goblet cells. The former represent immature cells found exclusively in the crypt whereas the later are mature cells that have migrated onto the villous epithelium. The oligomucous cells are able to divide, and differentiate into non-dividing goblet cells, ascend towards the villous tip and be lost at the extrusion zone after about 3 days (Cheng 1974a).

The mucous cells may be readily distinguished from other types of epithelial cells by their possession of mucous granules. It has been suggested (Trier 1963) that most small intestine goblet cells secrete their mucous into the gut lumen gradually by merocrine secretion. The function of the mucus elaborated by the goblet cells has not been clearly defined, even though casual reference to a lubricating and protective function is mentioned.

(iv) Paneth Cells.

Unlike the other three types of crypt cells, Paneth cells at no stage of its life has the ability to proliferate, they do not migrate up the villus, not are they lost from the extrusion zone (Cheng 1974b). At the end of their life span they undergo degeneration in the crypt base and are eventually phagocytosed by an adjacent crypt base columnar cell (Cheng 1974b).

They have been shown to contain zinc (Ham & Leesen 1961) and their function appears to be to secrete lysozyme and to phagocytose crypt micro-organisms (Erlandsen & Chase 1972). The small intestinal epithelium of many species including man and rat exhibit abundant numbers of these cells (Wheeler & Wheeler 1964), being predominantly located in the lower crypt (Cheng 1974b).

The columnar epithelial cells are by far the most abundant cell type present in the crypt. Since it has been shown that these cells represent the progenitors of new villous absorptive cells, by virtue of their physiological role, they undergo constant rapid mitotic activity. Various hormones have been shown to influence intestinal cell proliferation (Table 2); however most authors revert to a chalone to try to explain the control of intestinal cell replacement (Tutton 1973; Clarke 1974).

In view of the demonstration of the physiological control of cell proliferation in lymphopoietic and haemopoietic tissues, under the control of the calcium homeostatic system (see section 1.3), it seemed pertinent to elucidate just how tissue specific this degree of regulation was. The small intestine was chosen as a renewal tissue with the aim that control of intestinal cell replacement might be governed by physiological fluctuations in extracellular calcium. Support for this hypothesis came from earlier workers (Urban & Shedl 1969; Farley, Spielvogel & Norman 1972; Birge & Alpers 1973) who had shown mitogenic and growth properties exhibited by vitamin D_3, a hormone which is known to be closely associated with calcium ion homeostasis (see section 3.3).

3.1 Calcium Homeostatic System - Parathyroid hormone.

The average mammaliam total plasma calcium lies within the range 8.5-11.0mg%. Within any one strain of a given species, the daily variability falls within much narrower limits; in the Wistar rat this has been shown to be of the order of 0.6mg% (Hunt & Perris 1974). Such remarkable consistancy implies the existence of a fine control system and led McLean & Hastings to coin the phrase 'one of nature's physiological constants'. Calcium homeostasis is regulated by three hormones; a) parathyroid hormone; b) thyrocalcitonin (section 3.2) and c) vitamin D (section 3.3).

Parathyroid hormone (PTH) is virtually essential for life. If the source of the endogenous hormone, the parathyroid glands, is removed the plasma calcium falls to dangerously low levels (5-6mg%) and if the appropriate therapy is not administered the animal may die. In order to protect against hypocalcaemia, there is a comtinual secretion of PTH (Potts, Buckle, Sherwood, Ramberg, Mayer, Kronfeld, Deftos, Care & Aurbach 1968). The first direct proof that hypocalcaemia stimulated hormone release from the parathyroids was obtained by Patt & Luckhart in 1942 and later confirmed by Copp & Davidson in 1961.

Recent studies have also shown a close involvement of the magnesium ion in parathyroid activity; a more detailed discussion of magnesium homeostasis is presented in section 3.4. Both secretion (Sherwood, Herrmann, & Bassett 1969; Sherwood, Herrmann &Bassett 1970; Targovnik, Rodman & Sherwood 1971) and synthesis (Hamilton & Cohn 1969; Sherwood, Rodman & Lundberg 1970; Sherwood, Abe, Rodman, Lundberg & Targovnik 1972), of PTH have been shown to be inversely related to the combined calcium + magnesium concentration in the plasma.

The major influence on parathyroid activity is the ionised calcium concentration in plasma which is itself influenced by at least four factors. Both the dietary calcium and phosphate concentrations, the ratio of these

two components and the availability of vitamin D (section 3.3) all play a part (Kramer & Howland 1922; 1931). A positive linear relationship has been shown between dietary calcium and absorbed calcium, with no restriction to the quantity absorbed (Clark & Rivera-Cordero 1973). The influence of diet upon parathyroid activity has been discussed by Clark (1968) and Kemm (1972).

Calcium in the plasma exists in at least three forms: protein bound, complexed (e.g. to citrate or phosphate) and ionised (Moore 1970). It is the ionised fraction that is physiologically active (Toribara, Terepka & Dewey 1957) and the actions of PTH are important in the maintenance of the ionised fraction in the plasma. This is brought about through its action upon three tissues - the kidney, the bone and the gastrointestinal tract. PTH increases tubular reabsorption of calcium (Talmage 1956; Nordin & Peacock 1969), enhances bone resorption, i.e. the active dissolution of the bone mineral apatite, (Barnicot 1948; Chang 1951; Raisz & Trummel 1971) and augments calcium absorption from the gastrointestinal tract (Robinson, Matthews & MacIntyre 1969; Winter, Morava, Simon & Sos 1970; Wills 1973).

There is controversy as to the primary target tissue involved in calcium homeostasis. It is generally agreed that calcium absorption from the intestine does not have a great effect on serum calcium levels (Peacock, Knowles & Nordin 1968). The early work of Barnicot in 1948 demonstrated direct resorption of bone by parathyroid hormone and as a result it was assumed the main target was the skeleton. However quantitative considerations refute a significant role for bone formation or bone resorption in the minute to minute regulation of calcium homeostasis; such changes in bone metabolism are much too small and slow to account for any significant change in plasma calcium (Baylink 1971; Baylink & Wergedal 1971; Neuman 1972; Neuman & Ramp 1971). Only when bone turnover rate is very rapid in the young animal, may bone resorption play a role

in calcium homeostasis (Kalu, Hadji-Georgopoulos, Sarr, Solomon & Foster 1974). Since calcium ions can be almost quantitatively reabsorbed by the kidney tubules Peacock & Nordin 1973 believe that this organ is the primary controller of rapid fluctuations in calcium levels. The role played by the kidney in the maintainance of plasma calcium probably increases and surpasses that of bone with age (Kalu, Hadji-Georgopoulos, Sarr, Solomon & Foster 1974). More recent evidence however suggests that although bone resorption may be too slow and imprecise a mechanism for minute to minute calcium regulation, PTH-mediated calcium fluxes in the readily exchangeable bone mineral pools, may be of greater significance (Talmage, Cooper & Park, 1970; Kalu, Hadji-Georgopoulos, Sarr, Solomon & Foster 1974). Furthermore the possibility that the effect of the hormone is due in part to inhibition of bone accretion (Milhaud & Perault-Staub 1971) cannot be ruled out.

Whatever the precise contribution of the different mechanisms and target tissues, negative feedback of PTH synthesis and secretion by way of plasma calcium (Copp & Davidson 1961; Copp 1969b)can readily counteract large changes in plasma calcium. However in view of the demonstration of a diurnal rhythmicity of the plasma calcium in the rat (Hunt & Perris 1974), it has been proposed there could be an anticipatory release of PTH to explain this rhythm (Perault-Staub, Staub & Milhaud 1974). Control of plasma calcium by PTH or some endogenous rhythm is not the only hormonal factor involved as detailed below.

3.2 Calcium Homeostatic System - Thyrocalcitonin.

The discovery of a second endocrine vector (thyrocalcitonin) which, in contrast to PTH's ability to raise plasma calcium, lowered plasma calcium (Copp, Cameron, Cheney, Davidson & Henze 1962) opened new frontiers in the maintainance of blood calciostasis. In the bird the ultimobranchial gland is responsible for the elaboration of this hypocalcaemic agent (Copp, Cockroft & Kueh 1967; Moseley, Matthews, Breed, Galante, Tse & MacIntyre 1968). Indeed, it was once thought that the calcitonin-secreting ('c' cells) of the mammalian thyroid originated from the ultimobranchial region in the embryo. However it is now certain that all the 'c' cells come originally from the neural crest (Pearse & Polak 1971). The hormone is principally found in the thyroid gland in mammalian species (Munson, Hirsch, Brewer, Reisfeld, Cooper, Wasthed, Orimo & Potts 1968) and not the parathyroids as Copp originally believed, hence the terminology thyrocalcitonin (TC) is adopted.

One of the fundamental stimuli for TC release is hypercalcaemia (Care, Duncan & Webster 1967). Care, Cooper, Duncan & Orimo 1968a have demonstrated a direct linear relationship between hypercalcaemia and TC secretion rate over the calcium range 12-20mg%. In the rat it was first suggested (Talmage, Neuenschwander & Kraintz 1965) and later substantiated (Klein & Talmage 1968; Minkin & Talmage 1968), that TC is secreted continuously even during normocalcaemia. Bronner, Sammon, Nichols, Stacey & Shah 1968, on the other hand, present evidence suggesting that TC is only secreted above plasma calcium levels of 10mg%. In the pig, Care, Cooper, Duncan & Orimo 1968b have shown that TC is secreted at normocalcaemic levels and below.

The prevention of hypocalcaemia (the function of PTH), is an ever-present problem both in the young and adult, due to varied intake of calcium. Hypercalcaemia rarely occurs to any marked extent, except in pathological conditions. Since thyroidectomy in contrast to parathyroid-

ectomy, does not result in any acute change in the blood calcium in rats maintained in the fasting state (Cooper, Hirsch & Munson 1970) several workers have sought for a physiological role for TC under normal conditions.

Before anticipating the role played by TC in calcium homeostasis, it must be emphasised that there is a species difference, which may reflect altered function. Most mammalian species (including rat) have undetectable amounts of TC whereas birds and especially fish have comparatively vast quantities (Kenny, Boelkins, Dacke, Fleming & Hanson 1971). Bijvoet, Van der Sluys Veer, de Vriis & van Koppen 1971 have suggested that TC has a sodium regulating function in fish. In high doses TC has been claimed to be natriuretic (Nielsen, Buchanan-Lee, Mathews, Moseley & Williams 1971); however the natriuretic dose of TC is about one hundred times its hypocalcaemic dose in rats (Aldred, Kleszynski & Bastian 1970), thus in the rat the regulation of sodium balance seems unlikely to be its physiological role.

It has been concluded that both short term (Krawitt 1967) and long term administration (Wase, Peterson, Rickes & Solewski 1966) of TC in the rat has no appreciable influence upon calcium absorption from the intestine. However, in the pig following long term administration there was shown to be a significant reduction in calcium absorption (Swaminathan, Ker & Care 1974). The principal action of TC is clearly on bone and bone metabolism. Munson & Hirsch 1966 obtained a hypocalcaemic response following TC injection, even in the absence of kidney and intestine. The main effect is an early transient hypercalcaemia (Copp & Kuczerpa 1970) and later a block of bone resorption especially that induced by PTH (Raisz & Niemann 1967).

Age is ofparamount importance since adult rats do not respond as well as rapidly growing animals (Care & Duncan 1967; Orimo & Hirsch 1973; Roycroft & Talmage 1973). With increasing age the situation is complicated by a resistance to TC and general impairment of calcium tolerence due to

low bone turnover rate (Copp 1969a). Thus calcium tolerence is reduced in all mature rats compared to growing ones irrespective of the presence or absence of TC (Kumar & Sturtridge 1973). The TC content of the thyroid is much higher in adult rats, thus since hypercalcaemia triggers TC secretion, the decreased calcium tolerence of intact mature rats is due to refractoriness of their bones to endogenous TC rather than a deficiency of circulating hormone (Kumar & Sturtridge 1973).

TC deficiency can not be implicated in the aetiogenesis of any serious bone disease since it has just been concluded that it plays very little role in the adult. However its usefulness in treating the hypercalcaemia of Paget's disease is well established (Bordier, Rasmussen & Dorfmann 1974; Queener & Bell 1975). The high circulating levels of TC are used as a diagnostic tool in medullary carcinoma of the thyroid (Delorme, Kerceron, Raymond, & Klotz 1974; Goltzman, Potts, Chester Ridgway & Maloof 1974; Queener & Bell 1975; Triggs, Hesch, Woodhead & Williams 1975).

One possible hypercalcaemic threat to the body is after a meal. Following intra-gastric administration of calcium there was shown to be no rise in plasma calcium (Cooper, Schwesinger, Mahgoub, Ontjes, Gray & Munson 1972) while circulating TC levels rose. Clearly hypercalcaemia was not the trigger for the hormone release. Although in intact animals Cooper has shown a direct correlation between intra-gastric calcium and plasma TC, in the absence of the thyroid there was post-prandial hypercalcaemia. Probably the presence of calcium in the gastrointestinal tract evokes secretion of a gastrointestinal hormone capable of signalling the thyroid to secrete TC. Several workers (Care, Brue, Boelkins, Kenny, Conaway & Anast 1971; Cooper, Schwesinger, Ontjes, Mahgoub & Munson 1972; Swaminathan, Bates, Bloom, Ganguli & Care 1973) conclude that gastrointestinal hormones alone or in concert with blood calcium, may well be involved in the physiological regulation of secretion of TC, and may be of special importance in protecting against post-prandial hypercalcaemia. It has also been

suggested that an equally important physiological role of TC is the protection of the skeleton against excessive PTH-induced resorption during pregnancy (Lewis, Rafferty, Shelley & Robinson 1971).

Since TC has reduced effectiveness in adult animals it seems unlikely that it plays much part in calcium regulation in the adult (Kumar & Sturtridge 1973). It may be concluded that the prime importance of TC is protection against post-prandial hypercalcaemia and protection of the skeleton in pregnancy; the dual antagonistic actions of PTH and TC on bone metabolism are probably only of importance and significance in the young growing animal (Swaminathan, Bates & Care 1972; Kumar & Sturtridge, 1973).

Apart from the prime controlling hormones PTH and TC, intricate hormonal interactions make the endocrine regulation of calcium homeostasis complex. Thus both the influence of vitamin D and the adrenal steroids in the male and the additional factor of the oestrogens in the female need to be considered.

3.3 Endocrine modulation of calcium homeostatic hormones.

Apart from the principal hormones concerned with the maintenance of calcium homeostasis (PTH and TC), various other hormones exert an influence on this finely controlled system. Those of prime importance are:-

(i) Vitamin D.

(ii) The adrenal steroids.

(iii) The sex steroids.

(i) Vitamin D.

It has long been known that not only lack of PTH, but also Vitamin D deficiency, can result in hypocalcaemia and tetany (Thompson & Collip 1932). Indeed, Vitamin D has recently been accorded hormonal status (DeLuca 1973), and is intimately involved in calcium homeostasis (Harrison 1964; Melancon & DeLuca 1969; Borle 1974). The two main vitamins of the 'D' group, Vitamin D_2 (ergocalciferol) and Vitamin D_3 (Cholecalciferol) are equipotent in the rat (Norman 1968); in this review reference to Vitamin D implies cholecalciferol.

It is now generally accepted that Vitamin D must undergo sequential hepatic (Ponchon, Kennan & DeLuca 1969) and renal (Holick, Schnoes & DeLuca 1971; Lawson, Fraser, Kodick, Morris & Williams 1971) hydroxylation, as a prerequisite to the formation of the principal active metabolite. In the liver, cholecalciferol is converted to 25-hydroxycholecalciferol (25-HCC) and then transformed, depending on the calcium status of the animal to either 24,25 dihydroxycholecalciferol (24,25-DHCC) (Holick, Schnoes, DeLuca, Gray, Boyle & Suda 1972) or 1,25 dihydroxycholecalciferol (1,25-DHCC) (Lawson, Fraser, Kodick , Morris & Williams 1971) by two distinct renal hydroxylase enzymes (DeLuca 1974). No physiological role has yet been attributed to 24, 25-DHCC, and indeed was wrongly characterised by earlier workers as 21,25-DHCC (Boyle, Gray & DeLuca 1971; Holick, Schnoes, DeLuca Gray, Boyle & Suda 1972).

The amount of 1,25 DHCC generated by the action of the renal hydrox-

ylase depends on the calcium status of the animal. Animals maintained on adequate dietary Vitamin D, but deficient in calcium, for about three weeks exhibit marked stimulation of the production of 1,25 DHCC (Boyle, Gray, & DeLuca 1971). On the other hand, the feeding of diets high in calcium inhibits or turns off the production of 1,25 DHCC and the 24,25-DHCC metabolite is synthesised instead (Omadl & DeLuca 1973; DeLuca 1974).

Other polar metabolites of Vitamin D have been identified, such as 25,26 DHCC (Suda, DeLuca, Schnoes, Tanaka & Holick 1970) and 1,24,25 tri-HCC (Holick, Kleiner-Bossaller, Schnoes, Hasten, Boyle & DeLuca 1973) They have some activity in promoting intestinal calcium transport, but do not as yet have any biological function attributed to them (Norman 1974a).

1,25 DHCC is considered to be the most active form of the vitamin, increasing both calcium absorption from the intestine (Boyle, Miravet, Gray Holick & DeLuca 1972) and stimulating bone resorption (Reynolds, Holick & DeLuca 1973). 1,25 DHCC stimulates intestinal calcium absorption even in Vitamin D-deficient thyroparathyroidectomised rats (Holick & DeLuca 1974), and is probably the most important agent controlling intestinal calcium transport (Nicolaysen, Eeg-Larsen & Malm 1953); PTH is able to modify the Vitamin D-induced calcium mobilization (Olsen, DeLuca & Potts 1972). In contrast, although 1,25 DHCC is the most potent bone resorbing agent known, both 1,25 DHCC and PTH are required for maximal bone mobilization (Harrison, Harrison & Park 1958; Rasmussen, DeLuca, Arnaud, Hawker & von Stedingk 1963; Holick & DeLuca 1974). The action of TC is not likewise dependent on the Vitamin D status of the animal (Morii & DeLuca 1967).

The regulation of 1,25 DHCC production has been a controversial issue over the past few years, and even less is known regarding the hepatic 25-hydroxylase regulation (Bhattacharyya & DeLuca 1973; Tucker, Gagnon & Haussler 1973; Horiuchi, Suda, Sasaki, Ezawa, Sano & Ogata 1974). With respect to the 1,25 DHCC regulation, Fraser & Kodick (1973) concluded

that the level of circulating PTH (which would rise during hypocalcaemic episodes or when animals are kept on a low calcium diet), was directly responsible for activating the renal 1-hydroxylase enzyme. The picture was clouded when MacIntyre and his co-workers showed that when thyro-parathyroidectomised rats were given low calcium, low phosphate diets, a pronounced increase in 1,25 DHCC production resulted (Galante, Colston, Evans, Byfield, Mathews & MacIntyre 1973; Larkins, Colston, Galante, MacAuley, Evans & MacIntyre 1973). They suggested that the determining factor governing the production of 1,25 DHCC was the intracellular renal calcium concentration. Tanaka & DeLuca (1973) emphasised the importance of the renal cellular phosphate, and postulated that PTH was acting via its effects upon the phosphate ion. A more extensive survey of this problem may be found in the review by DeLuca (1974). Thus, it seems likely that the renal cell levels of phosphate and calcium, of specific Vitamin D metabolites and perhaps PTH, interact in a complex fashion to determine which of the hydroxylase enzymes is synthesised and which is degraded (DeLuca 1974).

Whatever the mechanism governing its formation, 1,25 DHCC has been highlighted as the prime controlling factor responsible for intestinal calcium transport. The discovery of a Vitamin D-dependent (Wasserman & Taylor 1966), PTH-independent (Bar, Hurwitz & Cohen 1972) intestinal calcium-binding protein (CaBP) opened new frontiers in the intestinal action of cholecalciferol. A vitamin D-dependent CaBP has been found in several species, including rat (Kallfelz, Taylor, & Wasserman 1967); dog (Taylor, Wasserman & Jowsey 1968); chick (Taylor, & Wasserman 1967); cow (Pullmer & Wasserman 1973); pig (Hitchman, Kerr & Harrison 1973; Dorrington, Hui, Hofmann, Hitchman & Harrison 1974); and monkey (R.H. Wasserman, unpublished results). There is a high correlation between CaBP and calcium absorption under a variety of physiological conditions

known to alter circulating 1,25 DHCC levels (Wasserman & Taylor 1968; Taylor & Wasserman 1969; Wasserman, Corradino & Taylor 1969), indicating a major role for CaBP in vitamin D-stimulated calcium transport across the small intestine (Emtage, Lawson & Kodicek 1974).

A cautionary note is necessary at this stage with respect to the species studied. Since chicks readily become rachitic on Vitamin D-deficient diets, the majority of CaBP work has been done on this species. In the chick it has been shown that the concentration of CaBP at different locations along the small intestine correlates well with the regional ability to absorb calcium; CaBP and calcium absorption are greatest in the duodenum and lowest in the ileum (Taylor & Wasserman 1967). In the rat CaBP activity and calcium transport along the length of the gut are also closely correlated (Schachter, Kowarski & Reid 1967). However in the hamster, calcium transport is maximal in the ileum, whereas CaBP activity is greatest in the duodenum (Schachter, Kowarski & Reid 1967).

The basic action of 1,25 DHCC is to promote intestinal movement of calcium from the lumen to the blood - a process which in chick takes between 9 - 12 hours for maximal effect (Norman 1974b). It is believed that in common with other steroid hormones 1,25 DHCC travels to the nucleus of the intestinal epithelial cell to direct the formation of new mRNA and eventually to influence the synthesis of CaBP (Brumbaugh & Haussler 1973; Emtage, Lawson & Kodicek 1973) (See Fig 5). The enhanced calcium absorption which follows would tend to diminish the hypocalcaemia which initiated the enhanced 1,25 DHCC elaboration, and thus decrease the 1-hydroxylase and increase the 24-hydroxylase activity in the kidney. Such a sequence obviously provides a complete negative feedback control for calcium absorption (Bar & Wasserman 1973). A similar system controlling bone resorption has not yet been established.

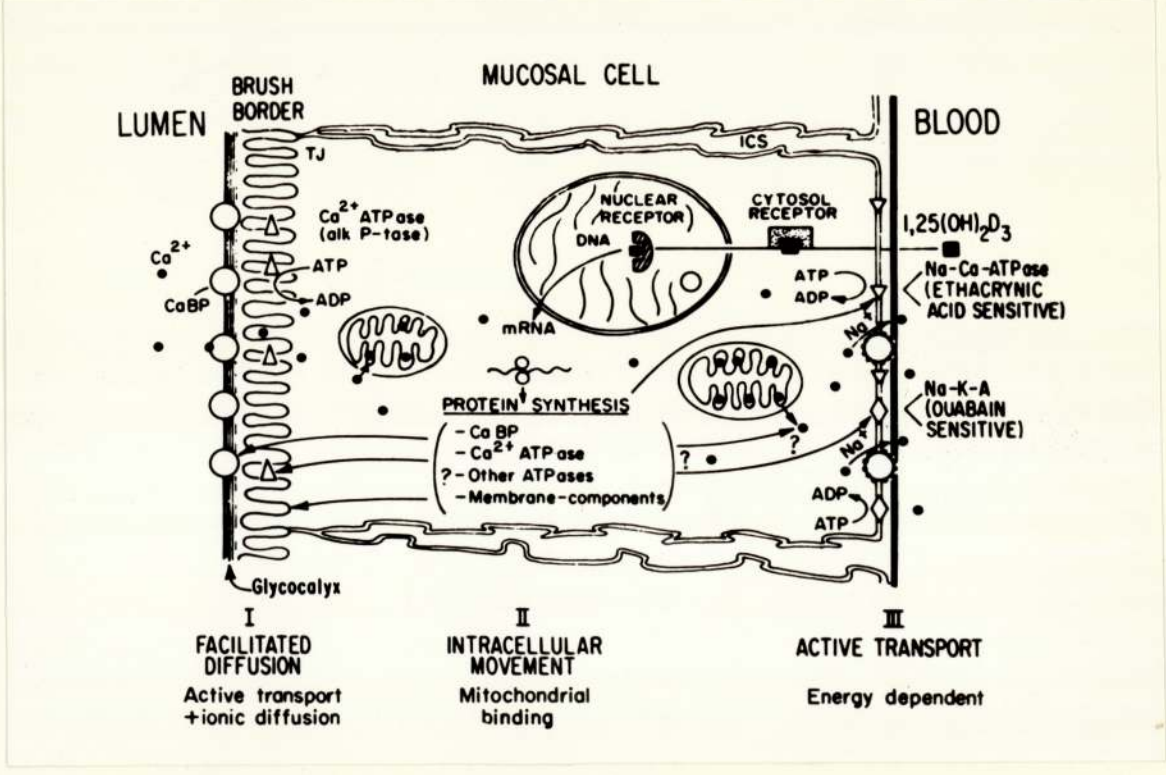


Fig. 5: Proposed mechanism of action of 1,25 DHCC in the intestine and its effects on intestinal calcium translocation. The essential features include a two-step binding process; first the 1,25 DHCC is associated with a cytoplasmic receptor, and this is followed by movement of the steroid to the intestinal nucleus. Here the vitamin directs synthesis of various proteins - the CaBP (O) travels to the cell membrane adjoining the lumen and promotes the transport of calcium ions (●), across the cell and into the blood.

(ii) The Adrenal Steroids.

Any stressful situation involves the adrenal steroids, and in the rat results in corticosterone secretion (Dallman & Jones 1973). The adrenal steroids play an important role in calcium homeostasis. There appears to be general agreement that adrenal steroids oppose the action of PTH in several species including guinea-pig (Marney-Gulat, Dam Trung Tuong, Labitte & Raoul 1972); rat (Williams, Peterson, Bowser, Henderson, Hargis & Martinex 1974) and in humans (Eliel, Palmier, Thompson, Bird & Hawrylko 1971). Corticosteroids are also known to inhibit calcium transport, but probably not via an antagonism of Vitamin D (Lukert, Stanbury & Mawer 1973).

(iii) The Sex Steroids.

The parathyroid glands of female rats have been reported to be heavier than males of the same weight (Jackson & P'An 1932; Pappenheimer 1936; Blumenfeld & Rice 1938), an observation which led to the investigation of the role played by the sex hormones in calcium metabolism. Sex differences in calcium metabolism undoubtedly exist. Apart from the work of Ogata, Shimazawa, Suzuki, Yoshitoshi & Ando (1970), the androgenic steroids are not well documented in their possible interrelationship with calcium balance, in contrast to the oestrogenic steroids to which much literature has been devoted.

A number of studies have shown that treatment of growing rats with oestrogens depresses bone resorption (Day & Follis 1941; Budy, Urist & McLean 1952; Lindquist, Budy, McLean & Howard 1960). It is also well established that PTH acts to resorb bone calcium (Raisz & Niemann 1967), whereas TC inhibits this process (Mittleman, Chausmer & Bellavia 1967; Friedman, Au & Raisz 1968; Reynolds & Dingle 1968).

Hypocalcaemia has been observed following oestrogen treatment, but administration of a small dose of TC did not decrease plasma calcium in the oestrogen-treated rat (Shai & Wallach 1973). This may be ascribed to an already inhibited resorptive system, in interpretation which is consistent with a decreased responsiveness to TC in mature females (Schlueter & Caldwell 1967; Sorensen & Hindberg 1971). In contrast to these observations Shai & Wallach (1973) showed that the presence of oestrogen promoted the skeletal effects of TC as manifest by a decrease in bone resorption.

It is well established that PTH-induced bone resorption (Raisz & Niemann 1967) is inhibited by oestrogens (Lafferty, Spencer & Pearson 1964; Eisenberg 1966; Stott 1968; Young, Jasani, Smith & Nordin 1968; Anderson, Greenfield, Posada & Crackel 1970; Atkins, Zanelli, Peacock & Nordin 1972; Orimo, Fujita & Yoshikawa 1972). As a consequence therefore, post-menopausal females tend to develop osteoporosis (Gallagher & Nordin 1973). Indeed Manunta, Saroff & Turner (1957) have hypothesised that an antagonism exists between PTH and the oestrogens, such that a proper balance between the two is necessary for normal calcium equilibrium.

Since increased plasma magnesium ion concentrations will, like calcium, stimulate cell division in some tissues, and since its metabolism is also influenced by PTH and TC, a brief consideration of magnesium homeostasis follows.

3.4 Magnesium Homeostasis.

In addition to the intimate involvement of calcium in the control of thymic and bone marrow proliferation (section 3.5), the magnesium ion has also been shown to enhance thymic lymphoblast proliferation both *in vivo* and *in vitro* (Perris, Whitfield & Rixon 1967; Morgan & Perris, 1974). It is thus necessary to briefly outline the relationships that exist between magnesium and calcium ion homeostasis. In some of their actions, calcium and magnesium are similar, whereas in other aspects they are antagonistic (Goodman & Gilman 1970).

In spite of the fairly close chemical relationship between calcium and magnesium, their relative distribution in body tissues is widely divergent. The ratio of extracellular:intracellular calcium is of the order of 10^2 to 10^5 (Rasmussen 1970). Under normal conditions magnesium tends to be higher than calcium in the cells of soft tissues, while calcium is higher than magnesium in the extracellular fluid (Tibbetts & Aub 1937). Thus, in contrast to calcium, magnesium is mainly found inside cells, the ratio of extracellular:intracellular magnesium being of the order of $4.0\text{-}5.0 \times 10^{-2}$ (Bell, Davidson & Scarborough 1968).

It has been mentioned (section 2.3) that both calcium and magnesium are actively transported primarily by the ileum. Earlier workers (Alcock & MacIntyre 1962; Hendrix, Alcock & Archibald 1963) imply that a competition exists between these two cations for the site of absorption, whereas this is refuted by Pointillart & Gueguen (1973). Vitamin D (section 3.3) is of utmost importance in the intestinal absorption of calcium; one of the factors invovled in the absorption of magnesium has also been shown to be the Vitamin D intake (Meintzer & Steenbock 1955), even though Vitamin D administration causes hypomagnesaemia (Richardson & Welt 1963). This apparent paradoxical situation has been explained by internal redistribution of cations, since there is a correlation between hypomagnesaemia and an augmented urinary

excretion of potassium (Richardson, Huffines & Welt 1963).

Despite increasing knowledge about calcium homeostasis (sections 3.1 and 3.2) and the factors influencing this (section 3.3) a surprising ignorance remains with respect to magnesium homeostasis. There is considerable data which supports the concept that plasma magnesium concentration plays a role in PTH secretion (Sherwood, Herrmann & Bassett 1969; Sherwood, Herrmann & Bassett 1970; Targovnik, Rodman, & Sherwood 1971). Magnesium balance in the rat may be a unique control system, since although magnesium depletion in man (Shils 1969); monkeys (Dunn 1971); sheep (L'Estrange & Axford 1964); dog (Levi, Massry, Coburn, Llach & Kleeman 1974); chick (Reddy, Coburn, Hartenbower, Friedler, Brickman, Massry & Jowsey 1973); calf (Smith 1959) and pig (Miller, Ullrey, Zutant, Baltzer, Schmidt, Hoefer & Luecke 1965) are associated with hypocalcaemia, similar depletion in the rat (Alcock & Shils 1974) results in hypercalcaemia. The involvement of the parathyroid gland in magnesium depletion has been strengthened by the observation that parathyroidectomised rats made deficient in magnesium did not develop hypercalcaemia (Gitelman, Kukolj & Welt 1968a). Despite the fact that hypomagnesaemia provokes PTH release (Gitelman, Kukolj & Welt (1968b) this hormone does not seem to influence normomagnesaemia. Thus although PTH elevated plasma calcium in the young rat, it was ineffectual upon the plasma magnesium concentration (Garel & Barlet 1974). Low doses of TC that produced hypocalcaemia and hypophosphataemia, had no effect upon plasma magnesium, whereas high doses caused hypomagnesaemia (Garel & Barlet 1974). Parathyroidectomy also causes slight hypomagnesaemia (Clark & Rivera-Cordero 1975). Thus although calcium homeostatic hormones appear to be involved in some way, there has not been any demonstration of magnesium-controlling analogues equivalent to PTH or TC.

In the male rat there has been shown to be a slight circadian variation of plasma magnesium between 1.9 - 2.1mg% (Hunt & Perris 1974). The most extensive study of magnesium concentration in body fluids is in man,

where Raut & Viswanthan (1972) showed serum magnesium values of around 2.5mg% irrespective of age or sex. These figures indicate the presence in the body of some mechanism responsible for maintaining stable magnesium homeostasis; Heaton (1969) concludes that in man this is achieved by the kidney. Even so, the physiological function of magnesium and the necessity for its homeostatic control in healthy individuals still remains ill defined, although its intimate involvement with many enzyme systems has long been recognised (Wacker 1969).

3.5 Physiological control of cell proliferation by calcium.

In addition to affecting such functions as nerve excitability, muscle contraction and glandular secretion (See General Introduction), evidence is accumulating to justify a role for the calcium ion as a regulator of cell proliferation in haemopoietic and lymphoid tissues. A detailed analysis of the discoveries leading to this postulate follows:-

a) Elevation of extracellular calcium by injection of $CaCl_2$

Observation	Reference
(i) Stimulates thymocyte proliferation *in vitro*.	MacManus & Whitfield, 1971. Morgan & Perris 1974 Whitfield, Perris & Youdale 1968. Whitfield, Perris & Youdale 1969. Whitfield, Rixon, Perris & Youdale 1969.
(ii) Stimulates thymoctye proliferation *in vivo*.	Perris, Weiss & Whitfield 1970. Perris & Whitfield 1967. Perris, Whitfield & Rixon 1967. Perris, Whitfield & Tolg 1968.
(iii) Stimulates proliferation of bone marrow cells *in vitro*.	Hunt 1974. Morton 1968.
(iv) Stimulates proliferation of bone marrow cells *in vivo*.	Hunt & Perris 1973. Perris & Whitfield 1971. Perris, Whitfield & Rixon 1967. Rixon 1968
(v) Stimulates reticulocyte formation in bone marrow *in vivo*.	Perris & Whitfield 1971.
(vi) Stimulates ^{59}Fe incorporation into red blood cells *in vivo*.	Perris & Whitfield 1971.
(vii) Stimulates the proliferation of normal chicken fibroblasts *in vitro*.	Balk 1971. Balk, Whitfield, Youdale & Braun 1973.
(viii) Enhances the antibody response.	Braun, Ishizuka & Seeman 1970
(ix) Increases survival of irradiated rats.	Rixon & Whitfield 1963.
(x) Increases division of grasshopper neuroblasts.	St. Amand, Anderson & Gaulden 1960.

b) Elevation of extracellular calcium by injection of PTH.

	Observation.	Reference.
(i)	Stimulates proliferation of bone marrow cells *in vivo*.	Perris & Whitfield 1971.
(ii)	Stimulates reticulocyte formation in bone marrow *in vivo*.	Perris & Whitfield 1971.
(iii)	Stimulates ^{59}Fe incorporation into red blood cells.	Perris & Whitfield 1971.
(iv)	Stimulates thymocyte proliferation *in vitro*.	Whitfield, MacManus & Rixon 1970b. Whitfield, MacManus, Youdale & Franks, 1971.
(v)	Stimulates thymocyte proliferation *in vivo*.	MacManus, Perris, Whitfield & Rixon, 1970. Perris, Weiss & Whitfield 1970.
(vi)	Increases survival of irradiated rats.	Rixon & Whitfield 1961. Rixon, Whitfield & Youdale 1958.
(vii)	Enhances post-haemorrhagic erythropoiesis.	Perris, MacManus, Whitfield & Weiss 1971.
(viii)	Implicated in liver regeneration.	Rixon & Whitfield 1972b.

c) Reduction of extracellular calcium by parathyroidectomy.

	Observation.	Reference.
(i)	Lowers thymocyte proliferation *in vivo*.	Hunt & Perris 1974. Perris, Weiss & Whitfield 1970.
(ii)	Causes thymic atrophy.	Perris, Weiss & Whitfield 1970.
(iii)	Lowers bone marrow cell proliferation.	Hunt & Perris 1973, 1974. Perris, MacManus, Whitfield & Weiss 1971. Perris & Whitfield 1971. Rixon 1968. Rixon & Whitfield 1972a.
(iv)	Causes bone marrow hypoplasia.	Rixon & Whitfield 1972a.
(v)	Lowers reticulocyte production.	Perris & Whitfield 1971.
(vi)	Inhibits ^{59}Fe incorporation into red blood cells.	Perris & Whitfield 1971.
(vii)	Increases time required for post haemorrhagic restoration of haematocrit.	Perris, MacManus, Whitfield & Weiss 1971.

c) cont... Reduction of extracellular calcium by parathyroidectomy.

	Observation.	Reference.
(viii)	Reduces and delays DNA synthesis and cell proliferation in the regenerating liver.	Rixon & Whitfield 1972b.
(ix)	Abolishes the mitogenic action of erythropoietin on bone marrow.	Hunt & Perris 1974.
(x)	Abolishes circadian changes in bone marrow and thymus cellular proliferation.	Hunt & Perris 1974.

The effects of these increases in calcium ion concentration were exerted within the physiological range, indicating the importance of this ion in the physiological control of thymic lymphoblast proliferation *in vivo* (Whitfield, Rixon, Perris & Youdale 1969; MacManus & Whitfield 1971). As can be seen from the above literature, the majority of the findings have been restricted to two tissues, namely the thymus gland and the cells of the bone marrow. These tissues readily lend themselves to both *in vitro* and *in vivo* study; there is a large cell population at any one time, and the mitotic activity of these tissues is such that both decreased and increased division is demonstrable without exhausting mitotic capabilities.

Having detailed the situations *in vitro* and *in vivo* in which calcium has been shown to be mitogenic, it is necessary to discuss briefly the cellular mechanism by which this effect is believed to be achieved.

3.6 Calcium, cyclic-AMP, and the control of cell proliferation.

The thymic lymphoblast has been used as a model for the elucidation of the cellular mechanisms underlying cell proliferation for the reasons mentioned overleaf. Even though it is difficult to draw analogies between rat and man, it is anticipated that a knowledge of the fundamental control systems in rodents will result in a greater insight into normal and neoplastic mammalian cell division.

The integrated requirement for both calcium and cyclic adenosine-3' 5' monophosphate (cyclic-AMP) has been recognised in many hormonally-mediated processes (Rasmussen 1970). In these systems it has been established that calcium is not required for the production of cyclic-AMP, whereas the physiological response is calcium-dependent. Elevation of the extracellular calcium concentration both *in vitro* and *in vivo* (section 3.5) has been shown to increase cellular proliferation in thymic lymphoblasts and bone marrow cells. Bridging these two processes a complex biochemical series of events occur in the cell membrane involving cyclic-AMP. The formation of cyclic-AMP from adenosine 5' triphosphate (ATP) and degradation to adenosine 5'monophosphate (5'AMP) are catalysed by the enzymes adenylate cyclase and phosphodiesterase (PDE) respectively (Robison, Butcher & Sutherland 1971). The cyclic-AMP levels in tissues may be influenced by agents which potentiate or inhibit either of these two enzymes. A number of hormones (e.g. glucagon, ACTH) are known to be adenylate cyclase stimulators (Robison, Butcher & Sutherland 1971).

Parathyroid hormone, via its intermediary action of elevating the extracellular calcium ion concentration, causes DNA synthesis in thymic lymphoblasts by raising their cyclic-AMP levels (Whitfield, MacManus & Rixon 1970b; Whitfield, MacManus, Youdale & Franks 1971). There has been shown to be a striking parallel between the extracellular calcium concentration, the cyclic-AMP content, the level of DNA synthesis, and the resul-

ting cell proliferation (MacManus & Whitfield 1971; Whitfield, MacManus, Youdale & Franks 1971). The elevation of intracellular cyclic-AMP by the calcium ion has been attributed to its ability to inhibit the enzyme PDE (Whitfield, MacManus, Youdale & Franks 1971).

Since PTH increases the entry of calcium into cells (Borle 1967; Rasmussen 1971; Whitfield, MacManus, Youdale & Franks 1971) it has been postulated (Whitfield, Rixon, MacManus & Balk 1973) that a sudden rise in the extracellular calcium concentration, or an increase in the membrane's permeability could lower the activity of the surface PDE. The feasibility of such a system is substantiated by the observation that various membrane-active agents stimulate thymic lymphoblast proliferation by suddenly sensitising the cells to extremely low and normally non-mitogenic calcium levels (Whitfield, Perris & Youdale 1968).

As a result of this influx of calcium ions into the cell, quiescent G^{o} cells are recruited from the G_1/S boundary into the DNA synthetic (S) phase of the cell cycle (Whitfield, Rixon, Perris & Youdale 1969). Calcium also interacts with the cell membrane in order to bring about an elevation of cyclic-AMP. Cyclic AMP probably turns on an activation site in the cell membrane (See Fig. 6). Since exogenous cyclic-AMP's mitogenic action is inhibited by imidazole (a PDE activator), and promoted by caffeine (a PDE inhibitor) (Whitfield, MacManus & Gillan 1970), there must be a PDE associated with the surface activation site. It is thus postulated (Whitfield, Rixon, MacManus & Balk 1973) that calcium having gained access to this surface PDE site, raises intramembranal cyclic-AMP levels, which then switches on the activation site. The stimulated site would then produce the ultimate mediators which enter the cell and start the DNA-synthetic and mitogenic processes.

Several hormones require the presence of extracellular calcium in order to manifest their mitogenic potentialities in thymic and bone marrow cell

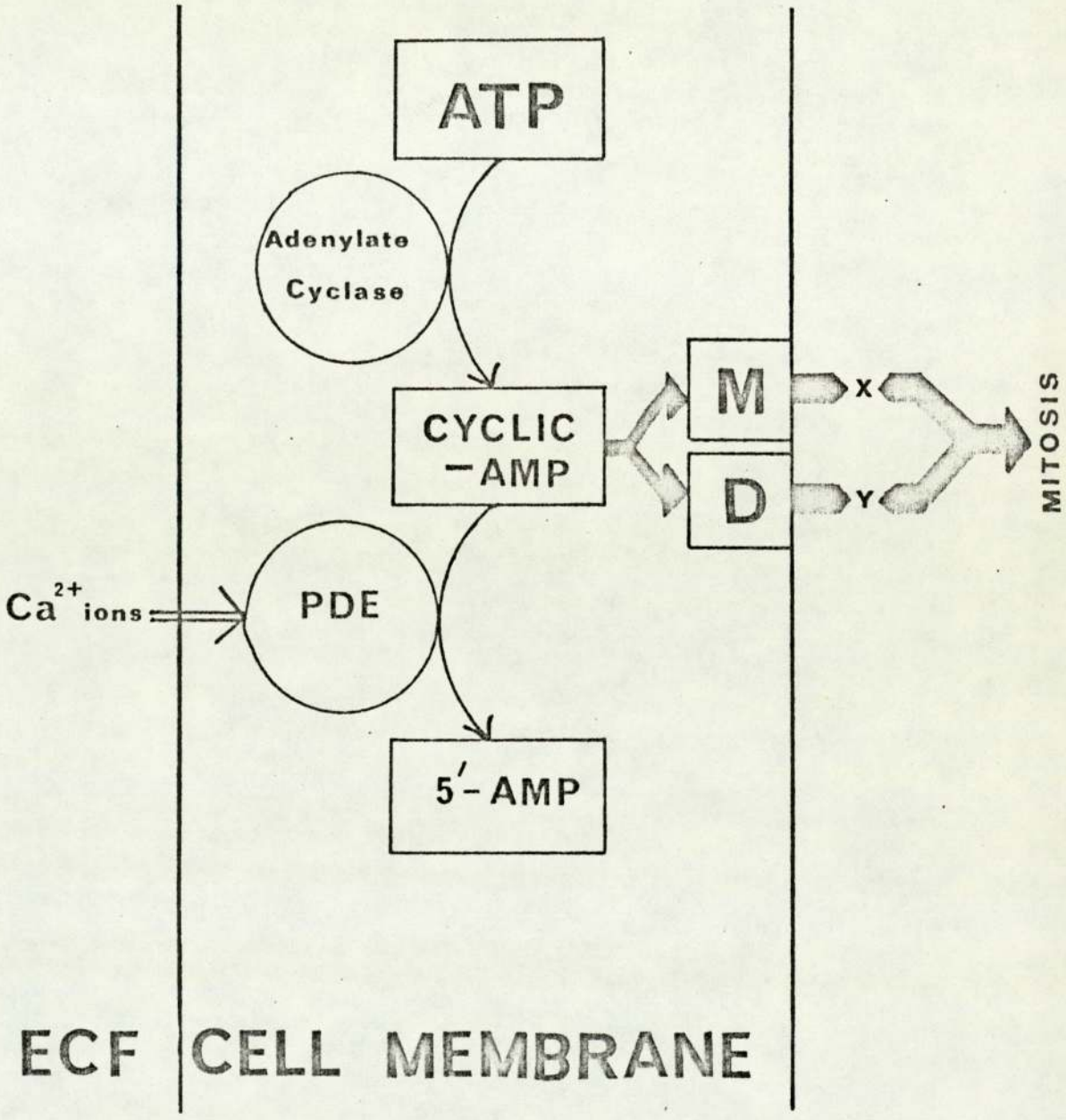


Fig. 6: Proposed mechanism for the mitogenic action of calcium in the extracellular fluid (ECF) upon thymic lymphoblasts. Exposure of the cell to calcium concentrations > 1.0mM inhibits the action of the membrane-bound phosphodiesterase (PDE) of cyclic adenosine 3'5' monophosphate (cyclic-AMP) which degrades cyclic-AMP to 5'-adenosine monophosphate (5'AMP). Intramembranal concentrations of cyclic-AMP, which is formed from ATP by the action of adenylate cyclase are thus elevated. These raised levels of cyclic-AMP stimulate the mitogenic (M) and the DNA-synthetic (D) components of a membrane activation site to produce initiators (X and Y respectively) which enter the cell and trigger the mitogenic and DNA-synthetic processes.

populations. This is true for PTH (Whitfield, Perris & Youdale 1969; Whitfield, MacManus, Youdale, & Franks 1971); growth hormone, neurohormones and prolactin (Whitfield, Perris & Youdale 1969; Whitfield, Rixon & Youdale 1969); cortisol (Whitfield, MacManus & Rixon 1970a; Whitfield, MacManus & Gilliam 1973a) and acetylcholine, histamine and insulin (Perris & Morgan 1975).

The action of cyclic-AMP on proliferative events in thymic and bone marrow cell populations is concentration dependent, the response at any one instant being governed by the extracellular calcium concentration. Thus at cyclic-AMP concentrations between 10^{-8} to 10^{-6} M a stimulatory response is observed (Rixon, Whitfield & MacManus 1970; Whitfield, MacManus & Gillan 1970; Whitfield, MacManus, Youdale & Franks 1971), whereas at concentrations around 10^{-4} an inhibitory response is observed (Abell & Monahan 1973; Whitfield, MacManus & Gillan 1973b). The inhibitory action of high concentrations of cyclic-AMP has been attributed (Whitfield, Rixon, MacManus & Balk 1973) to a reversible inhibition of the mitogenic process as a consequence of elevated intracellular calcium. Thus, depending on its own intracellular concentration and the extracellular calcium level, cyclic-AMP can be either a positive or a negative regulator of cell proliferation.

METHODS

Experimental Animals

Animals used were highly inbred Wistar rats obtained from Bantin & Kingman Ltd, Hull. They were maintained under constant temperature and natural 24-hour fluctuations of illumination in the Animal House, Department of Pharmacy, until required. The animals are normally asleep during the day and consume the majority of their dietary intake (Pilsbury's 41B - see Appendix for composition) during the night.

From their inherent nocturnal habit, it is obvious that any experiment performed during the day is likely to upset their endocrine balance to some extent, and induce a stress reaction. Nevertheless, the rat is an excellent animal to employ for these experiments because:

(i) inbreeding reduces animal variability

(ii) the size of the animal (and thus of its organs) readily permits the required surgery

(iii) at least 5mls of blood may be removed from the heart, for later biochemical analysis, without causing death

(iv) the rats are remarkably resistant to infection, as shown by Ingle & Griffith (1942); Lambert (1965).

1. Analysis of Plasma Constituents

1.1. Preparation and storage of plasma

The method of cardiac puncture was routinely adopted for blood sampling. This technique is far superior to others such as decapitation or by 'milking' the tail vein where there is a high probability of dilution by extravascular fluids. Only by cardiac puncture is one able to obtain a volume of blood up to 5mls; even though a small volume of 1ml would suffice for the biochemical requirements, if the experiment was terminal a larger volume was withdrawn to obtain clearer demarcation of the haematocrit following centrifugation.

Plasma was taken in preference to serum because:

(i) loss of the protein clotting factors in serum might affect the ionised calcium concentration.

(ii) complete formation of the blood clot requires several minutes which increases the possibility of haemolysis.

(iii) the volume of serum is not as great as that of plasma, which is an important practical consideration when dealing with small animals from which only limited volumes of blood may be obtained.

Although Hunt (1974) has shown that large concentrations of heparinised saline reduce the plasma ionised calcium values, the concentrations used in the present studies (50mg%) were insufficient to cause such a reduction. Thus blood was routinely withdrawn by cardiac puncture into 5ml heparinised syringes fitted with 21 gauge needles. After first removing the needle (to prevent haemolysis) the blood samples were slowly ejected into

glass centrifuge tubes, covered with a thin layer of light fraction liquid paraffin to prevent pH fluctuations affecting ionised calcium concentrations (Hunt 1974) centrifuged at 500g for 15 mins and then stored at 4°C until analysed.

1.2. Measurement of plasma total calcium

Plasma total calcium concentration was determined by the method of Copp, Cheney & Stokoe (1963), using an EEL Titrator. The principle of the method is that when calcium is added to an alkaline solution of murexide a colour change occurs, due to formation of a calcium-murexide complex. When ethylene diamine tetra-acetic acid (EDTA), a more powerful calcium chelating agent, is added via micrometer screw, this produces a gradual reversion to the original colour due to the liberation of free indicator.

4ml glass cuvettes equipped with a magnetic stirrer were employed for the calcium estimations; into each was placed 2.5ml of murexide solution, 0.2ml 1N NaOH, and 0.1ml calcium standard or plasma. The change in colour intensity following additions of EDTA was measured by a photo-electric cell attached to a galvanometer. All readings were made with the incorporation of the spectrum filter No.606 (Peak 5750-5800Å). Volume of EDTA added to the cuvette, by successive additions of lul aliquots, was plotted against the galvanometer reading. When finally two aliquots produced no further deflection, the intersect of a line drawn between these latter points and of the line joining the points where changing deflections did follow EDTA addition, represents the end point of the titration as shown in Fig.1.

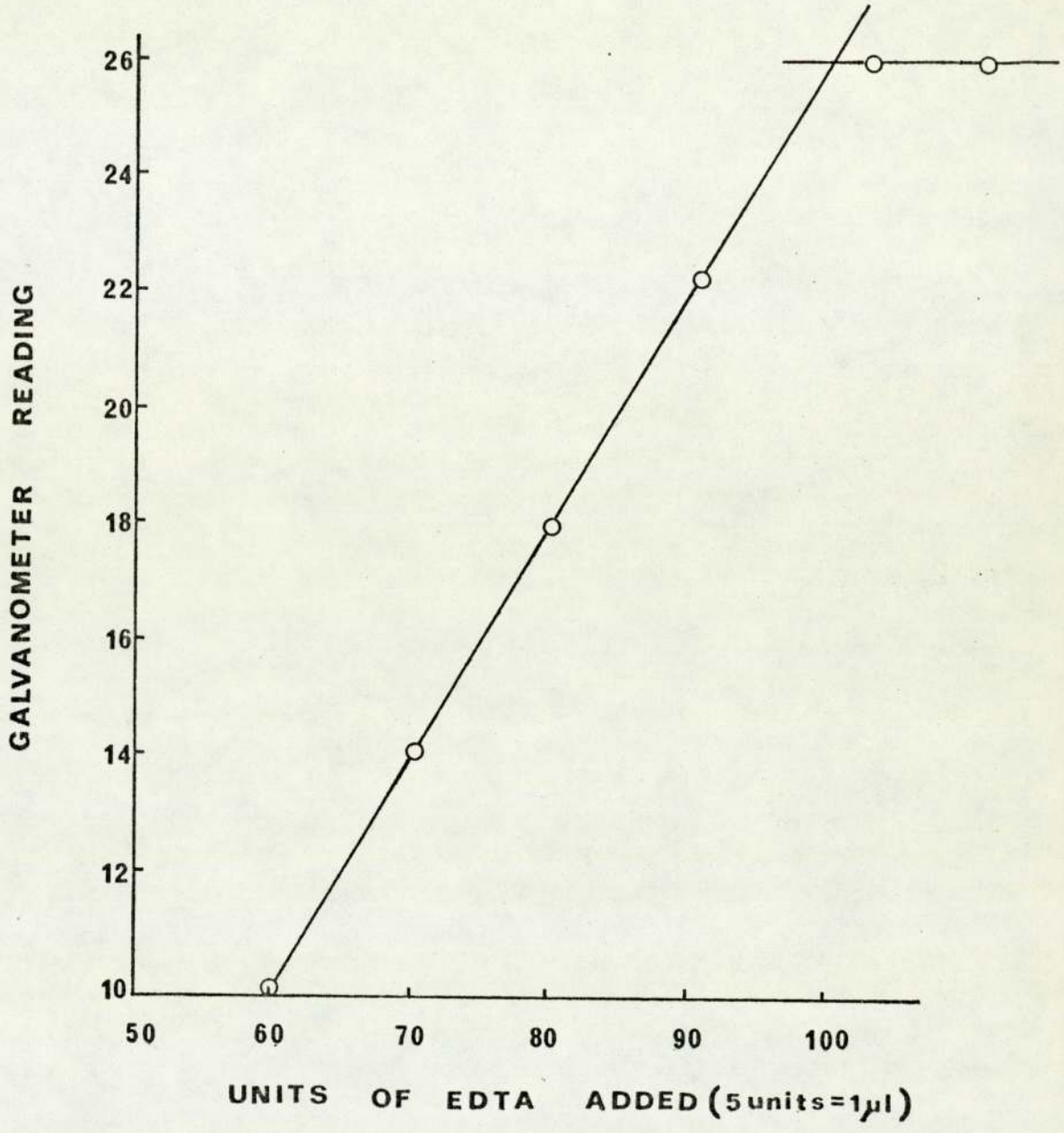


Fig. 1: Titrometric evaluation of plasma total calcium and conversion to mg%. Standard calcium solution containing 10mg% added to cuvette and typical graph constructed as indicated in text. The intersect of the two lines should approximate 100 EDTA units. Lines for unknown concentrations of calcium solution are likewise constructed. Plasma total calcium in mg% is determined relative to the standard.

i.e. Typical results:-

Units of EDTA of standard = 120

Units of EDTA of unknown = 113.5

Concentration of unknown = 113.5 x 100/120

= 94.5 = 94.5/10 = 9.45mg%

Calcium standard (concentration in mg%)	Units of EDTA titrated to end point
10	100 ± 1.2 (3)
9	86 ± 2.0 (3)
7	68 ± 0.8 (5)
5	48 ± 0.8 (5)
3	30 ± 1.1 (5)

Table 1: Relationship between the concentration of commercial calcium chloride standard (in mg%) and the units of EDTA titrated to reach the end point, shown as means ± s.e.m. Number of determinations for each standard shown in parentheses.

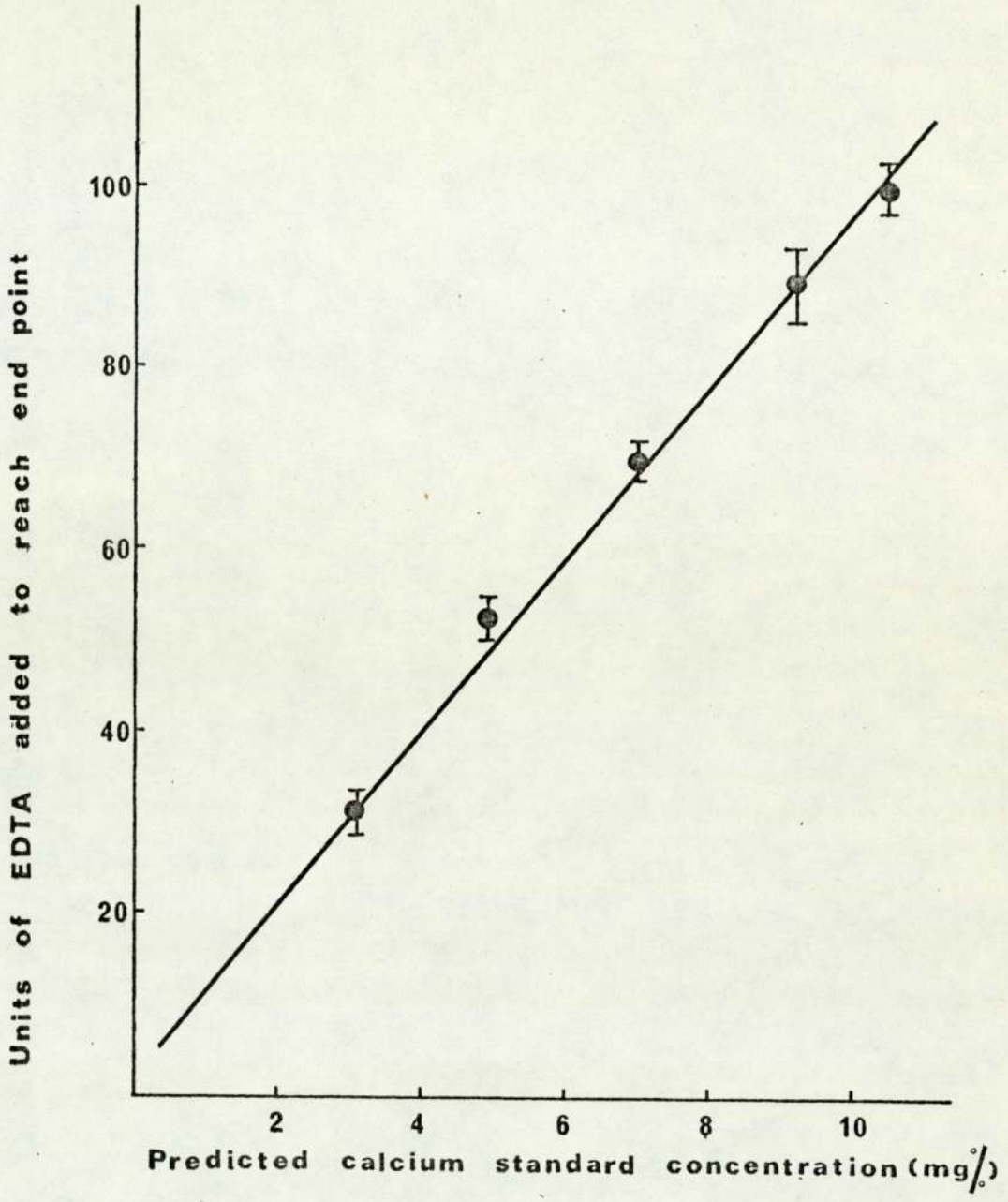


Fig. 2: Linearity between concentration of calcium in mg% (from accurate $CaCl_2$ solution for atomic absorption spectroscopy i.e. 1ml = 1.00mg Ca) and units of EDTA added to reach the end point of the titration. Each point represents the mean ± s.e.m. of 3-5 readings.

	Young Rats	Old Rats
Ether anaesthesia	9.89 ± 0.10 (10)	10.03 ± 0.05 (12)
Pentobarbitone (1hr duration)	9.87 ± 0.06 (9) (NS)	10.02 ± 0.06 (12) (NS)

Table 2: Influence of ether and pentobarbitone anaesthesia upon plasma total calcium in groups of young (200-250g) and old (375-450g) male rats. Values in mg% and expressed as means ± s.e.m. Number of animals in each group shown in parentheses. Significance compared between groups of the same age. NS, Not significant.

The validity of this method for assessing plasma total calcium concentrations over the required range of study was evaluated by employing a series of dilutions of accurate commercial calcium standard (BDH Ltd). These were titrated against EDTA and a direct linear relationship between calcium concentration and EDTA volume was obtained (Table 1; Fig.2). Test samples were thus routinely compared with a freshly prepared 10mg% calcium standard solution. Where up to 5 separate determinations were made on a given plasma sample, values were found to differ from each other by only 0.1mg per 100mls indicative of the accuracy and reproductability of the method.

In most experiments, ether was used as the anaesthetic, and in others due to the nature of the experiment (e.g. slow infusion into the jugular vein) a barbiturate (Nembutal) was used. Thus it was necessary to show if there was any significant influence of the anaesthetic upon total calcium concentration. Both ethanol and urethane have been shown to have a dramatic hypocalcaemic effect in rats (Peng & Cooper 1971; Peng, Cooper & Munson 1972). On the other hand ether and halothane were found not to be hypo calcaemic (Munson, Schwesinger, Cooper, Gray & Peng 1971). The results using a barbiturate show that even after one hour of anaesthesia no significant effect upon total calcium levels was apparent (Table 2).

1.3. Measurement of plasma ionised calcium

Calcium exists in plasma in three forms (Moore 1970). These are:

(i) non-diffusible, protein bound calcium (30-50% of

the total depending upon pH and temperature).

(ii) diffusible non-ionised calcium (5-15% of the total) in the form of complexes and chelates

(iii) ionised calcium.

It has long been established that the ionised form is the physiologically active species (Toribara, Terepka & Dewey 1957), and many important physiological processes are known to be solely dependent upon the calcium ion activity (see General Introduction and Introduction 3.5). The development of an electrode specific for the calcium ion has progressed from the unsatisfactory glass electrode (Truesdell & Christ 1967), through a dip-type ion-exchange electrode (Ross 1967) to the present flow through ion-exchange system developed specifically for the measurement of ionised calcium in plasma and serum (Moore 1970).

The apparatus used in this study was an Orion 99-20 flow through electrode coupled to an Orion 801 digital mV/pH meter. (Orion Research Inc. Mass. U.S.A.) The advantages of this specific electrode have been documented by Moore (1970). In brief they are:

(i) more rapid equilibration,

(ii) greater stability of potential,

(iii) smaller sample valume requirement,

(iv) anaerobic conditions for measurement

which maintains blood pH by preventing loss of CO_2.

The principle of the ion-exchange electrode is that a chemical exchange of calcium for another ion occurs at the interface of a test solution and an organic ion-exchange resin, thereby generating a potential. The electrode thus measures calcium ion activity and not calcium ion concentration. However, in dilute solutions of

calcium, such as in biological fluids, the activity of the ion is proportional to its concentration, and it is therefore possible to calibrate the electrode in terms of ionised calcium concentration (Moore 1970).

Standard solutions supplied by Orion containing 0.5, 1.0 and 2.0 x 10^{-3} M $CaCl_2$ in 1.5 x 10^{-1} M NaCl were used to calibrate the electrode. The standard containing 1.0 x 10^{-3} M $CaCl_2$ was allowed to flow slowly through the electrode system for an hour in order to obtain stable readings. Such readings were considered to be stable when the fluctuation was only $\pm$ 0.2mv.

Small quantities of trypsin and triethanolamine were initially added to the standard solutions. Triethanolamine was stated by Orion to retain membrane flexibility; the function of the trypsin was to clear possible deposits of plasma proteins which might precipitate on the membrane. Although addition of these substances causes a small error in electrode response (Li & Piechocki 1971; Lindgarde & Zettervall 1971; Schwartz, McConville & Christopherson 1971) several authors (Hattner, Johnson, Berstein, Wachman & Bracknam 1970; Li & Piechocki 1971; Lindgarde & Zettervall 1971) have claimed that both additives are necessary to preserve membrane stability.

The main disadvantage of this method is the short lifespan of the membrane, which is coupled with the expensive reagents required at each fresh membrane change. It was noticed that when trypsin was added to the standards, a white deposit was formed on the membrane, which might indeed have been the enzyme itself, and consequently reduced membrane life. Omission of trypsin was found to extend membrane life, with no loss of membrane stability, and was dispensed with in subsequent analyses.

Days of storage (4°C)	0	1	2	3	4
Plasma	10.35	10.20	9.70	9.55	9.50
total	10.00	9.90	9.73	9.45	9.30
calcium	10.35	10.25	10.00	10.05	9.80
(mg%)	10.38	10.20	10.20	10.30	10.20
	10.22	10.20	10.01	10.20	10.00
Plasma	5.20	4.10	4.20	3.72	3.61
ionised	5.40	4.00	3.76	3.20	3.72
calcium	5.10	4.30	4.30	4.00	3.60
(mg%)	5.60	4.40	4.40	4.30	3.60
	5.31	4.18	4.20	3.98	3.80

Table 3: Influence of storage (4°C) on oil-covered plasma in contact with the red blood cells upon plasma total and ionised calcium values, studied for up to 4 days following removal by cardiac puncture. Each value is the mean of 3 individual results.

The repeatability of this method was found to be good, if all readings were taken at a fixed time (1 min) after introduction of the sample.

A number of blood samples were taken by cardiac puncture from male rats, covered with a thin layer of light fraction paraffin oil, centrifuged, and stored at 4°C. Total and ionised calcium were measured daily, to show the effect of storage under these conditions. As shown in Table 3, the total calcium remains constant for at least three days, but the ionised calcium falls rapidly, and must be measured on the day of sampling.

1.4. Measurement of plasma total magnesium concentration

Plasma total magnesium concentration was measured by atomic absorption flame spectro-photometry. Plasma samples were diluted twenty-fold and deproteinised with trichloroacetic acid in the presence of 2×10^3 p.p.m. strontium chloride; strontium reduces the interference of other ions, particularly phosphate in solution (Murdoch & Heaton 1968). After centrifugation at 350g for 5 minutes the supernatent was analysed in a Unicam SP900A atomic absorption flame spectrophotometer. The flame was an acetylene (10.5 lb/sq. in.)/air (27 lb/sq.in.) mixture. Wavelength was 285.2 mμ, slit width 1.0mm and a lamp current of 8mA.

At least an hour was set aside for the machine to attain equilibrium and the background electrical noise to reduce to negligible levels, during which time distilled water was flushed through the tubing. Samples were then read in comparison with a blank solution and magnesium standards prepared from a commercial standard (BDH Ltd). Strontium and trichloroacetic acid were

added to these solutions in the same proportion as to the plasma samples.

The absorbance for any given standard or sample showed remarkable consistancy, the variation being no greater than $\pm$ 1 unit. The method was thus considered to be accurate and reliable for the estimation of plasma magnesium concentration.

1.5. Measurement of plasma inorganic phosphorus

The method of Chan, Toribara & Warner (1956) was employed and although this is often stated to measure plasma phosphate concentration, it actually measures inorganic phosphorus. Thus in this study the term phosphate is used with the assumption that essentially all inorganic phosphorus is present in plasma as phosphate.

After deproteinisation with trichloracetic acid, a sample of supernatent was incubated at 37°C for 1½ hrs, with an acidic ammonium molybdate solution, to allow the blue colour to develop fully. Standard solutions, prepared from a stock solution of potassium dihydrogen orthophosphate, were treated in a similar manner.

Following the incubation period, absortance was read against a blank at 820 mμ in a Unicam SP500 spectrophotometer. The repeatability and reproductability of individual plasma samples was determined, and since very little variability was found the method was thus considered to be accurate and reliable for the estimation of plasma inorganic phorphorus concentration.

1.6. Measurement of plasma glucose concentration

Plasma glucose was determined by the glucose oxidase method, employing a Beckman Glucose Analyser (Stevens 1971). A critical survey of this method has been documented by Morrison, Scotland & Fleck (1972).

1.7. Measurement of plasma insulin concentration

Plasma insulin was assayed by a modification of the double antibody method of radioimmuno assay (Hales & Randle 1963), using the kit supplied by the Radiochemical Centre, Amersham. Human insulin was used as the standard, obtained from Wellcome.

2. Estimation of Mitotic Activity

2.1. General Considerations

The presence of mitotic figures in histological sections of organs and tissues of the body indicates that cell proliferation is occuring. At least three methods have been used in this study to visualise cell proliferation, each having limitations and disadvantages. These have been reviewed in detail by Leblond & Walker (1956) and Cameron (1971); brief consideration of the salient points will be discussed here with reference to:

(i) mitotic index;

(ii) metaphase arresting agents; and

(iii) tritiated thymidine (^{3}H-tdR).

(i) Mitotic index

Probably the simplest method of estimating the mitotic activity of a tissue is to make an assessment of its mitotic index, since this involves no exogenous pertubation that may interfere with the cell cycle. The mitotic index is defined as the percentage of cells in the population which is in some stage of mitosis at any one time. This normally encompasses all the cells in prophase, metaphase, anaphase and telophase. However, in these cell populations, very early prophase figures were not readily distinguishable from the non-dividing cells. The mitotic index in this study therefore encompasses the percentage of nucleated cells which are in any stage of mitosis from late prophase to the end of telophase.

Even though mitotic index may represent the simplest approach, it is the least informative since no measurement of time is involved, and therefore the rate at which the cells of a tissue are dividing cannot be calculated, An equally important disadvantage is the fact that the mitotic index is low for most tissues (in the bone marrow this represents about 1%) and requires considerable numbers of cells to be counted to obtain statistically significant results. In thymic and bone marrow cell polulations it has been estimated that about 2000 cells need to be counted in a given population.

An increase in the mitotic index of these cell populations could be interpreted in various ways.

(i) There may be the destruction or extrusion of mitotically incompetent non cycling cells from within the tissue. This would artificially enrich the percentage of the remaining mitotically competent nucleated cells which were undergoing mitosis, without affecting the absolute numbers of cycling cells. It would seem unlikely that cell loss of this nature would be responsible for observed increases in mitotic index. Indeed, it has previously been shown that increases in bone marrow mitotic index following calcium ion injections are not associated with any reduction in the numbers of non-dividing cells (Perris & Whitfield 1971). Furthermore, a number of hormones and ions which increase bone marrow and thymus mitotic index are also active <u>in vitro</u> (Perris 1971; Whitfield, Rixon, MacManus and Balk 1973) which would preclude the possibility of nucleated cell extrusion, though not of destruction. Thus a decrease in non-dividing nucleated cells without a concomitant decrease in cells undergoing mitosis seems an unlikely explanation for an increased mitotic index.

(ii) It may be that some pertubation of the cell cycle is the underlying mechanism responsible for an elevation of the mitotic index. Thus, there could be a prolongation of the mitotic (M) phase without a corresponding increase in the entire cell cycle time. If this was the case, the porportion of the cell cycle occupied by dividing cells would be increased, and although the rate of entry of cells into M would be normal, their extended duration in this phase would lead to an increase in mitotic index which would be wrongly interpreted as an increased mitotic activity. In all experiments where mitotic index was measured, parallel measurements of the true rate of entry of cells into M using colcemid metaphase accumulation would preclude such erroneous interpretations.

On the other hand, a shortening of the overall cell cycle time without a decrease in M would also lead to an increased proportion of cycling cells in M at a given time. Such a situation would truly represent an increase in the rate of production of new cells. It has previously been stated (Introduction 1.2) that it is the G_1 phase of the cell cycle that shows most variability, and the G_2 and S periods remain relatively constant under most conditions (Cameron 1970). In a number of rapidly dividing tissues (Cameron 1970) the G_1 phase constitutes about 10-15% of the total cell cycle time. Thus large decreases in the duration of the G_1 would have to be postulated to account for the observed variability in the mitotic index. It would seem unlikely therefore that a change of the overall cell cycle time, without a concomitant change in M is the factor responsible for the mitotic index changes observed in this study.

(iii) Resting G^{o} cells may be recruited into the actively dividing cell population, while the cell cycle time remains constant. Thus at any instant of time an increased percentage of the total nucleated cell population would be engaged in proliferative activity. This is the most feasible explanation for an elevation of the mitotic index.

(ii) Metaphase arresting agents

A number of compounds have been used to arrest dividing cells at metaphase in the cell cycle (Gelfant 1963; Tannock 1967). In essence, these so called mitotic spindle poisons, or stathmokinetic agents are relatively specific in their action by preventing the flow of dividing cells past metaphase. The low number of dividing cells observed by the mitotic index method is overcome since with time, the dividing cells enter the mitotic phase of the cell cycle and accumulate here. Spindle poisons are generally allowed to act for 3-6 hrs. In consequence, therefore, the percentage of dividing cells in a given cell population is much increased as compared to that observed by mitotic index.

An effective stathmokinetic agent should possess the following characteristics:

(i) There must be an optimum dose at which it arrests all metaphases in the tissue over a certain time period.

(ii) The arrested metaphases should not degenerate into an unrecognisable state before the tissues are fixed and examined.

(iii) The agent itself does not disrupt the normal entry of cells into mitosis. Thus, if the number of prophases stay constant while the percentage of metaphases increase linearly, one

may reasonably assume that no detrimental effect has occurred prior to spindle disruption.

Colchicine has been used as an investigative tool in problems concerning cell proliferation since the early 1930's. Some of the most comprehensive studies on the qualitative and quantitative effects of this drug in causing the arrest in metaphase of cells undergoing division were made by Brues (1936); Ludford (1936); Eigsti & Dustin (1955). These workers showed that colchicine acted only to arrest cells in metaphase and did not interfere with the normal rate of entry of cells into mitosis. Furthermore, these effects were achieved with extremely low doses, in the order of 1mg/kg. A smaller dose will arrest only a fraction of the cells entering metaphase. Conversely, when the dose of colchicine is too high, disintegration of the dividing cells is produced. This effect increases with time (Ludford 1945) and in addition, high doses of colchicine cause a depression of the rate of DNA synthesis (Fitzgerald & Brehaut 1970).

A derivative of colchicine, N-desacetyl-N-methylcolchicine (Colcemid, Ciba Ltd) is also widely used. It is reportedly less toxic (Schar, Loustalot & Gross 1954) and more effective than colchicine (Meier, Schar & Neipp 1954). The depression of the rate of DNA synthesis is less marked with an equivalent dose of Colcemid (Hell & Cox 1963). For these reasons, colcemid is preferred for <u>in vivo</u> work.

The appearance of colcemid-arrested metaphases ('C' metaphases) is readily distinguishable since the chromosome pairs fail to separate at the equatorial plate, and cytologically appear as a highly concentrated mass of densely staining material, devoid of a

nuclear membrane (see Plates 3 & 4). Thus, it is possible to score the percentage of metaphase-arrested cells within a population after a lapse of x hrs following the colcemid administration. When comparing various treatments the variations in colcemid-metaphase accumulation may reasonably be interpreted as a direct fluctuation of the mitotic activity of that tissue.

(iii) Tritiated-thymidine

An alternative way of studying cell proliferation is by the use of isotope precursors that label the nuclear DNA. In contrast to colcemid, which acts at the M phase of the cell cycle, the current isotopic labels ^{14}C- or ^{3}H- thymidine, have their effect specifically in the S phase. Apart from the far greater counting efficiency obtained with ^{14}C compounds, tritiated compounds are often employed since they are much cheaper and less hazardous.

Thus when animals are given a solution of tritiated thymidine (^{3}H-tdR), the label is rapidly distributed throughout the body and specifically incorporated into the DNA of those cells which are replicating their nuclear DNA in preparation for cell division. This single pulse label is specific for these cells only, the remainder of the ^{3}H-tdR which is not immediately used in the synthesis of DNA is rapidly catabolized. This process is independent of the route of administration whether it be oral, s.c. or i.p. injection, the labelled DNA being stable until that cell dies.

A number of assumptions have to be made concerning the specificity of ^{3}H-tdR as well as the stability of nuclear DNA. These have been dealt with in great detail by Cleaver (1967).

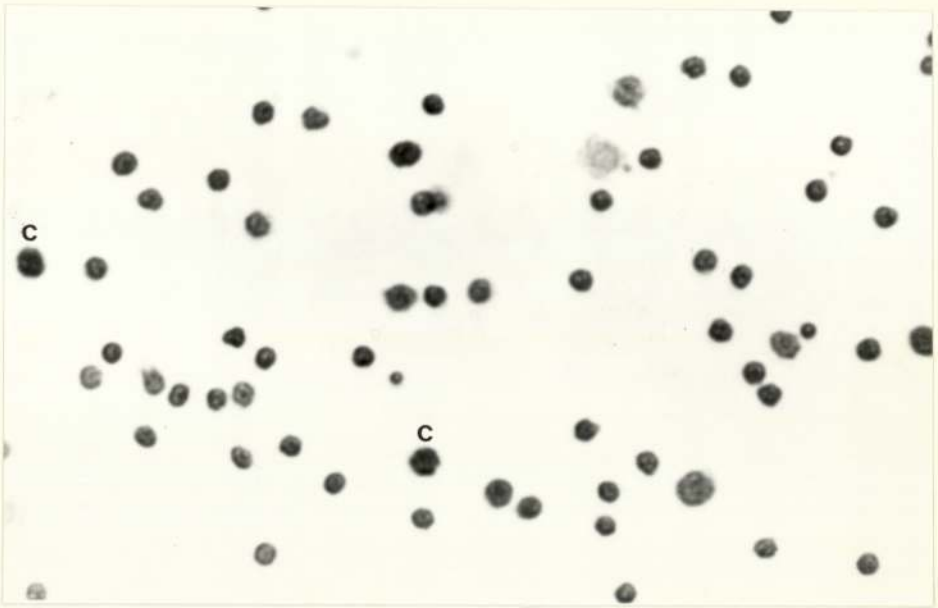


Plate 3: Typical appearance of cells from the thymus arrested at metaphase with colcemid; such densely staining cells are labelled (c).

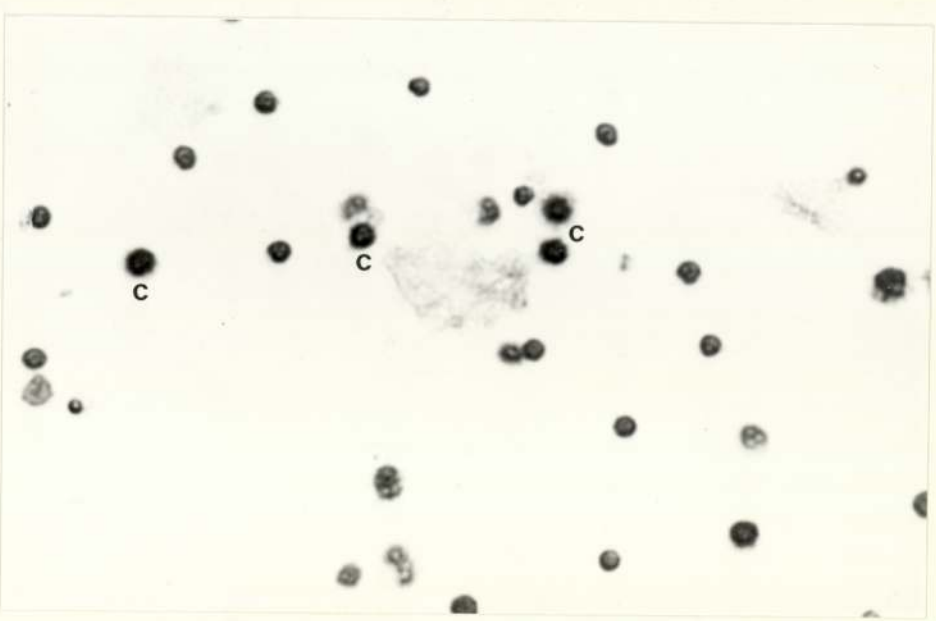


Plate 4: Typical appearance of cells from the bone marrow arrested at metaphase with colcemid; such densely staining cells are labelled (c).

Briefly, these are:

(i) Cells which replicate DNA proceed through the cell cycle and eventually divide.

(ii) The amount of ^{3}H-tdR used as a tracer does not alter the normal proliferation kinetics, and is not toxic to the animal.

(iii) Every cell which is synthesising DNA during the time the animal is given the ^{3}H-tdR is labelled.

(iv) The method of ^{3}H-tdR administration does not alter the cell proliferation and renewal characteristics of the animal.

Although high doses of ^{3}H-tdR can cause deleterious radiation damage, when tracer doses are employed (usually not greater than 100µc /100g), ^{3}H-tdR is a very useful tool in studying cell proliferation in rapidly dividing tissues. Indeed, one advantage of the use of ^{3}H-tdR is the rapidity by which results may be obtained.

If the above technique is coupled with liquid scintillation counting, this procedure suffers from an inherent disadvantage. Results are expressed as disintigrations per minute (d.p.m.) and does not quantify the number of dividing cells. However, if it is first demonstrated for the tissue concerned, that there is a direct relationship between DNA content and the d.p.m.'s, then it may reasonably be inferred that an increase in the d.p.m. per µg of DNA is a consequence of an increased incorporation of the thymidine label into newly synthesised DNA. In tissues where the cells readily lend themselves to microscopic examination, this assumption may be overcome by the use of autoradiography. However, if autoradiography were employed in the intestine, this would have to be coupled to microtomal sectioning, which has its own disadvantages which will be discussed subsequently.

2.2. Methodology Employed for Bone Marrow and Thymus Cell Preparations

a) in vivo

The mitotic index of the above tissues was assessed after rapid removal of the thymus gland and the right femur from anaesthetised rats. The thymus gland was washed in 0.9% saline, and then the tissue dispersed in 3mls of saline by mincing with curved scissors. This suspension was squeezed through four layers of moistened cheesecloth into a further 2mls of saline. One drop of this solution was mixed evenly with two drops of calf serum, (Wellcome Reagents Ltd). The slides were dried in a current of warm air at 37°C, fixed, and stained with Delafields Haematoxylin, as described by Whitfield, Brohee & Youdale (1964b). After extracting the plug of bone marrow cells, the cells were dispersed in 2mls of saline. Since cells undergoing division may be more fragile than non-dividing cells (Lala, Maloney & Pratt 1964) this was achieved by gentle application of a Pasteur pipette. Two drops of this suspension were mixed with one drop of calf serum, and the slides treated as for the thymus preparations.

The characteristic appearance of late prophase, metaphase, anaphase and telophase are shown in Plates 1 & 2. With each slide, the percentage of dividing cells within the entire population was estimated, by at least 1000 cells being counted by two independent workers. All the estimations were performed within a given time period, usually between 10.00 a.m. and 11.00 a.m. to counteract variation due to the circadian rhythm in these tissues (Hunt & Perris 1974).

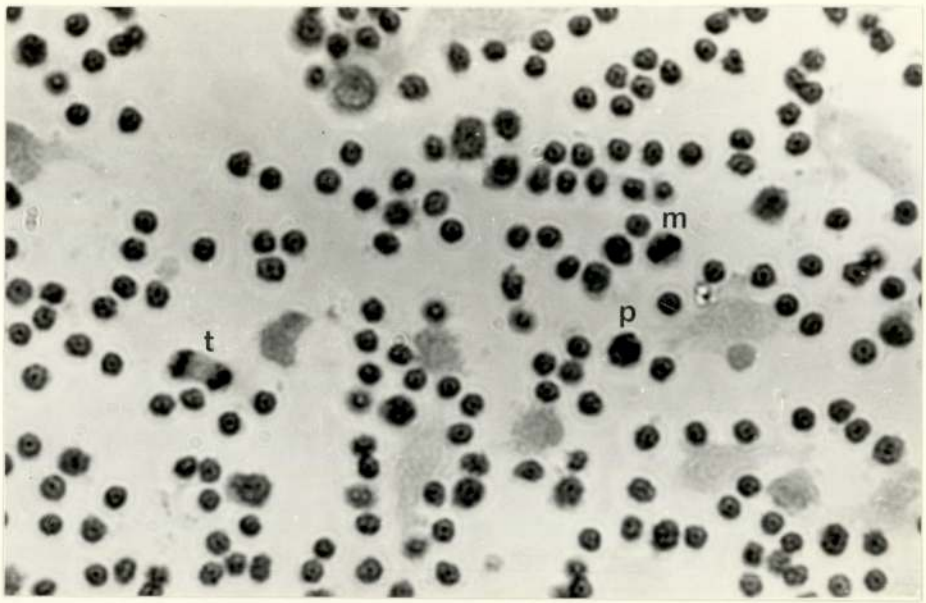


Plate 1: Typical appearance of dividing cells from the thymus gland, stained with Delafield's Haematoxylin. p (prophase); m (metaphase); t (telophase).

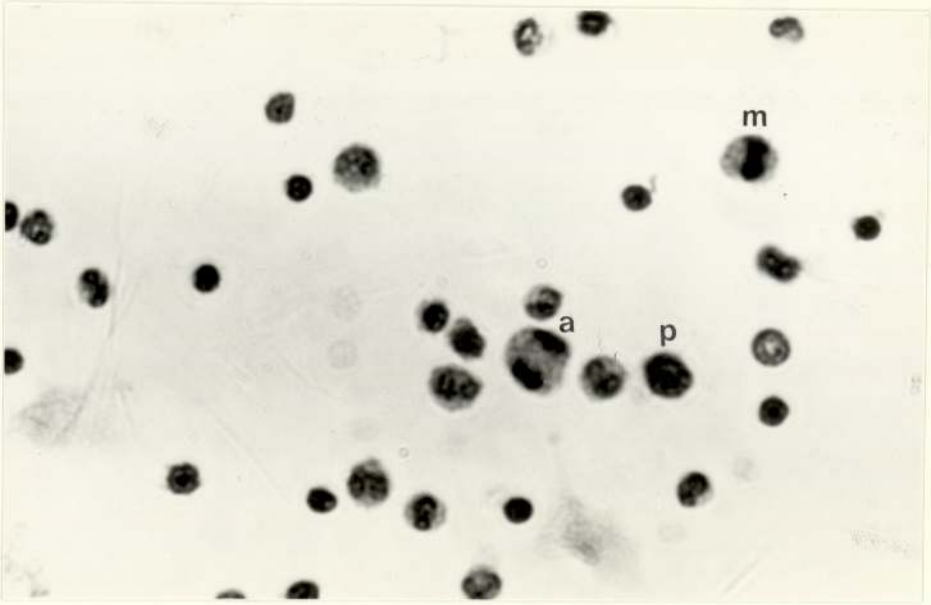


Plate 2: Typical appearance of dividing cells from the bone marrow, stained with Delafield's Haematoxylin. p (prophase); m (metaphase); a (anaphase).

In in vivo experiments employing a metaphase arresting agent, colcemid was chosen for the reasons discussed earlier. Since the closely related alkaloid colchicine at a dose of 0.2 mg/100g has been shown to depress plasma calcium concentrations in young rats within 4-6 hrs after injection (Rixon 1968; Heath, Palmer & Aurbach 1972) as a result of inhibiting bone resorption, careful consideration had to be given to the dose employed within the time that the drug was being studied. The concentration of colcemid used was 0.4 mg/ml 0.9% NaCl. Over a 4 hr period, one i.p. injection of 0.2 mg/100g body weight administered at time zero has been shown in preliminary experiments to produce complete metaphase arrest in bone marrow and thymus cells, and this dose also has no influence upon total plasma calcium concentration (Hunt & Perris 1974). If two i.p. injections administered at zero and 2½ hrs (Perris, Whitfield & Rixon 1967) over a 6 hr period of study, a slight but significant decrease in plasma calcium is observed (Hunt 1974). Regardless of whether the hypocalcaemic action of colcemid significantly affected the flow of cells into metaphase, the use of the alkaloid over a 6 hr period was not invalidated as a means of comparing mitotic activity, since the effect would be manifest in both control and test animals.

It has been shown in vivo that over a 4 or 6 hr period that there is a linear progression of bone marrow and thymic cells into mitosis and hence into the arrested metaphase configuration, in the presence of colcemid (Perris, Whitfield & Rixon 1967). Thus by scoring the percentage of cells which were arrested in the colchicine-metaphase configuration over a 4hr or 6hr period, it is possible to compare the effect of various treatments upon

the level of mitotic activity in these tissues. At least 1000 cells were counted by two independent workers.

The in vivo treatments that the animals were subjected to were mainly those involved with an alteration in the extracellular calcium concentration. A decrease in extracellular calcium was achieved by parathyroidectomy (Section 3.5), whereas a temporary elevation of extracellular calcium was obtained by injection of a 62.5 mmol/l $CaCl_2$ solution. Rats (weighing approximately 200g) received a series of 1ml i.p. injections at 0, $\frac{1}{2}$, 1, 2, 3, 4 and 5 hrs after the start of the Colcemid treatment. To elevate plasma magnesium, an identical routine was followed, as for calcium.

b) in vitro

In some experimental situations, it was necessary to isolate thymic lymphocytes to understand more fully the division process in these cells. The thymus gland from one or more rats was removed from the body, placed in a petri dish containing a few mls of balanced glucose salts (BGS) medium, and rapidly dispersed as described above. After squeezing this suspension through four layers of moistened cheesecloth, an aliquot was added to the incubation tube, in the presence of 0.062 mM colchicine, and the agent being studied for its mitogenicity. The calcium concentration, for example, could be altered by appropriate additions from a concentrated stock solution. The basic composition of the BGS was 5.5 mM-glucose, 5.0 mM-KCl, 0.6 mM $CaCl_2$, 1.0 mM-$MgSO_4$, 5.0 mM-Na_2HPO_4, 120 mM-NaCl and 5.0 mM-Tris (hydroxymethyl)amino methane buffer (Whitfield, Perris & Youdale 1968, 1969). The pH was adjusted to 7.2 with HCl.

Thus in the presence of colchicine, cells continue to flow into mitosis over a 6hr incubation period, and accumulate at metaphase as they do *in vivo* (Whitfield, Brohee & Youdale 1964a; Perris, Whitfield & Rixon 1967; Whitfield, Perris & Youdale 1969). At the end of a 6hr incubation period at 37°C, samples were taken from the suspensions, mixed with a small volume of calf serum on a slide, dried, fixed, stained and scored as described previously.

2.3. Methodology employed for assessment of mitotic activity in the small intestine

All the work on the small intestine was restricted to a specific area (the duodenum), to ensure minimal variation between animals. It is also the most rapidly dividing part of the small intestine (Clarke 1971) and thus any mitotic change would be more readily detectable. All of the animals used to study the intestinal responses were males, since earlier workers had implied a fluctuation of mitosis throughout oestrous in the skin (Bullough 1950; Ebling 1954), a fluctuation was shown in bone marrow and thymus gland (see Results, Part 1), and might also exist in the intestine of females.

a) Colcemid

Colcemid has been used *in vivo* for the study of bone marrow and thymic cell population kinetics with much success. The use of colchicine for the measurement of mitotic rate in the intestinal epithelium was first looked at critically by Stevens Hooper in 1961. She concluded that there was a linear accumulation of cells into the colchicine metaphase configuration over a 3hr period, the drug did not interfere with the progression of

cells into mitosis, and was thus a valid approach to study cell proliferation in the small intestine.

Since there is a circadian variation of mitotic activity in the intestine (Sigdestad, Bauman & Lesher 1969; Scheving, Burns & Pauly 1972), colcemid is preferred to mitotic index and division assessed over a fixed time period. The other major reason that led to the justification of using colcemid instead of mitotic index was the fact that the percentage of arrested cells in the presence of colcemid is much greater than in its absence. For example the mean mitotic index of rat ileal epithelium is 3.32%; in the presence of colchicine for 6hrs this becomes 17.6% (Leblond & Stevens 1948), a figure far less susceptible to counting errors.

Colcemid was administered at the usual concentration at a dose of 0.2 mg/100g at time zero and 2½ hrs. This dose had previously been shown to cause complete metaphase arrest in all intestinal crypts (Clarke 1971). Using this regime, a linear accumulation of arrested metaphases was demonstrated up to 4hrs, but by 6hrs little additional increase was observed, (see Results section). Thus all treatments performed were assessed by comparing the colcemid accumulation over a 4hr period.

Following the relevant in vivo treatment, the abdomen was opened, the duodenum removed, and the intestinal contents washed away by gentle flushing with isotonic saline. The intestinal segments were fixed in Bouins fluid; for the first 12hrs of fixation the segments were pinned out on fixative-covered wax trays to prevent excessive curling of the tissue. The prepared tissue was finally embedded in paraffin wax, and subsequently sections cut at 7μ (Leblond & Stevens 1948; McMinn 1954;

Bertalanffy 1960), and stained with haematoxylin and eosin. Each section that was used for counting was separated from the last by at least 100u of tissue to avoid counting the same mitotic figures twice (Lesher, Walburg & Sacher 1964). Crypts were carefully selected and only those where the entire tissue from the base of the crypt to the tip of the villus was clearly visible were used. Some initial preparations were sectioned at 3μ; however irrespective of microtome thickness the major difficulty encountered was elucidating precisely the position of the crypt-villus junction. Since this would obviously lead to a possible source of error if results were expressed as a percentage of arrested metaphases/crypt, it was decided to express the results as the number of colcemid metaphases/crypt (Clarke 1970a).

b) Tritiated thymidine (^{3}H-tdR)

^{3}H-tdR was employed on the small intestine to complement the results obtained by histology. Indeed, since the ^{3}H-tdR approach does not visualise cells, if the two methods reflected comparable results, then it could be reasonably assumed that the ^{3}H-tdR counts were in fact originating from dividing crypt epithelial cells, and not some other diverse cell population such as the lymphocytes of the lamina propria.

Cellular proliferation in the intestinal mucosa was assessed by determining the rate of thymidine incorporation into DNA. An aqueous solution of thymidine-6-H_3 having an activity of greater than 20c/mM (Radiochemical Centre, Amersham) was administered at a dose of 3μc/100g body weight by a single i.p. injection. One hour later, the animals were sacrificed and the duodenum (6cm

length) quickly removed and washed in iced 0.9% NaCl. The intestinal wall was slit lengthwise and opened, mucosa uppermost, on to a glass plate. The mucosa was then separated from the underlying muscle layers, and homogenised in 3mls of 7% ice cold perchloric acid (PCA). A 1ml sample was taken, centrifuged, and the resultant pellet washed three times with 7% ice cold PCA to remove any unincorporated isotope. The precipitate was then resuspended in 3mls of 7% PCA and heated at 80°C for 30 mins to extract the DNA. An aliquot (1ml) of the solublized DNA was counted on a Beckman Scintillation Counter employing NE 260 Scintillator (Nuclear Enterprises Ltd, Sighthill, Edinburgh). Total DNA was estimated according to Dische (1955). A further aliquot of solubilized DNA (1.5ml) was added to 3.0ml of Dische reagent (1g diphenylamine; 98ml glacial acetic acid and 2ml Conc. H_2SO_4), heated for 10 mins in boiling water, cooled, and the absorbance read at 595mμ and 650mμ on a Unicam SP600 spectrophotometer. DNA content was calculated using the formula:

Optical density (O.D.) = (O.D.595-Blank) - (O.D.650-Blank)

and the results subsequently expressed as d.p.m. per μg of total DNA.

c) Estimation of intestinal surface area and surface/volume ratio

In order to quantify which group of cells in the intestine were responsible for the increased mucosa:muscle ratio of the Streptozotocin-treated animals, an estimate was made of the surface area and surface:volume ratio. A modification of the method of Dunnill & Whitehead (1972) was employed by projecting histological sections on to a square template comprising of four hundred $\frac{1}{2}$" squares. The

number of times the horizontal and vertical axes cut the muscle layer was termed M. The number of times these axes intersected any part of the villus was termed E. Surface area was thus expressed as $^{E}/_{M}$. The number of times that the intersection of the horizontal with the vertical axis was found in the villous tissue was termed L. Surface:volume ratio was thus expressed as $^{E}/_{L}$.

3. Surgery and Related Procedures

3.1. Vaginal smears

Young adult females show a regular 4-day oestrous cycle (Long & Evans 1922) characterised by fluctuating levels of circulating oestrogens and progestogens (Butcher, Collins & Fugo 1974). The concentration of these circulating sex steroids is reflected in the cellular organisation in the target tissues (e.g. the vaginal epithelium). Four phases are characterised in the rat oestrous cycle:

phase of oestrous	vaginal architecture	duration (hr)
(i) pro-oestrus	dominated by nucleated epithelia, singly or in sheets	16
(ii) oestrus	cornified epithelium only	32
(iii) metoestrus	few cornified cells together with many leucocytes	24
(iv) dioestrus	predominantly leucocytes with few nucleated epithelia	24

The stage of the oestrous cycle was determined by the vaginal smear technique of Zarrow, Yochim & McCarthy (1964). In addition the dried smears were covered with Leishman's stain and immediately drained vertically. This resulted in characteristic staining and unquestionable recognition of the leucocytes and the nucleated epithelial cells (see Plate 5-8).

3.2. Induction of chronic hyperglycaemia

In some rats an experimentally induced hyperglycaemic state was achieved with the drug Streptozotocin (Upjohn Co, Kalamazoo,

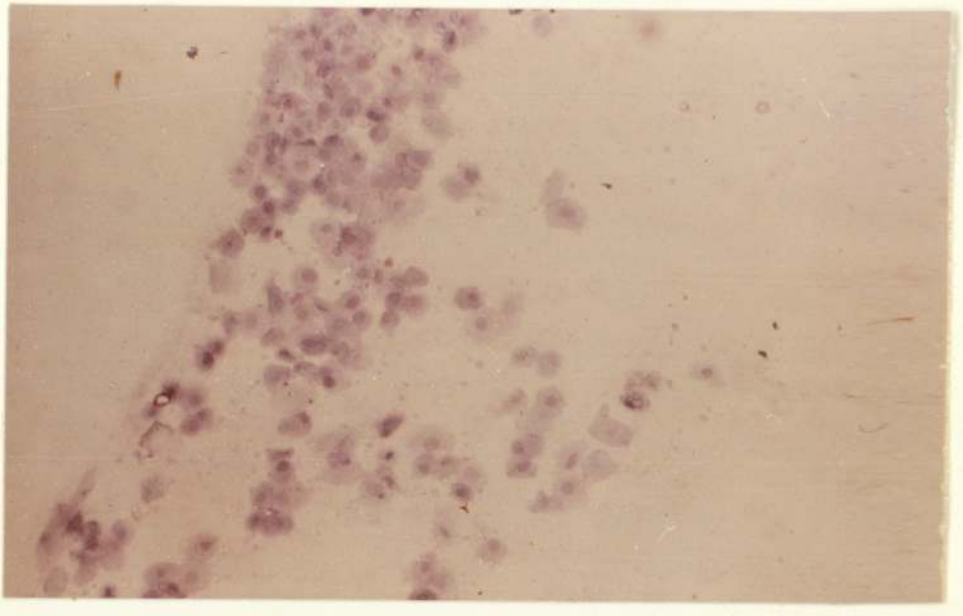


Plate 5: Appearance of vaginal epithelial cells (pro-oestrus). Predominantly nucleated cells. Stained with Leishman's.

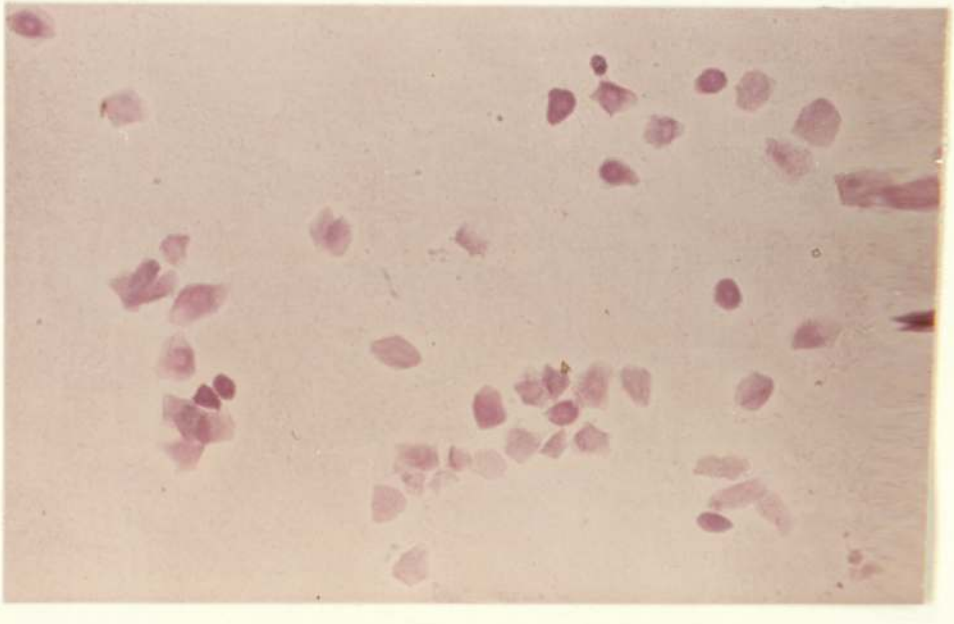


Plate 6: Appearance of vaginal epithelial cells (oestrus). Cornified cells only. Stained with Leishman's.

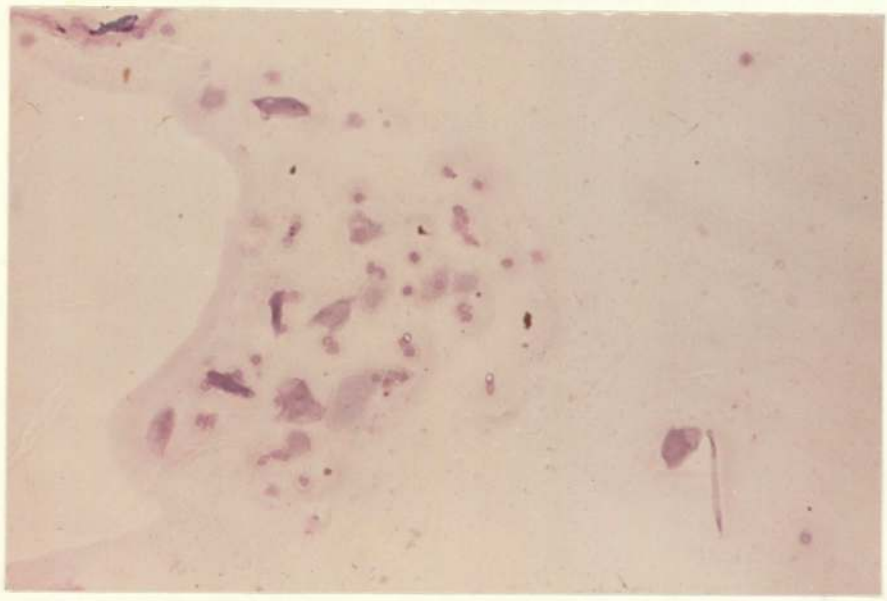


Plate 7: Appearance of vaginal epithelial cells (metoestrus). Few cornified cells and many leucocytes. Stained with Leishman's

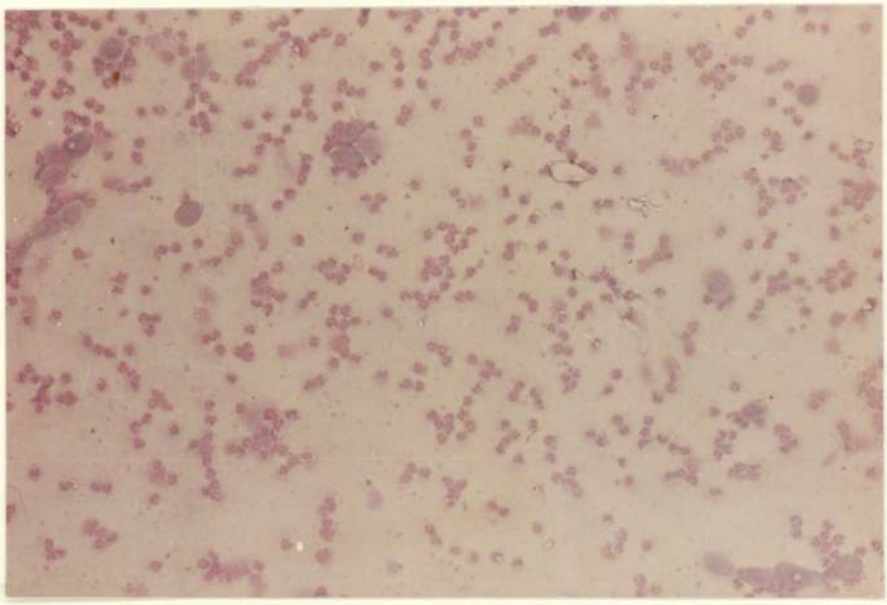


Plate 8: Appearance of vaginal epithelial cells (dioestrus). Predominantly leucocytes with few nucleated cells. Stained with Leishman's.

Mich., U.S.A.) This was chosen, since the older drug, alloxan, has been shown to influence calcium metabolism (Wang, Rogler & Doolittle 1973), has fiarly toxic side effects and the success rate in destroying the pancreatic β-cells is variable.

Streptozotocin produces its β-cytotoxic effects more consistently (Caspary 1973) and does not appear to influence calcium metabolism (see Results - Part 2). The drug was given in a minimal volume of 0.1M citrate buffer pH 4.5; the dose was 65mg/kg, dissolved rapidly (within 30 seconds) and administered by i.p. injection (Junod, Lambert, Stauffacher & Renold 1969).

3.3. Administration of Vitamin D3

Even though a physiologic dose of Vitamin D3 in the rat is 10-I.U. (Stohs & De Luca 1967), and the weekly requirement for the normal physiological development of a 100-150g rat is 100-200 I.U. (Carlsson & Lindquist 1955; Steenbock & Herting 1955) most workers (Avioli, Scott, Lee & De Luca 1969; Martin & De Luca 1969; Birge & Alpers 1973) have administered either 100 or 500 I.U. of Vitamin D3 orally to Vitamin D-deficient animals when studying the intestinal calcium transport in response to Vitamin D. A toxic dose of Vitamin D in rats is about 10,000 I.U./100g body weight (Hashim & Clark 1969).

The young (150g) rats were maintained on a Vitamin D deficient diet (Cooper Nutrition Products Ltd, Witham, Essex - full dietary composition in Appendix) for 3 weeks prior to the experimental day. 100 or 500 I.U. of Vitamin D3 (Sigma Ltd, London) was then given by oral intubation to lightly etherised rats, in 0.1ml of arachis oil. Control animals were given an equal volume of the vehicle.

To ensure the vitamin reached its site of action, the animals were starved for 48 hours prior to use and the sawdust changed on these days to prevent coprophagy.

3.4. Administration of Sex Steroids

In some experiments the relative contribution of the oestrogens and progestogens upon mitotic events was studied. From day 2 to 5 after ovariectomy rats received a daily injection of either oestradiol benzoate (Sigma Ltd, London) or progesterone (Sigma Ltd, London). Oestradiol dissolved in 0.1ml arachis oil was given s.c. at a dose of 1µg/100g body wt/day and progesterone dissolved in 0.1ml arachis oil was given s.c. at a dose of 1mg/100g body wt/day. These doses were chosen on the basis of the normal daily secretion of these steroids and adequately compensate for lost ovarian function (Hashimoto, Henricks, Anderson & Melampy 1968; Shaikh 1971). Preliminary experiments revealed the vehicle to be ineffectual towards mitotic activity.

3.5. Animal Operations

Surgical procedures which necessitated subsequent recovery were performed under semi-sterile conditions, such that the instruments and open wounds were frequently washed in an antiseptic solution of Lyseptol (Philip Harris Ltd, Birmingham). For short term surgery (i.e. not > 15 minutes duration) gaseous (ether) anaesthesia (Diethyl Ether - Fisons Ltd, Loughborough) was employed. Sufficient depth of anaesthesia could be obtained by firstly exposing the animals to the anaesthetic in a closed container, and subsequent maintenance of this level by the administration of an ether-cotton wool pad to the nasal region as the

need arose.

Experience has shown however that although ether facilitates rapid recovery, the dose has to be very carefully monitored since the animals may readily die, due to overdose. This was prevented by periodic pinching of the hind toes, and observing the degree of consciousness. To minimise post operative infection, the animals were placed in cages lined with fresh sawdust.

a) Ovariectomy

Bilateral ovariectomy of young female rats was performed according to D'Amour, Blood & Belden (1965). Following light ether anaesthesia, the lateral wall of the mid-abdominal region was shaved and washed with antiseptic solution. A small (1cm) vertical incision was made in the shaved area. By careful exploration of the exposed abdominal cavity, the ovary could be identified as a bright red globular mass embedded in much fat. Forceps were employed to exteriorise the ovary; the fallopian tube and its associated fat and blood vessels were doubly ligatured with suture thread, and then severed about 1cm distal to the ovary to ensure complete excision of the gland. The remaining tissue was washed in antiseptic and returned to the abdominal cavity, the muscle wall closed with suture thread using a curved triangular needle, and the body wall closed with surgical clips.

b) Parathyroidectomy and Thyroparathyroidectomy

Animals were thyroparathyroidectomised (TPTX) by the method of Ingle & Griffith (1942), and parathyroidectomised (PTX)

by electocautery. PTX by cautery was introduced by Erdheim in 1906, and since then has been employed extensively because of its convenience and effectiveness. Various methods of PTX (Hirsch, Gauthier & Munson 1963; Munson & Hirsch 1966) have been employed, and careful analysis of these results in fact led to the discovery of TC, the hypocalcaemic principle of the thyroid. Cautery quickly and completely destroys the parathyroid gland by brief application of a hot copper wire tip.

The two major hazards of the technique are firstly damaging the recurrent laryngeal nerve which is manifest by severe respiratory difficulties, and secondly haemorrhage, which may occur as a result of exposing the thyroid gland sufficiently from the surrounding connective tissue and blood vessels.

The serum calcium falls precipitously following cautery, and three hours later approaches values around 6mg% (Hirsch, Gauthier & Munson 1963). This low plasma level may be maintained if the animals are subsequently fed a calcium deficient diet. The low 1,25 DHCC level in aparathyroid rats (Norman 1974b) together with decreased dietary calcium minimises intestinal calcium transport. Any possible 1,25 DHCC induced bone resorption would also be minimal since this requires the participation of PTH (Harrison, Harrison & Park 1958; Holick & De Luca 1974). Thus both the intestinal and skeletal routes for possible elevation of plasma calcium are kept at a low level.

The parathyroid glands are small structures partially embedded in the upper area of each thyroid lobe (Plate 9). Sham PTX was performed by cauterising the lower areas of each thyroid lobe,

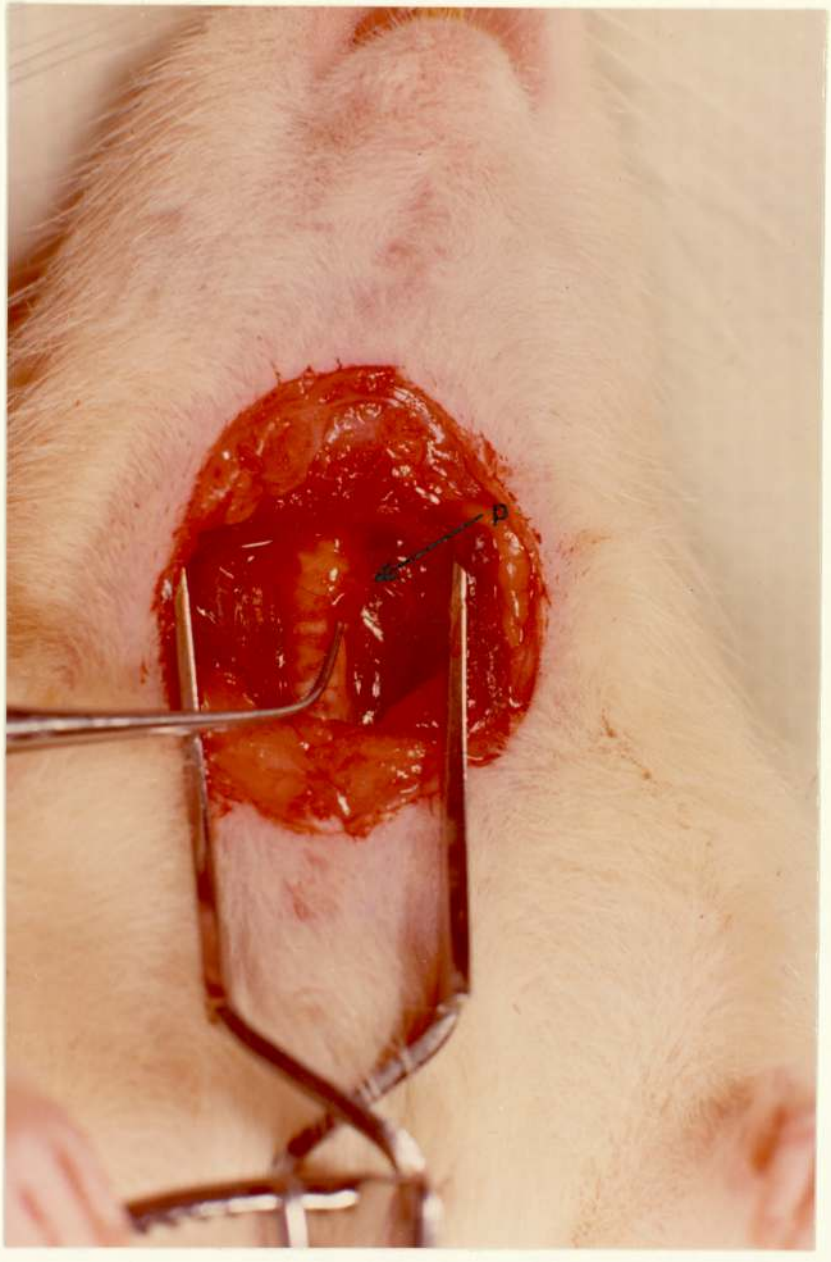


Plate 9: Location of the thyroid-parathyroid complex. The thyroid gland consists of two lateral lobes joined by a thin isthmus. The parathyroid gland (arrowed with p) is located in the anterior portion of each thyroid lobe. Forceps are holding one thyroid lobe.

since due to the anatomical location of the parathyroids, TC is released following PTX (Munson & Hirsch 1966).

The following regime (Hirsch, Gauthier & Munson 1963) was followed for PTX:

Day 1	-	PTX by electrocautery; normal diet and tap water
Day 2, 3 & 4	-	normal food and tap water
Day 5	-	animals transferred to a calcium deficient diet (Cooper Nutrition Products Ltd, Witham, Essex - full dietary composition in Appendix) and distilled water
Day 6	-	calcium deficient diet and distilled water
Day 7	-	experiment, together with blood sampling by cardiac puncture.

In all PTX animals, daily food intake was recorded and this amount of food subsequently given to the sham PTX animals. All the sham PTX animals were given standard 41B diet, from days 1-7.

c) Venous infusion technique

In some experiments it was desirable to maintain elevated plasma calcium levels for about an hour. This was achieved by slowly infusing a concentrated calcium solution into the jugular vein. Rats were anaesthetised with Nembutal at a dose of 0.1 ml/100g by i.p. injection. They were shaved in the throat region and the jugular vein exposed. After ligating the blood supply from the head, a small angled incision was made in the vein, and a short length of pp20 cannulation tubing (Portex Ltd, Hythe, Kent) filled with heparinised saline (50mg/) carefully inserted into the aperture. This was securely tied, and if the procedure had been accomplished

correctly, slight withdrawal of the syringe plunger would result in blood appearing in the tubing. Having replaced the heparinised saline syringe for a 5ml syringe containing a calcium concentration, (12mg $CaCl_2$/ml) sufficient to sustain elevated plasma calcium levels of 11mg%, the syringe and associated tubing was attached to a slow infusion apparatus (Scientific Research Instruments Ltd, Croydon, Surrey), and set at a rate of 0.025 ml/min for 1hr.

RESULTS

'STATISTICS'

Significance between given groups of parameters was determined by the Students t-test. The level of significance was taken as being that which gave $p < 0.05$.

The line of best fit through scattered points was constructed by the method of least squares; the correlation coefficient 'r' was determined as appropriate.

PART ONE

The role of the oestrogen molecule in cell division in lymphopoietic and haemopoietic tissues.

Introduction.

About twenty years have elapsed since Bullough (1950) and Ebling (1954) indicated that cell division in the skin of the female rodent might differ from that in the male. It has also been well characterised that young female rats have regular 4-day oestrous cycles (Long & Evans 1922) determined by fluctuating plasma levels of oestrogen and progesterone (Butcher, Collins & Fugo 1974).

Experiments were thus designed to make a detailed study of the possible significance that the oestrogen molecule may have upon mitotic events in the bone marrow and thymic cell populations of the female rat.

Results.

Previous work employing male rats has shown that thymic lymphopoiesis was enhanced by elevated calcium or magnesium concentrations (Perris, Whitfield & Rixon 1967; Whitfield, Perris & Youdale 1968; Morgan & Perris 1974) and cell division in the bone marrow was also enhanced by calcium (Perris, Whitfield & Rixon 1967). It was therefore necessary to establish if there were any sex differences in the normal plasma concentrations of these ionic mitogens before any exogenous pertubations were imposed on the female animal. In young animals (150-250g) total plasma calcium of males showed consistent values of around 10.0mg%; female rats on the other hand showed a variation throughout their oestrous cycle exhibiting peak concentrations at oestrus and minimal concentrations at pro-oestrus (Table 1.). Ionised calcium levels followed a similar pattern. However the plasma magnesium levels in the male did not differ significantly from those in the female at any stage of the oestrous cycle (Table 1.), in accordance with the human studies of Raut & Viswanathan (1972).

Several groups of virgin female rats were studied over a 6-day period, and it was concluded that these Wistar rats exhibited 4-day cycles, with a very short and often undetectable metoestrus. Due to the fluctuation of plasma total calcium during this cycle, all female rats used as controls in subsequent experiments were in dioestrus.

When male and female rats were compared for their mitotic responsiveness to these cations, it was shown that injections of $CaCl_2$ significantly increased the division of both bone marrow and thymus cells in the male rat (Fig. 1.). However, in the female rat, although the basal level of mitotic activity in both tissues was not significantly different from that in the male, cells were completely unresponsive mitotically to the calcium

	FEMALE				MALE
	Pro-Oestrus	Oestrus	Metestrus	Dioestrus	
Plasma total calcium	9.31 ± 0.22 (8)	10.18 ± 0.26 (10)	9.62 ± 0.24 (5)	9.54 ± 0.18 (11)	10.07±0.13 (14)
	$p = < 0.05$				
Plasma ionised calcium	4.00 ± 0.18 (8)	4.42 ± 0.09 (10)	4.23 ± 0.27 (5)	4.14 ± 0.10 (11)	4.45±0.07 (14)
	$p = < 0.05$				
Plasma total magnesium	2.49 ± 0.20 (8)	2.26 ± 0.11 (10)	2.38 ± 0.25 (5)	2.36 ± 0.14 (11)	2.39±0.12 (14)

Table 1: Comparison of plasma calcium and magnesium concentrations (in mg%) in young (150 - 200g) male and female rats. Number of animals in each group shown in parentheses.

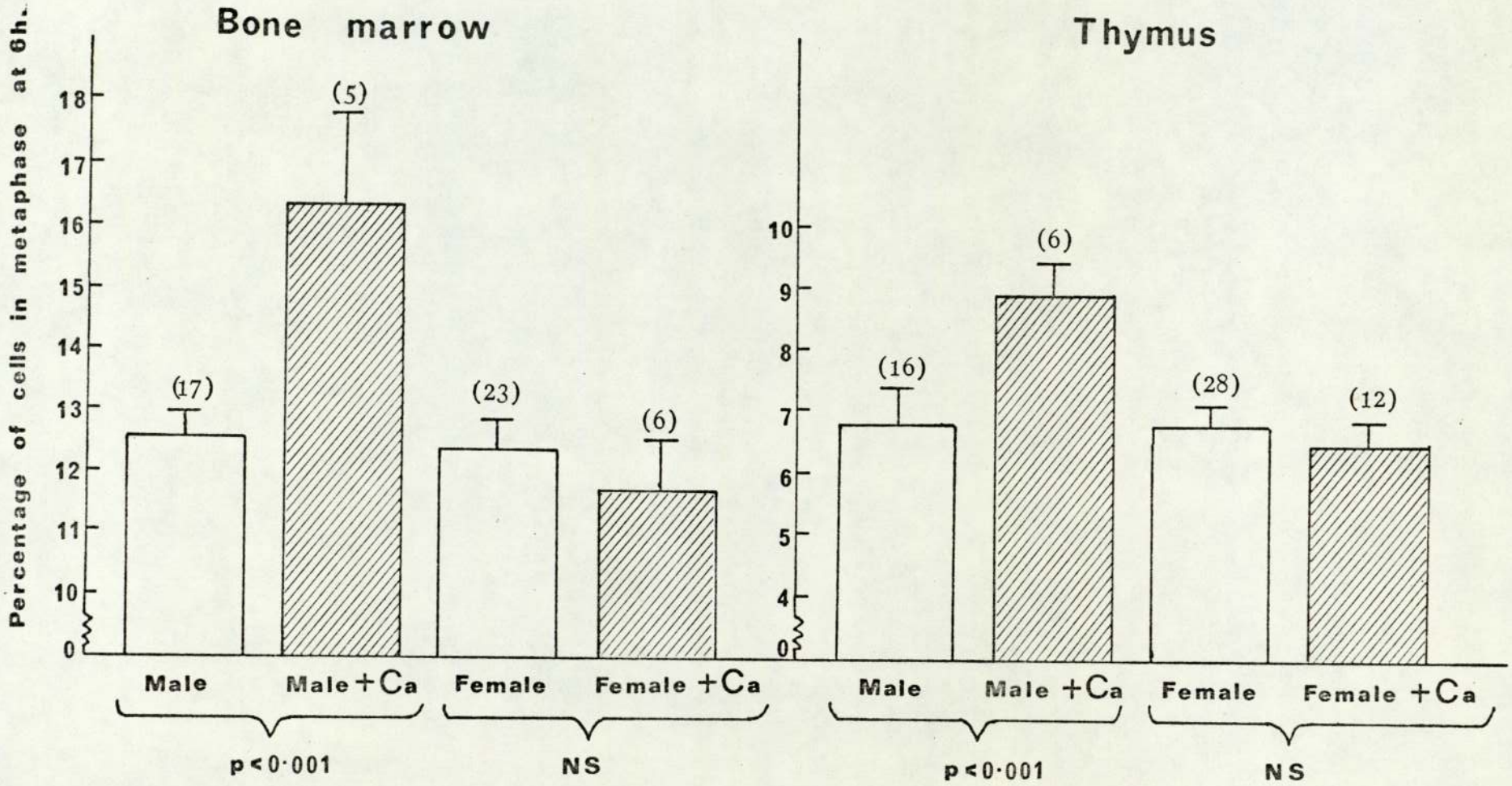


Fig.1. Effect of a series of i.p. injections of 62.5 mM-$CaCl_2$ (Ca) on mitotic activity in bone marrow and thymus tissue of male and female (dioestrus) rats over a 6h period. Number of animals in each group shown in parentheses. Vertical bars indicate ± s.e.m. NS, Not significant.

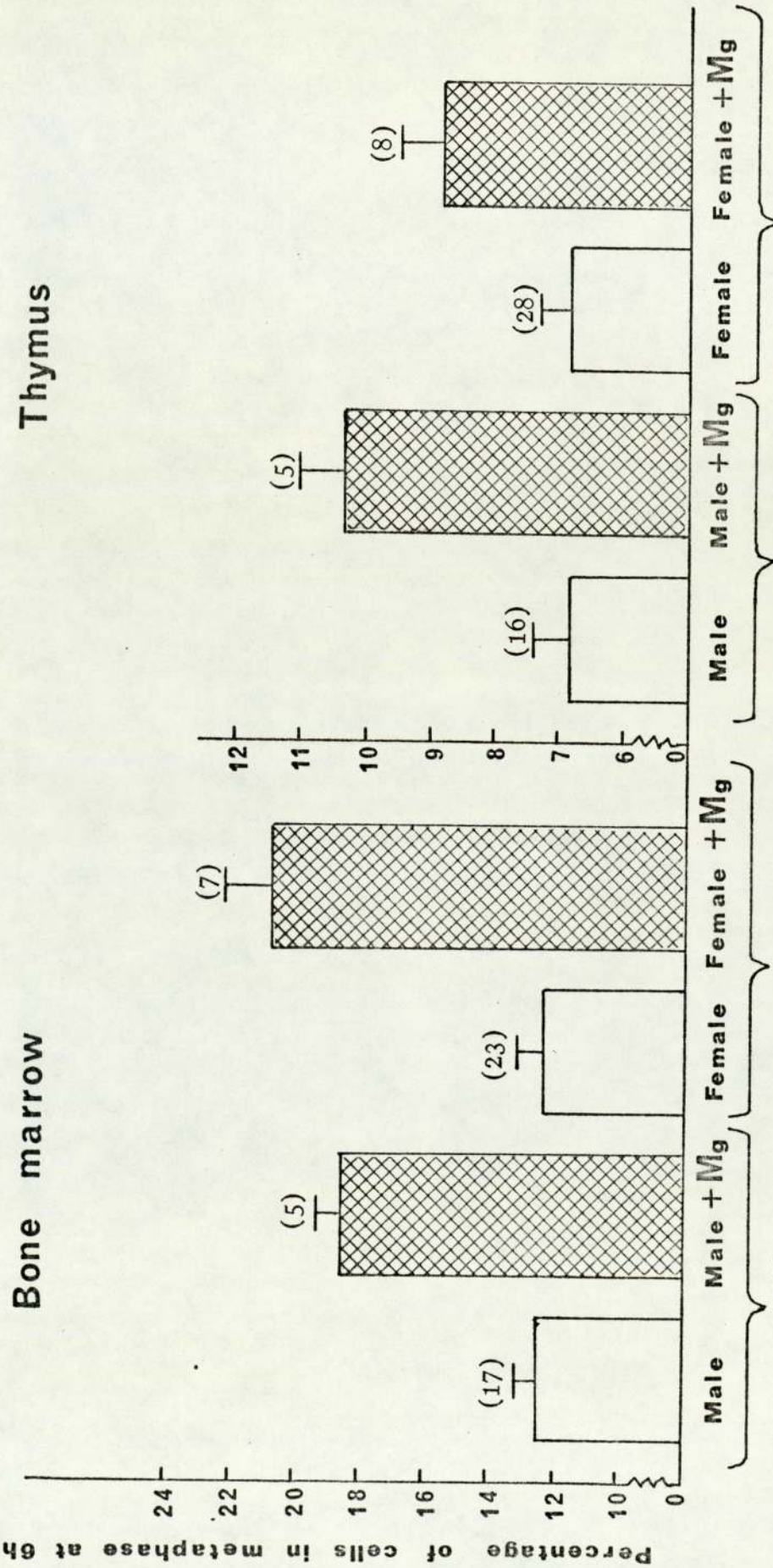


Fig.2. Effect of a series of i.p. injections of 62.5 mM-$MgCl_2$(Mg) on mitotic activity in bone marrow and thymus tissue of male and female (dioestrus) rats over a 6h period. Number of animals in each group shown in parentheses. Vertical bars indicate ± s.e.m.

challenge. On the other hand, $MgCl_2$ injections provoked a highly significant mitotic stimulation in bone marrow and thymus cells in both sexes (Fig. 2.).

The hypothesis that some ovarian secretion may have a restraining role and be responsible for the inhibition of calcium-induced mitogenesis in the female was strengthened by the observation that the basal level of mitotic activity in bone marrow and thymus tissue was increased when measured 6 days after ovariectomy (Table 2.). A significant increase in thymic weight was also observed following removal of the ovaries (Fig. 5.). Plasma concentrations of calcium and magnesium 6 days after ovariectomy did not differ from those in the sham-ovariectomoised controls (Fig. 5). Although initial food intake of the operated animals was much reduced, 6 days after ovariectomy the food consumption was again basal (Fig. 3.) and lack of the oestrogen molecule 6 days post ovariectomy did not influence the 24hr. feeding pattern (Fig. 4.). Indeed, to exclude any influence of possible dietary changes following ovariectomy (Kenney & Mook 1974) on mitotic activity, control rats were pair-fed with the operated animals. Thus neither extracellular divalent cationic mitogens nor dietary factors play any role in the enhanced cell proliferation following ovariectomy.

Furthermore, in the ovariectomised (Ox) rat, in contrast to intact females, calcium injections markedly stimulated proliferation in both bone marrow and thymic cell populations (Table 2.). When replacement therapy with 1 µg oestradiol benzoate/100g body weight/day was given to Ox rats, to restore oestrogen levels in the blood (Shaikh 1971), the mitogenic capacity of calcium was again abolished when observed 6 days after ovariectomy (Table 2.). A similar regime of injections of progesterone at a dose of 1mg/100g body weight/day, to restore progesterone levels in the blood

Treatment	Bone Marrow	P	Thymus	P
Normal female	12.2±0.6 (23)	NS	6.8±0.3 (28)	NS
Sham-Ox	12.4±0.9 (4)		6.8±0.8 (4)	
Ox	15.5±0.6 (18)	<0.05	8.9±0.4 (17)	<0.05
Ox + Ca	22.2±0.9 (17)	<0.001	11.5±0.4 (17)	<0.001
Ox + OB	15.2±1.7 (5)	NS	7.7±0.4 (6)	NS
Ox + OB + Ca	14.5±0.7 (6)		8.1±0.3 (6)	
Ox + Prog	14.6±1.1 (5)	<0.01	8.4±0.7 (5)	<0.001
Ox + Prog + Ca	21.1±0.9 (5)		13.0±0.3 (4)	

Table 2: Effect of $CaCl_2$ injections on mitotic activity in ovariectomised female rats expressed as the percentage of cells in metaphase 6h after the first injection (means ± s.e.m.). Control animals in dioestrus. Treatments were: ovariectomy (Ox); series of i.p. injections of 62.5mM $CaCl_2$ (Ca); replacement therapy with either oestradiol benzoate (OB) or progesterone (Prog) as described in Methods. Number of animals in each group is shown in parentheses. NS, Not significant.

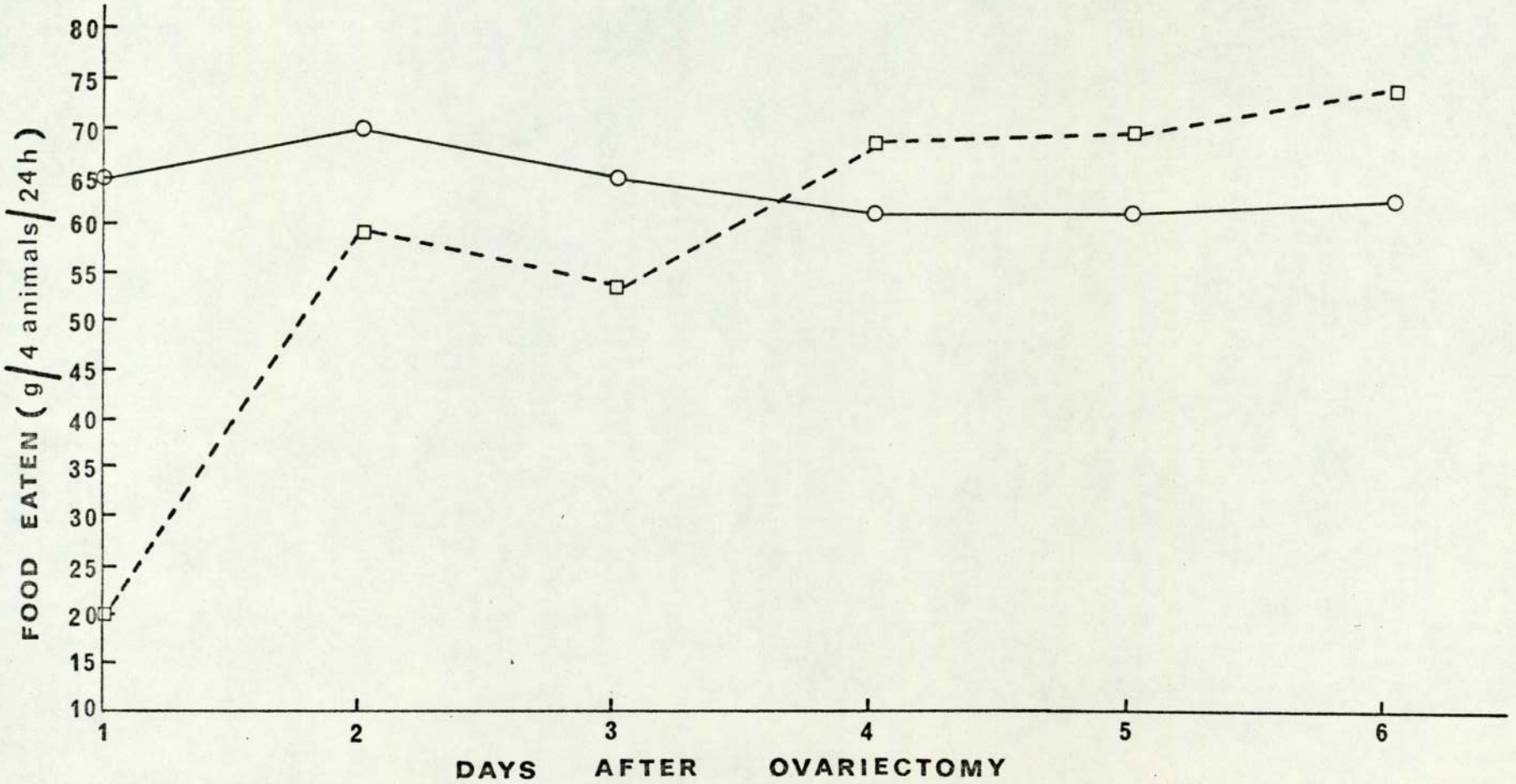


Fig.3: Post-operational recovery of food consumption following ovariectomy.
○——○ Control females. Each point represents the mean of 5 cages of unsynchronised animals.
□----□ Ovariectomised females.

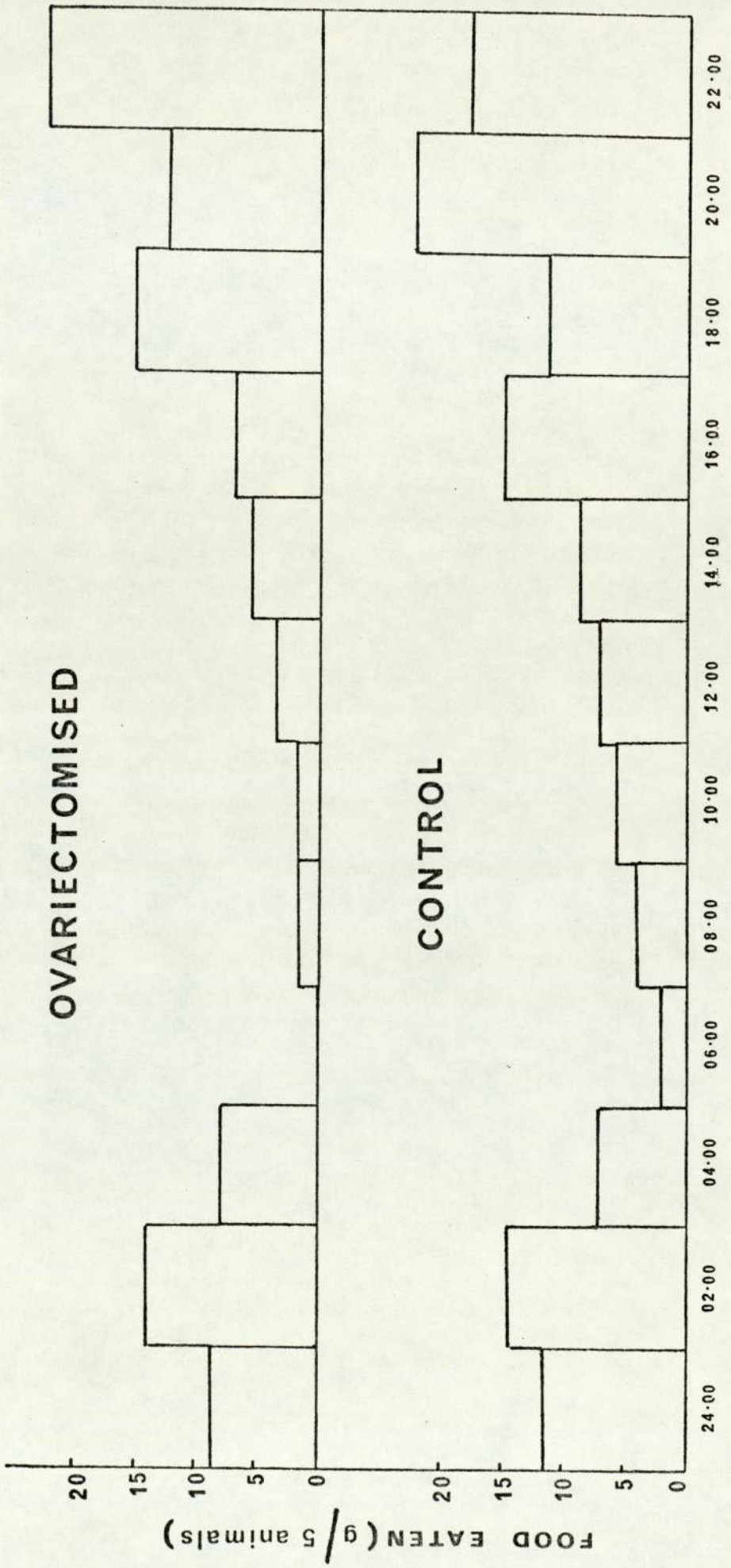


Fig.4: Food consumption from groups of ovariectomised and control female (dioestrus) animals recorded 6 days after ovariectomy. Each block represents a 2hr period, commencing from the time indicated.

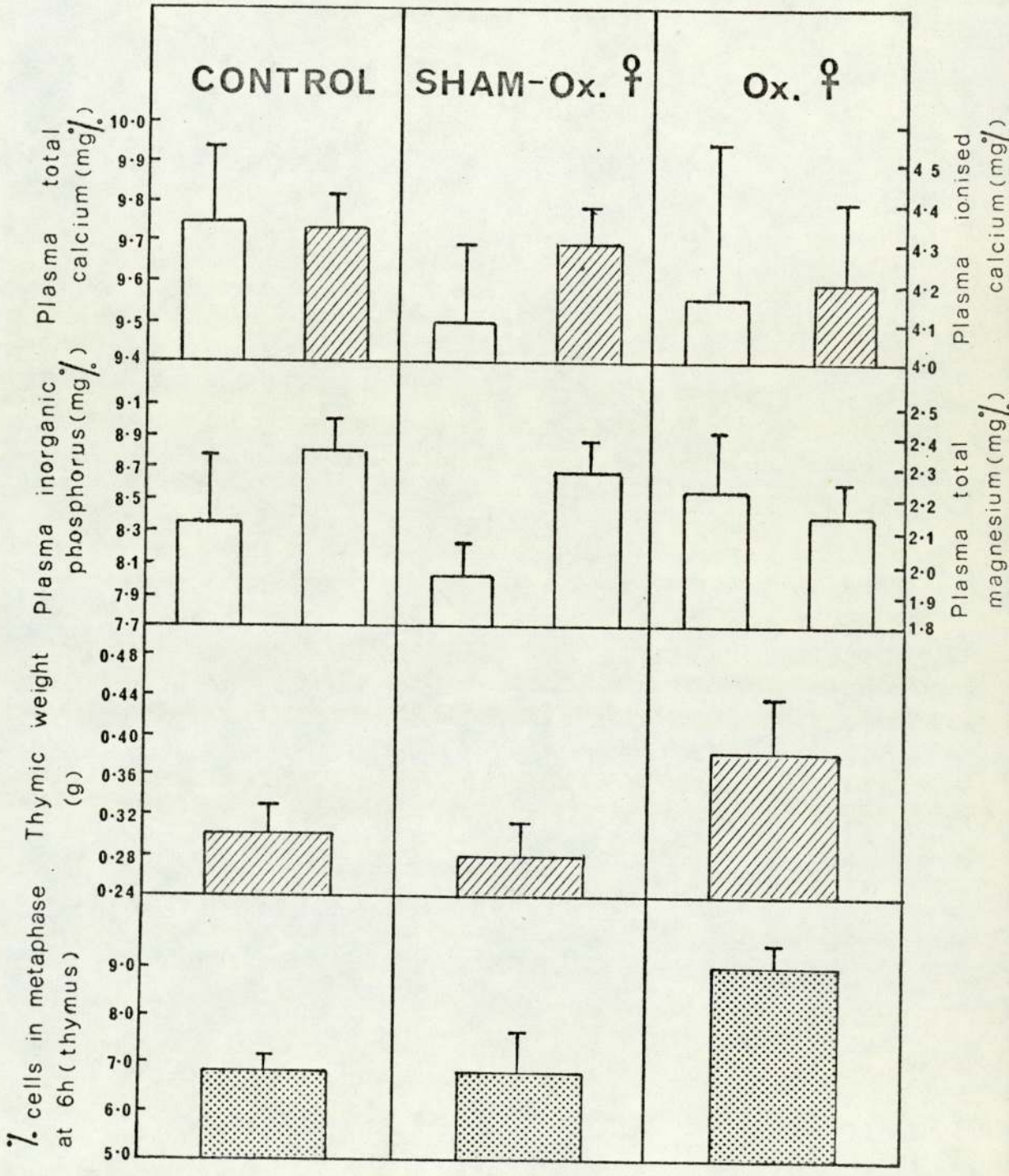


Fig. 5: Lack of change of cationic mitogens in control (dioestrus) and ovariectomised (Ox) female animals, 6 days following ablation of the glands. Significant ($p < 0.05$) elevation of the thymic weight was however paralleled by significant ($p < 0.05$) elevation of the rate of cell division. Number of animals in each group between 5-12. Vertical bars indicate ± s.e.m.

(Hashimoto, Henricks, Anderson & Melampy 1968) were without effect (Table 2.). Thus it is the presence of oestrogen and not progresterone that is the agent responsible for negating the mitogenic potential of calcium in the bone marrow and thymus tissue of the female rat.

This inhibitory action of oestrogen on the mitotically competent cells could be either a direct or an indirect effect. Thus if oestrogen was able to interact with the calcium homeostatic hormones in some way (Currie & Black 1972; Shai & Wallach 1973) the response to the calcium challenge in the female rat might be modified so that the usual mitotic response could not be elicited. For example, if oestrogen facilitated either the release of TC, or the inhibition of PTH output in response to $CaCl_2$ injections, the calcium load would rapidly be eliminated and mitogenic levels in the plasma never attained. However, when rats of both sexes were thyroparathyroid-ectomised, the basal level of mitotic activity in both tissues was reduced below that of the control values in accordance with previous observations (Perris, Weiss & Whitfield 1970; Rixon & Whitfield 1972a), calcium injections again stimulated cell division in the male rat, but had no effect on female bone marrow and thymus tissue (Table 3.). A more direct inhibitory action of oestrogen on the mitogenic capacity of calcium was thus considered.

In an attempt to clarify the mechanism of this direct action of oestrogen, attention was focused towards *in vitro* experiments employing isolated thymic lymphocytes. Such cells maintained in a BGS medium containing colchicine continue to flow into mitosis and accumulate at metaphase as they do *in vivo*. Thus when the calcium concentration in the medium was increased above 0.6 m mol/l there was a significant stimulation of cell division (Fig. 6.). This occurred whether the thymic lymphocytes were of male or female origin. Thus the cells taken from the oestrogen-rich environment of the female rat possessed no inherent resistance to the mitogenic

Treatment	MALE				FEMALE			
	Bone Marrow	P	Thymus	P	Bone Marrow	P	Thymus	P
Normal	12.4±0.5(17)	<0.001	6.8±0.5(16)	<0.001	12.2±0.6(23)	NS	6.8±0.3(28)	NS
Normal + Ca	16.3±1.5(5)		8.8±0.5(6)		11.8±0.7(6)		6.2±0.4(12)	
TPTX	6.6±0.5(7)	<0.001	4.9±0.5(7)	<0.05	9.6±0.7(5)	NS	5.0±0.5(6)	NS
TPTX + Ca	14.1±1.2(6)		7.1±0.7(6)		8.6±0.8(5)		5.1±0.6(5)	

Table 3: Effect of $CaCl_2$ injections on mitotic activity in normal and thyroparathyroidectomised rats, expressed as the percentage of cells in metaphase 6h after the first injection (means ± s.e.m.). Calcium response is ablated in the presence of relatively low oestrogen concentration in vivo of the dioestrus animals. Treatments were: a series of i.p. injections of 62.5mM-$CaCl_2$(Ca) and thyroparathyroidectomy (TPTX). Number of animals in each group is shown in parenthesis.
NS, not significant.

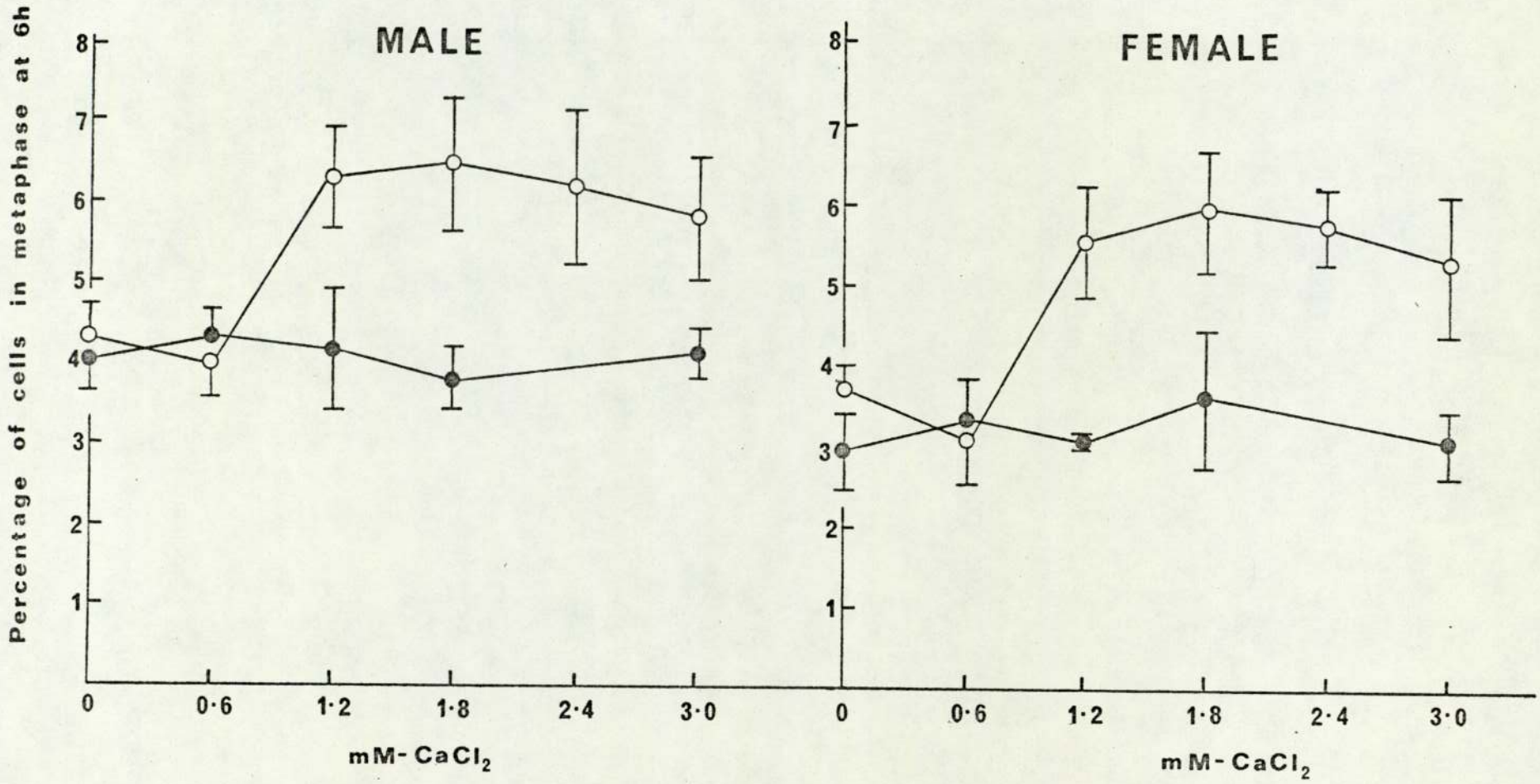


Fig.6: Influence of oestradiol benzoate (OB) on mitotic activity of isolated thymocytes from male and female rats exposed to varying concentrations of $CaCl_2$ in culture. ○ Control media: ● media containing 0.1μgOB/ml. Each value is a mean derived from at least five separate experiments. Vertical lines represent ± s.e.m. The magnesium ion concentration was maintained throughout at 1.0 mmol/l. A significant ($P<0.05$) elevation of mitotic rate of the control media was shown for all calcium concentrations of 1.2 mM and above.

action of calcium. However when oestradiol benzoate was added to the cultures of thymocytes taken from either male or female rats, the steroid again blocked calcium-induced cell proliferation, even when the calcium concentrations were as high as 3.0 m mol/l (Fig. 6.). The concentration of oestradiol benzoate employed in these culture experiments was 0.1 µg/ml, but previous experiments had shown that a concentration of 0.001 µg/ml was equally effective.

In line with the in vivo experiments illustrated in Figs. 1. & 2., it was shown that in other culture experiments if the calcium concentration was kept constant at 0.6 m mol/l, and the magnesium concentration increased above 1.0 m mol/l stimulation of division also resulted, irrespective of the sex of origin of the thymocytes. The level of mitosis was raised from a mean value of 3.7% to 5.5% when the magnesium concentration in the media wasincreased from 1.0 to 2.5 m mol/l. The presence or absence of an oestradiol benzoate concentration of 0.1 µg/ml was without effect on this magnesium-induced mitogenesis. Experiments also showed that this concentration of oestradiol was itself non-mitogenic. These in vitro experiments reinforce the original postulate of a direct inhibition by oestrogen at the cellular level of calcium-stimulated mitogenesis in bone marrow and thymic cell populations.

Since these female rats underwent regular 4-day oestrous cycles, which are characterised by fluctuating levels of plasma sex steroids (Butcher, Collins & Fugo 1974) it was anticipated that a fluctuation in mitotic activity throughout the oestrous cycle might well exist in bone marrow and thymic cell populations, since Bullough (1950) had demonstrated the presence of such a rhythm in skin. Indeed, a marked fluctuation in mitotic activity in both tissues was shown (Fig. 7.) with maximal activity at oestrus and minimal activity at pro-oestrus. There was a significant difference

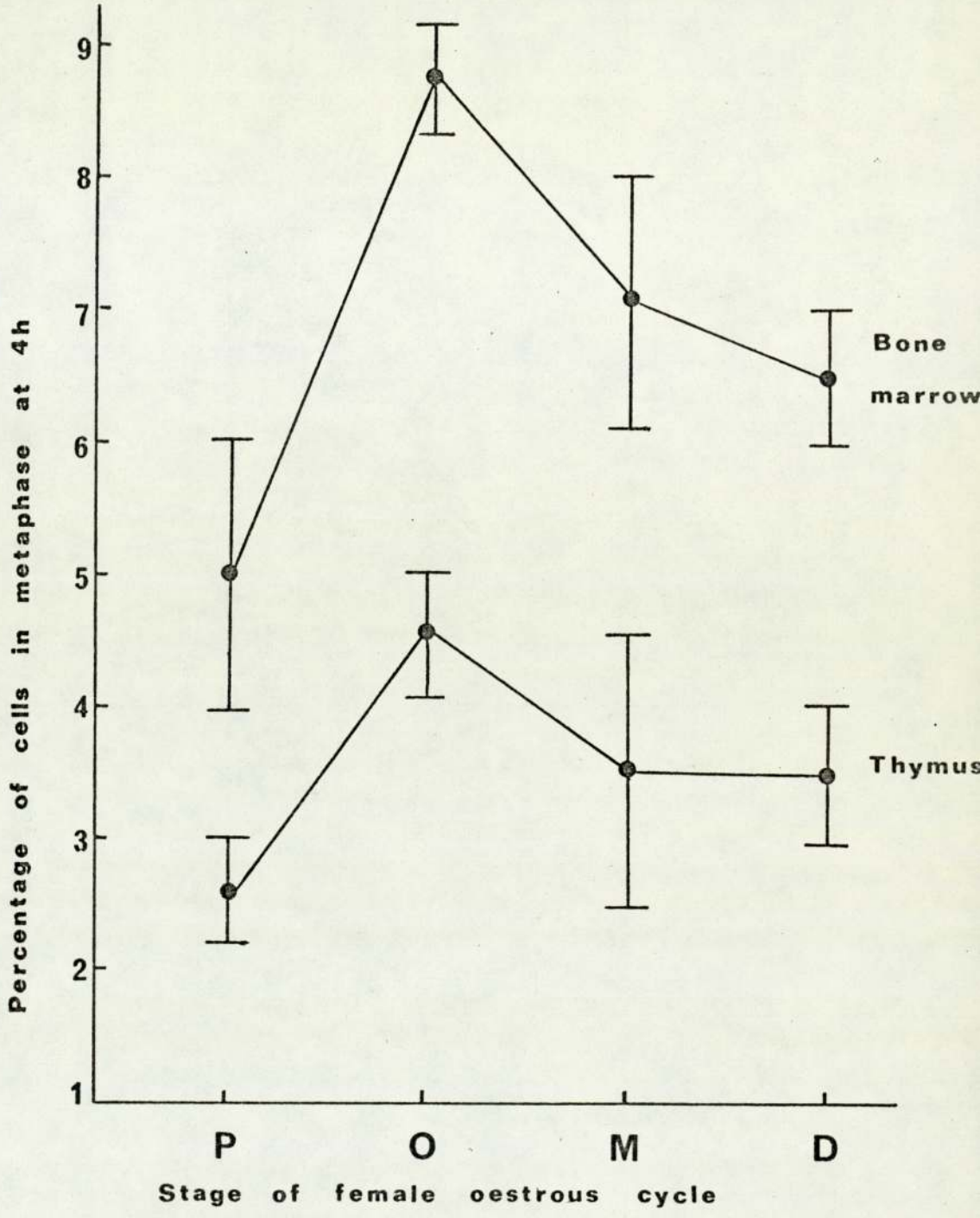


Fig. 7: Typical pattern of mitotic activity in bone marrow and thymic tissue in female animals throughout their oestrous cycle. P (Pro-oestrus); O (Oestrus); M (Metoestrus); D (Dioestrus). Minimal activity at pro-oestrus and maximal activity at oestrus; significance between these limits < 0.01 in both tissues. Between 3-8 animals per point shown as means ± s.e.m.

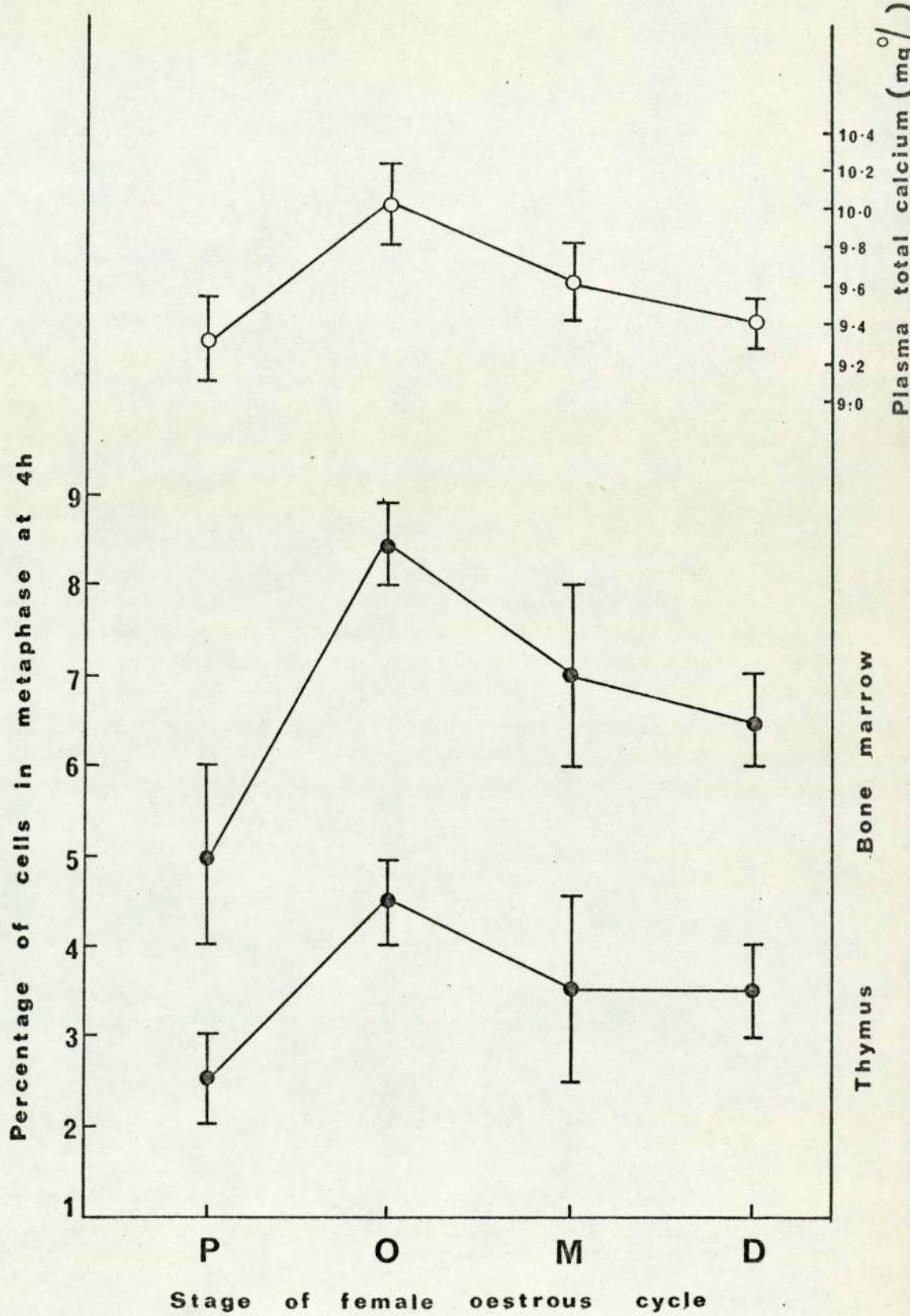


Fig. 8: Superimposition of the plasma calcium changes upon the mitotic activity pattern (Fig. 7) throughout the female oestrous cycle. Significance between minimal (pro-oestrus) and maximal (oestrus) plasma calcium < 0.05.

($p < 0.05$) in mitotic activity at these two times. In spite of the demonstration of a blockade of calcium-induced mitosis in the female due to the presence of oestrogen, this pattern of proliferation throughout the 4-day oestrous cycle still closely followed the plasma total calcium concentration (Fig. 8.; Table 1.).

This activity was subsequently studied at three different seasons throughout the year, namely Spring (January-February); Summer (March-April), and Winter (October). The same general pattern was shown at each seacon i.e. at oestrus plasma total calcium and cellular activity were highest, whereas at pro-oestrus these parameters were both minimal in bone marrow (Fig. 9.) and thymus (Fig. 10.) tissue. There was some seasonal fluctuation in this mitotic rhythm with minimal activity being at Summer; even so plasma total calcium closely followed this change (Figs. 9 & 10.). The main fluctuation of the plasma total calcium at different seasons was at pro-oestrus (Figs. 9. & 10.). Plasma total calcium did not always exhibit a direct relationship to the mitotic index, but did appear to parallel colcemid metaphase figures more closely. Thus, when this latter method of assessing division was employed, regression lines for both the bone marrow (Fig. 11b.) and the thymus (Fig. 11a.) showed a very highly significant ($p < 0.001$) correlation to the plasma total calcium throughout the 4-day cycle. However, despite this very highly significant relationship further elevation of the plasma calcium by i.p. injection of $CaCl_2$ was ineffectual in provoking an additional increase in cell proliferation at any stage of the oestrous cycle, in marked contrast to the male (Fig. 12.).

Even though a distinct correlation between plasma total calcium and cell proliferation in these tissues was apparent over this relatively short period Gallagher & Nordin (1973) showed that a more protracted loss of oestrogens in post-menopausal female patients was accompanied by hypercalcaemia. With an appreciation of the links between calcium and oestrogen gained from previous experiments it was of interest to persue this relation-

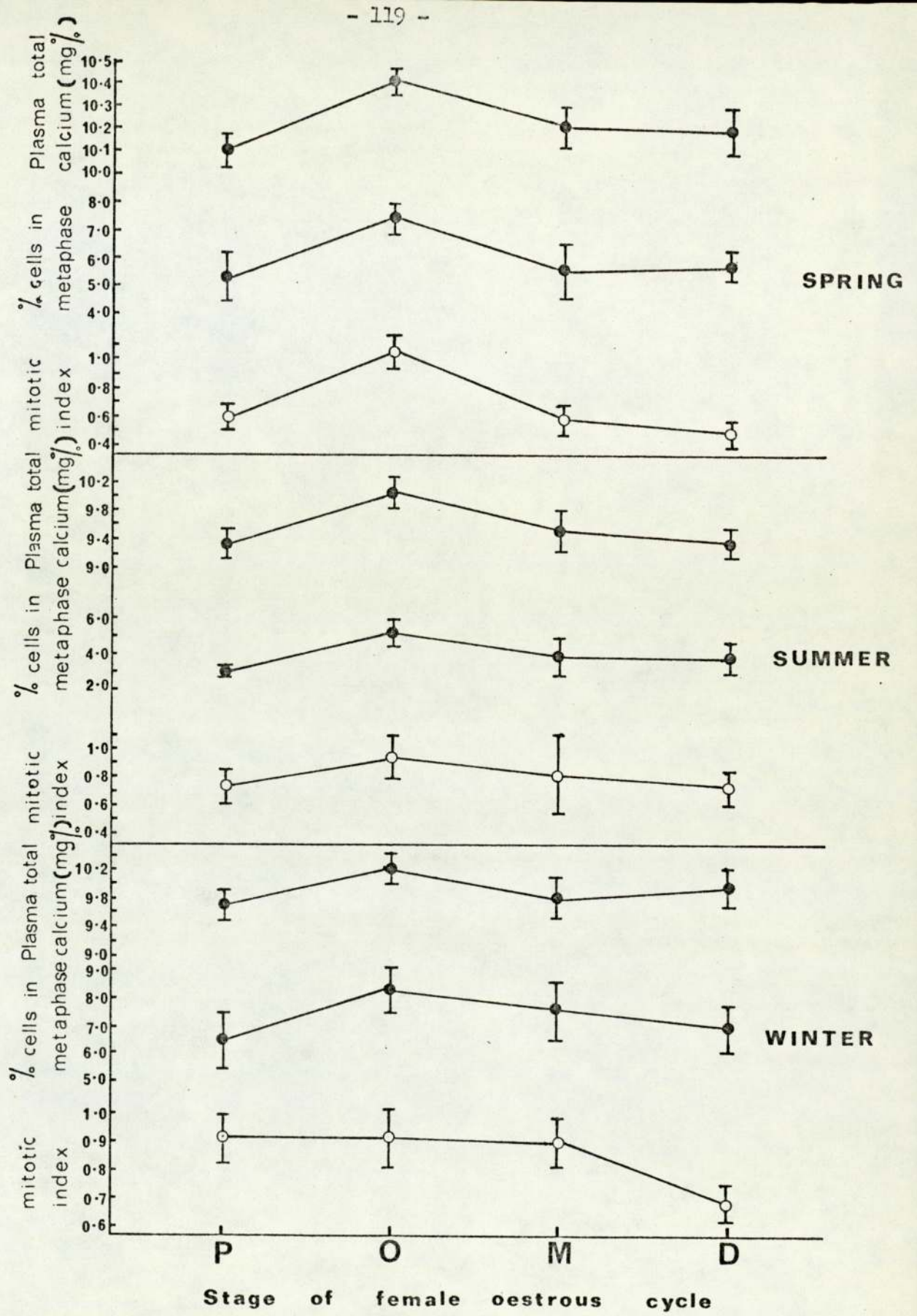


Fig. 9: Seasonal fluctuation in thymic mitotic activity and associated change in plasma total calcium throughout the female oestrous cycle assessed 4h after a single injection of colcemid.

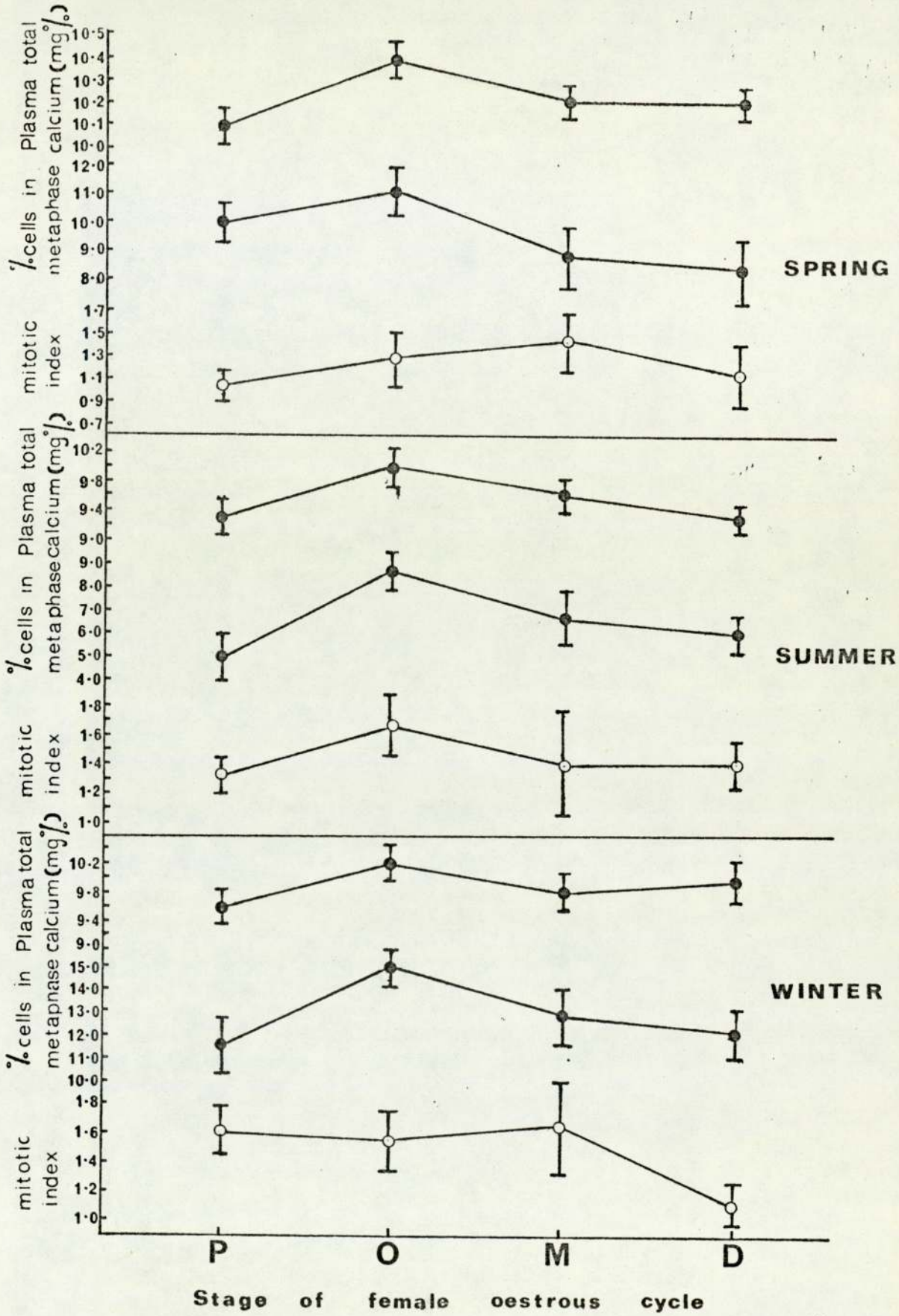


Fig. 10: Seasonal fluctuation in bone marrow mitotic activity and associated change in plasma total calcium throughout the female oestrous cycle assessed 4h after a single injection of colcemid.

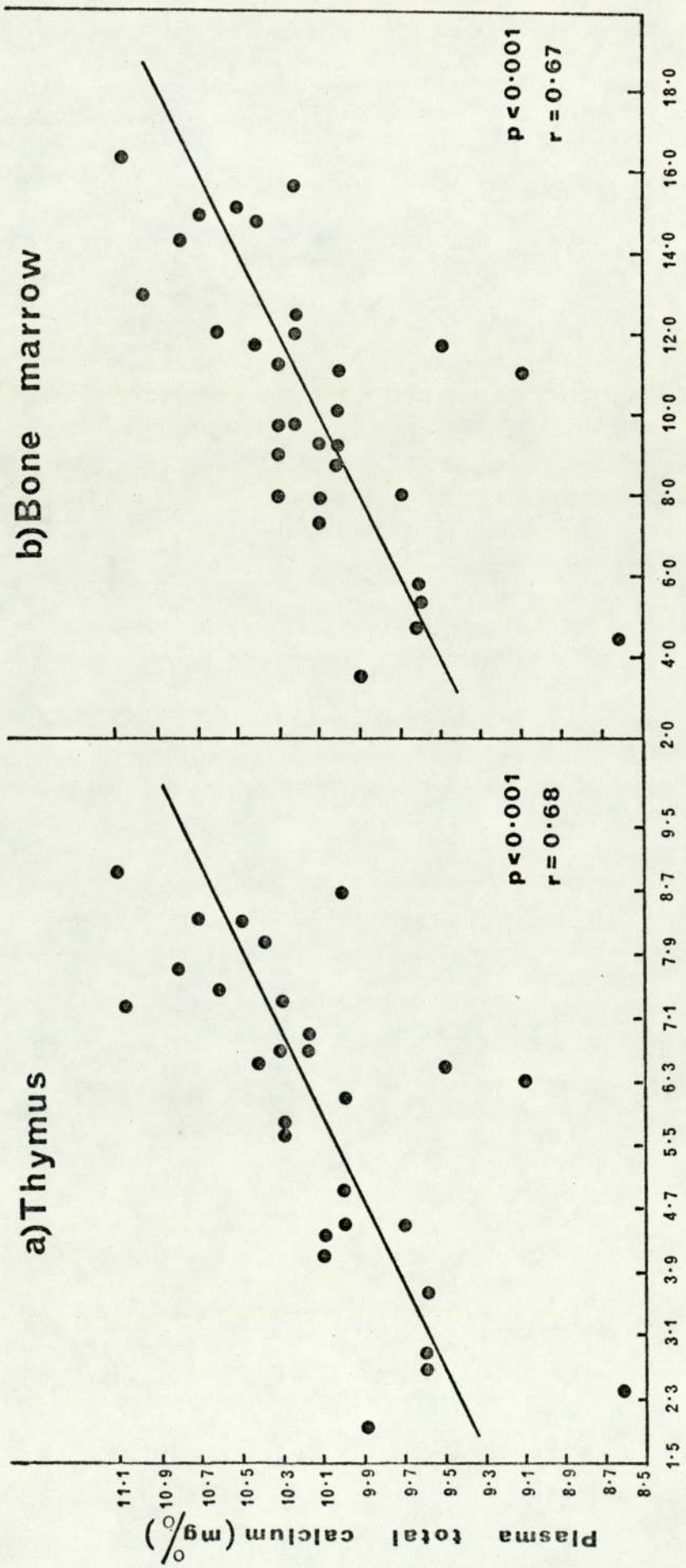


Fig.11: High correlation shown between the plasma total calcium concentration at any stage of the female oestrous cycle and the subsequent mitotic activity both in thymus and bone marrow tissue.

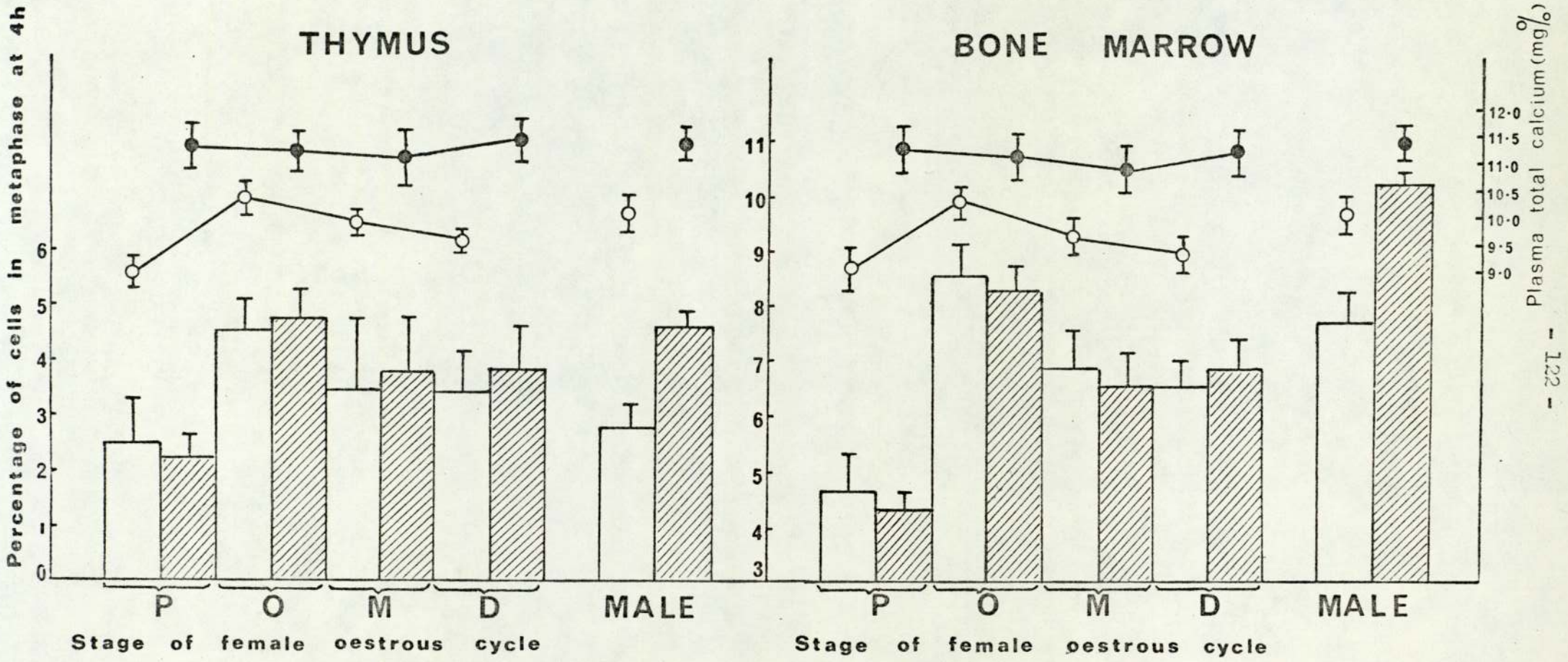


Fig.12: Unresponsiveness of female animals at any stage of oestrous cycle to enhanced cell division as a consequence of transient but highly significant elevation of plasma total calcium; unlike male animals where marked cell proliferation was shown ($P<0.001$) in both tissues.

□ Untreated animals

▨ Animals injected with a series of i.p. injections of 62.5mM-$CaCl_2$.

ship further to discover what calcium changes, if any, were apparent in female rats several months after ovariectomy, and how this might be reflected in cell proliferation in old female rats.

In the male thymic weight has previously been shown to be correlated with the concentration of extracellular ionised calcium (Perris, Weiss & Whitfield, 1970). In one group of long term Ox. female animals, both thymic weight and ionised calcium were significantly reduced (Fig. 13.); a significant ($p < 0.05$) correlation between these parameters was shown (Fig. 14.). However, in two subsequent experiments (Figs. 15. & 18.) removal of the ovaries for the same length of time (50 weeks) resulted in both the thymic weight and ionised calcium being significantly elevated; a significant ($p < 0.05$) correlation was shown between these parameters (Fig. 16.) from the latter two experiments. Due to age involution of the thymus gland some of the control rats were athymic.

Since food intake patterns of ovariectomised females are changed over a short time period (Kenney & Mook 1974) it was necessary to show what dietary changes were apparent in rats ovariectomised for 50 weeks. Food consumption studied over this period revealed that ovariectomy caused (Fig. 17.) a transient hyperphagia with a subsequent return towards control values (Mook, Kenney, Roberts, Nussbaum & Rodier 1972; Tarttelin & Gorski 1973). In terminal weeks the Ox. rats consumed about 30% less food than control females (Fig. 17.), but even so these animals showed highly significant increases in body weight (Figs. 13, 15. & 18.). When the mitotic activity of bone marrow and thymus from a group (Fig. 18.) of long term Ox. females with elevated ionised calcium was assessed, it was revealed that in contrast to the elevated mitosis in rats Ox. for 6 days (Table 2.), ovariectomy for 50 weeks resulted in a decreased mitotic activity (Fig. 18.).

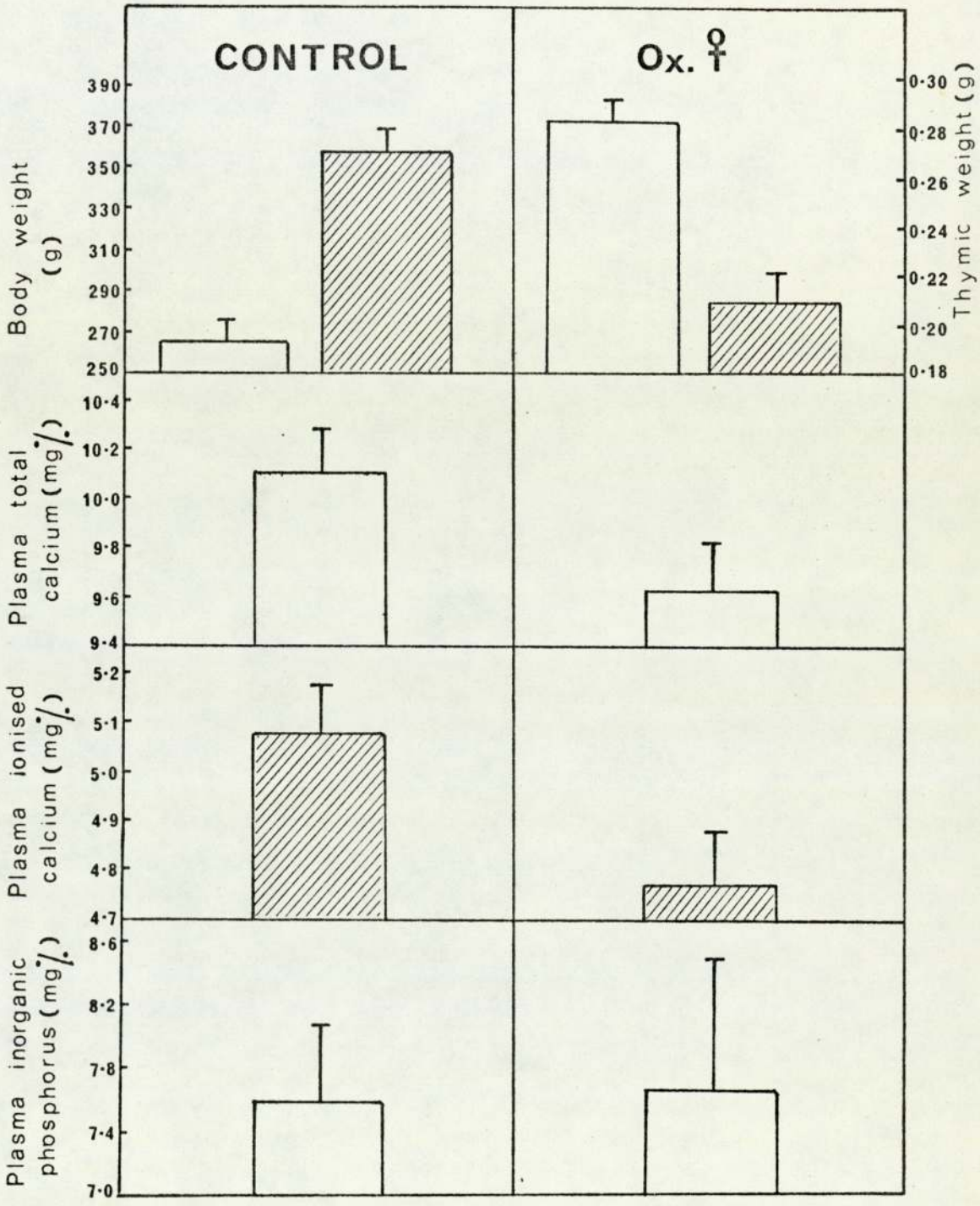


Fig. 13: Group of females ovariectomised (Ox) at 10 weeks old and studied at 50 weeks of age. All animals were in dioestrus. A significant ($p < 0.05$) decrease in the plasma ionised calcium concentration was accompanied by a highly significant ($p < 0.01$) decrease in thymic weight. Between 4-7 animals in each group.

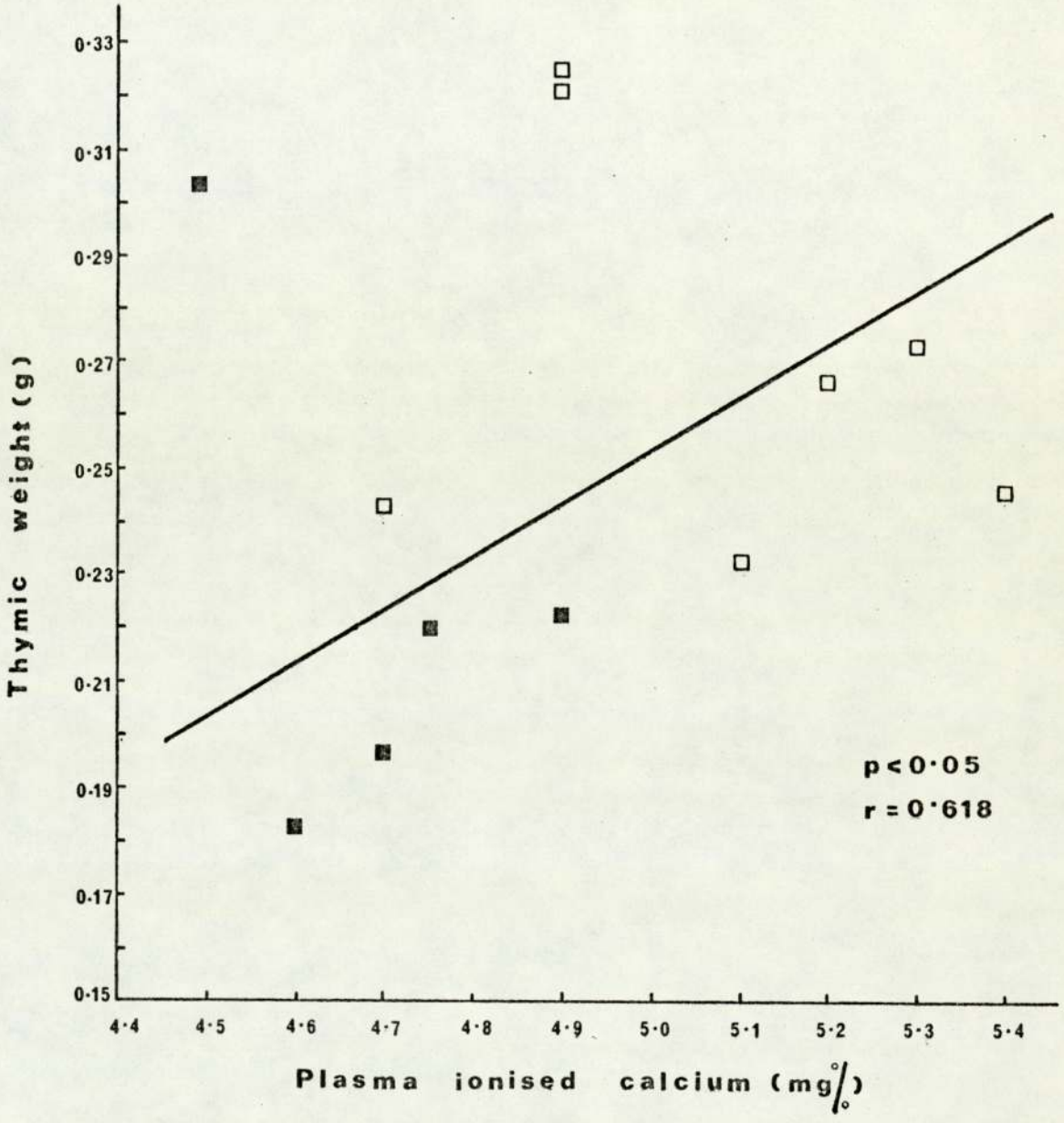


Fig. 14: Significant correlation shown between the plasma ionised calcium and the thymic weight from groups of control and ovariectomised females, both 50 weeks old. □ Control females; ■ Ovariectomised females.

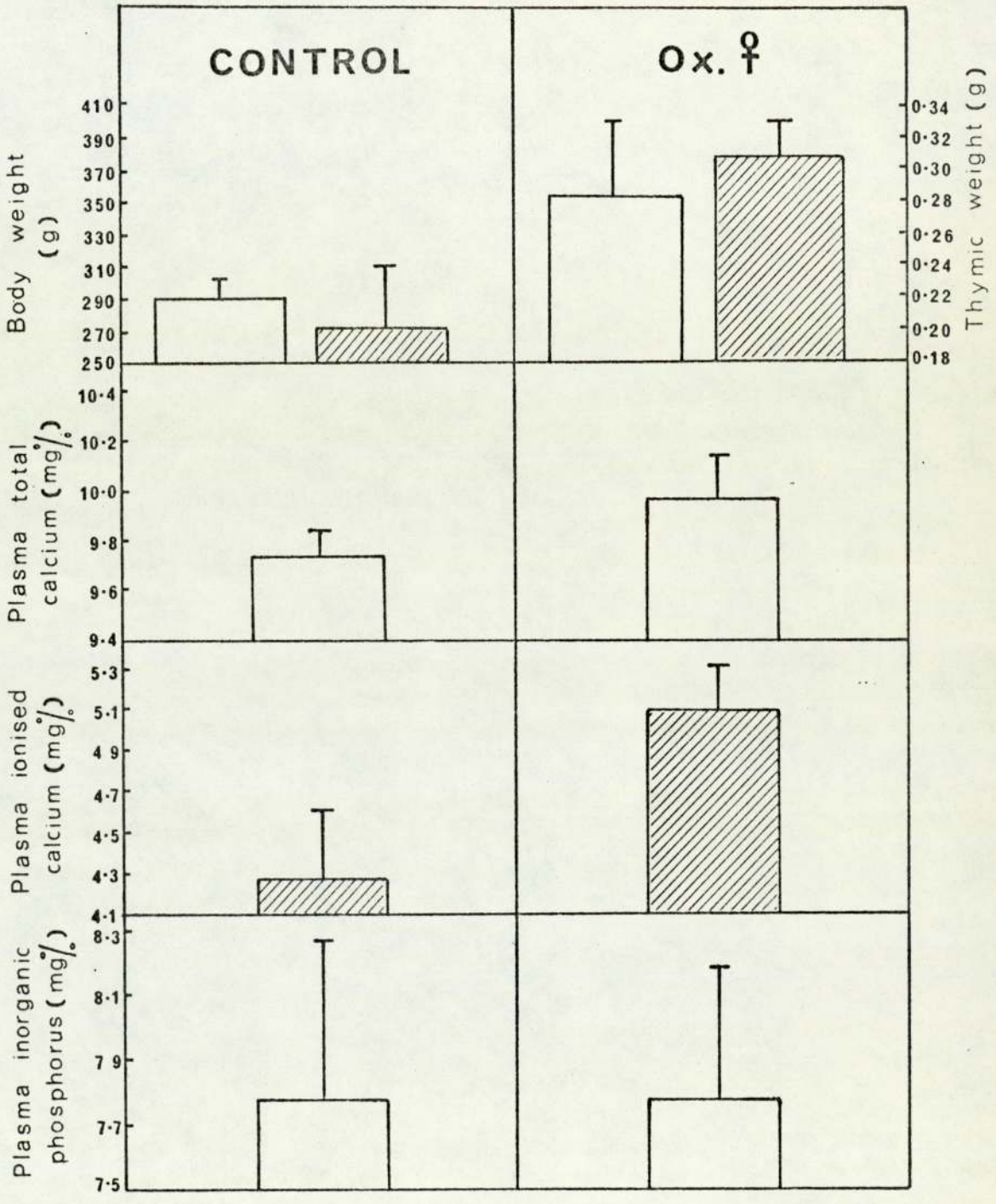


Fig. 15: Group of females ovariectomised (Ox) at 10 weeks old and studied at 50 weeks of age. All animals were in dioestrus. A significant ($p < 0.05$) increase in the plasma ionised calcium concentration was accompanied by a significant ($p < 0.05$) increase in thymic weight. Between 5-6 animals in each group.

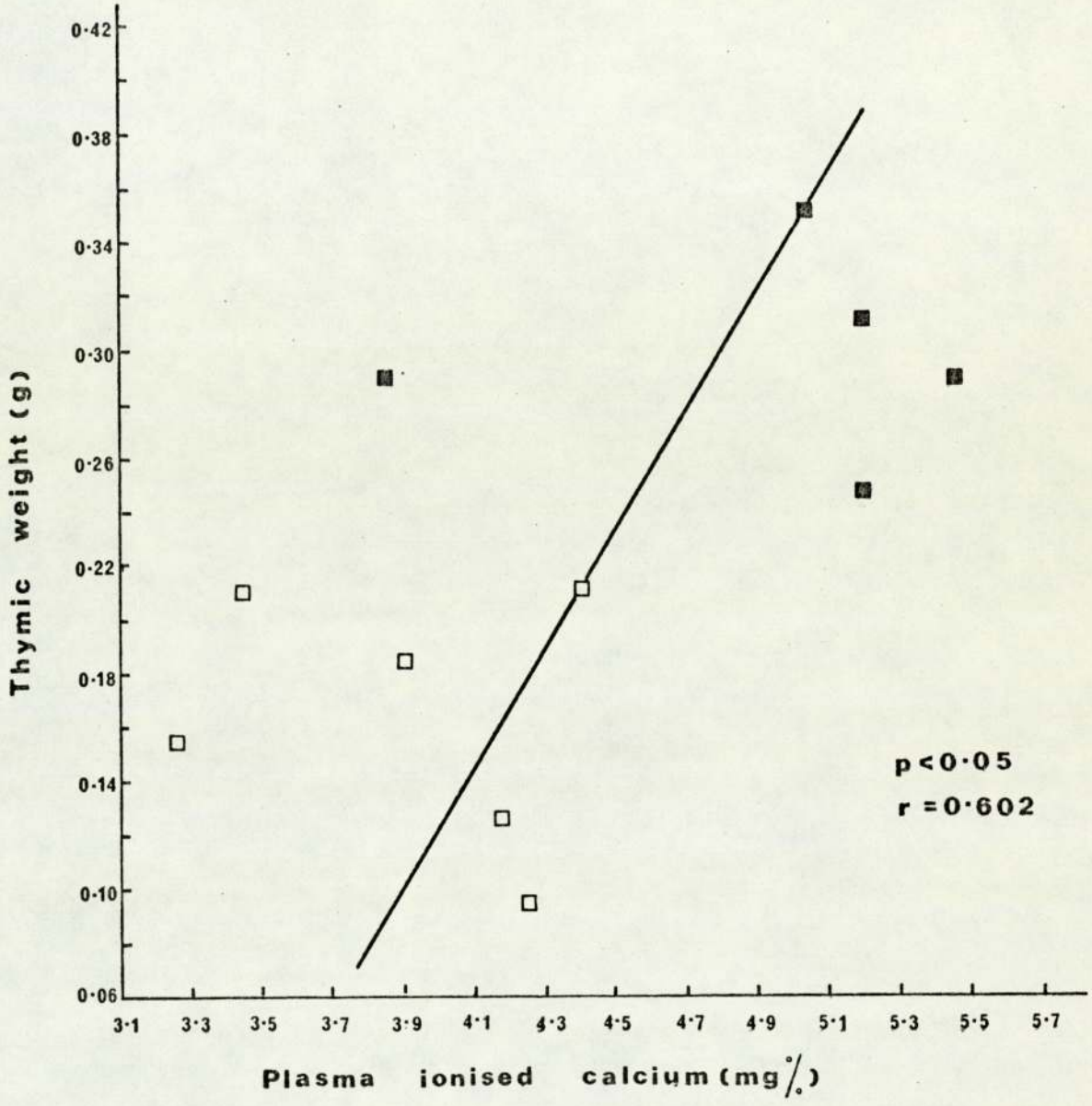


Fig. 16: Significant correlation shown between the plasma ionised calcium and the thymic weight from groups of control and ovariectomised females, both 50 weeks old. □ Control females; ■ Ovariectomised females.

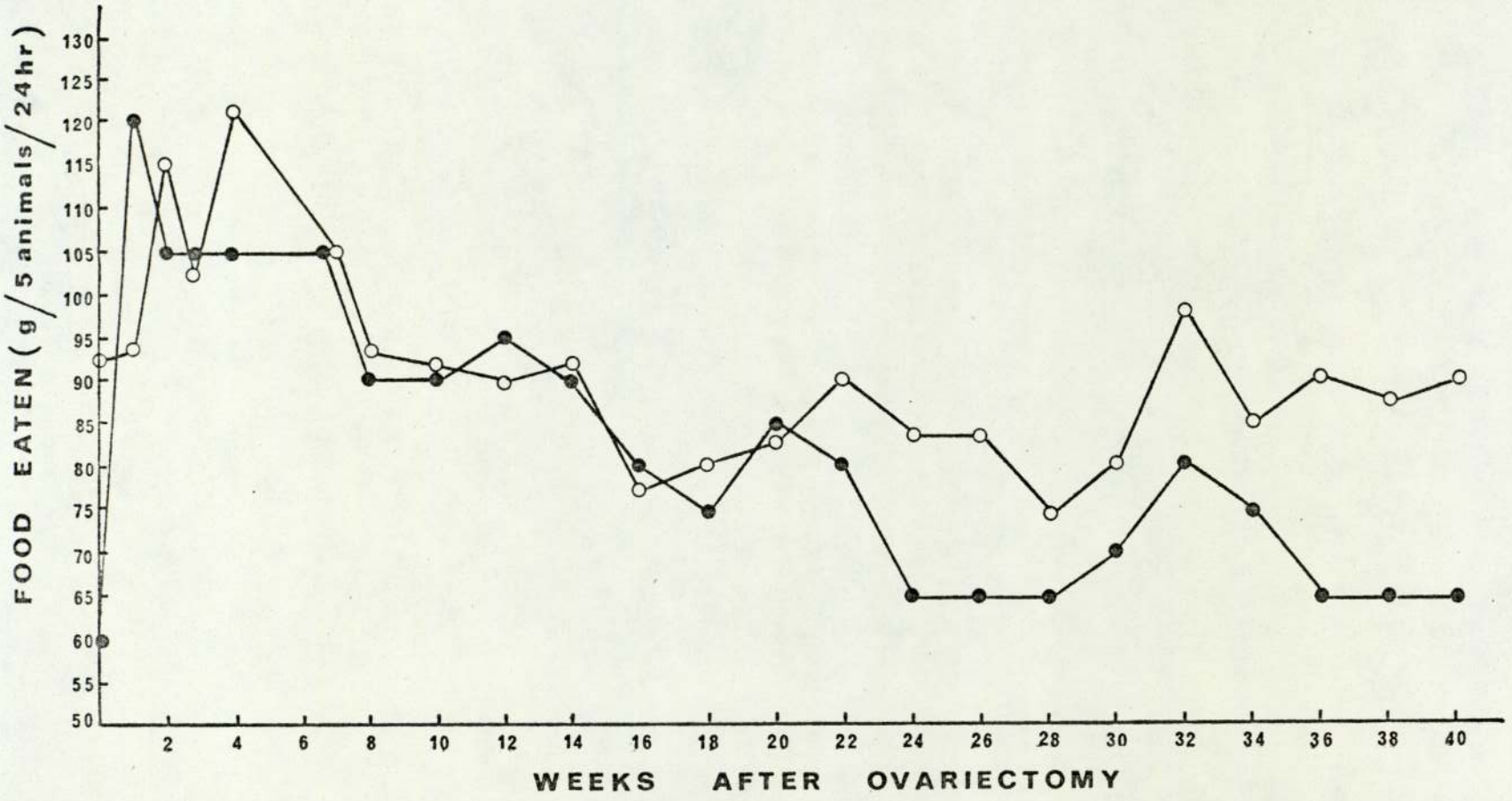


Fig.17: Effect of loss of ovarian hormones upon food consumption in animals studied up to 50 weeks of age. Control females (○) represent the mean food consumption from 3 cages of unsynchronised animals. Ovariectomised animals (●).

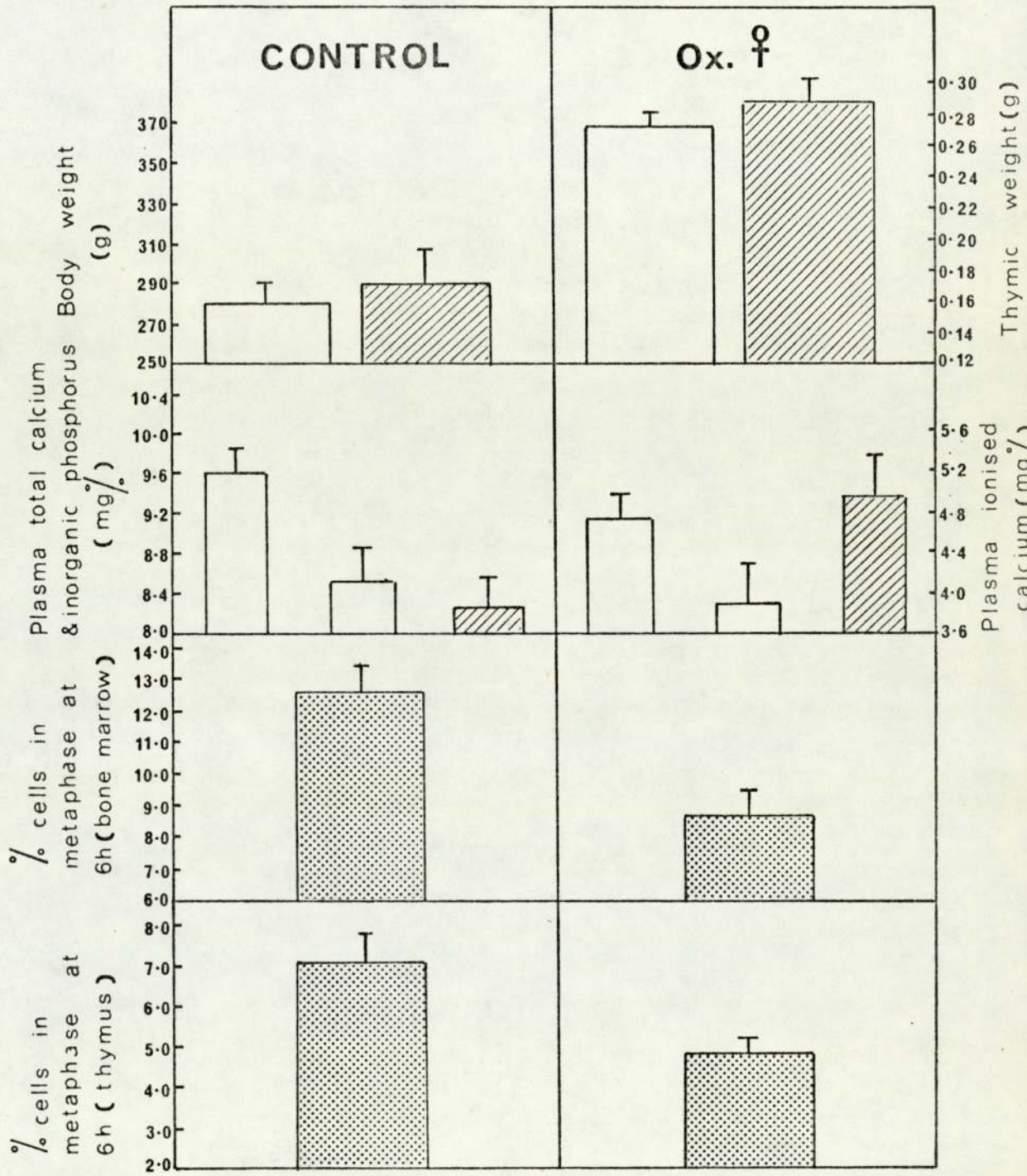


Fig. 18: Disparity between changes in plasma ionised calcium and those in mitotic activity in groups of control and ovariectomised (Ox) female animals. Animals Ox at 10 weeks and studied at 50 weeks of age. All animals were in dioestrus. Significant ($p < 0.05$) elevation of plasma ionised calcium, and highly significant ($p < 0.01$) elevation of the thymic weight; nevertheless very highly significant ($p < 0.001$) decrease in mitotic activity in both tissues. Between 5-8 animals in each group.

Discussion.

This study clearly demonstrates that an elevated extracellular calcium concentration serves as a trigger to initiate events leading to enhanced cell proliferation in bone marrow and thymic cell populations in male (Fig. 1,) rats, whereas a similar regime in female rats (Fig. 1,) was totally ineffectual. Oestrogen rather than progesterone was the ovarian steroid responsible (Table 2.) for this inhibition of mitosis. In contrast, elevation of the extracellular magnesium concentration was found to be effective in stimulating cell proliferation in these two tissues from either male or female animals (Fig. 2). These findings substantiate previous *in vitro* results of these divalent cations upon thymic lymphopoiesis (Morgan & Perris 1974), and imply that magnesium may stimulate cell division via a completely different mechanism.

The mechanism by which calcium stimulates proliferation of thymic lymphocytes and bone marrow cells has been discussed in detail in Introduction 3.6. Whitfield, Rixon, MacManus & Balk (1973) believed that an influx of calcium into the cell in some way inhibited the enzyme phosphodiesterase thus increasing the intracellular concentration of 3'5' cyclic adenosine monophasphate (cyclic-AMP). The cyclic nucleotide was then responsible for activating the chain of biochemical events that culminated in heightened DNA synthesis and ultimately in cell division. This occurs very quickly, since quiescent G° cells of the bone marrow (Lajtha 1962) and the thymus (Whitfield, Rixon, Perris & Youdale 1969) enter mitosis within 4h. of the calcium stimulation (Perris 1971).

The ability of oestrogen to block this sequence of events could occur in a number of ways. If oestrogen were to induce new RNA and protein synthesis in these cells as it does in uterine tissue (Tachi, Tachi & Lindner 1972) such a protein might inhibit one or more of the biochemical

stages which links the calcium-stimulus to mitosis. If such an inhibitor exists it must have an exceedingly short lifespan since thymocytes derived from the relatively oestrogen-rich environment of the female rat responded strongly to the mitogenic challenge of increased ambient calcium levels when cultured *in vitro*. In the whole animal and *in vitro*, oestrogens had to be present at the time the mitotic stimulus was applied in order to exert their inhibitory effect. Clearly (Table 3.; Fig. 6.) oestrogen is dictating subsequent mitotic events at the cellular level, rather than an interference with the calcium homeostatic hormones PTH and TC. Perhaps the simplest explanation, therefore, would be to ascribe to oestrogen the ability to prevent the entry of the calcium ion into sites on or in the cell where the initiation of the co-ordinated chain of events which lead to mitosis occurs. The low ambient plasma calcium concentration in the TPTX female rat, coupled with the presence of oestrogen, which would presumably inbibit diffusional entry further, could account for the diminished mitotic activity in the bone marrow and thymus of the aparathyroid female (Table 3.).

The role played by the oestrogen molecule in these mitotic events is believed to be one of preventing access of extracellular calcium ions to some membranal receptor. The relationships between divalent cations, cyclic nucleotides and the sex steroids has recently been thoroughly investigated (Morgan, Hall & Perris 1975a, b; Perris & Morgan 1975) and there is strong evidence to suggest that the divalent cations themselves, calcium and magnesium, may well be the ultimate agents promoting DNA synthesis in the cell nucleus.

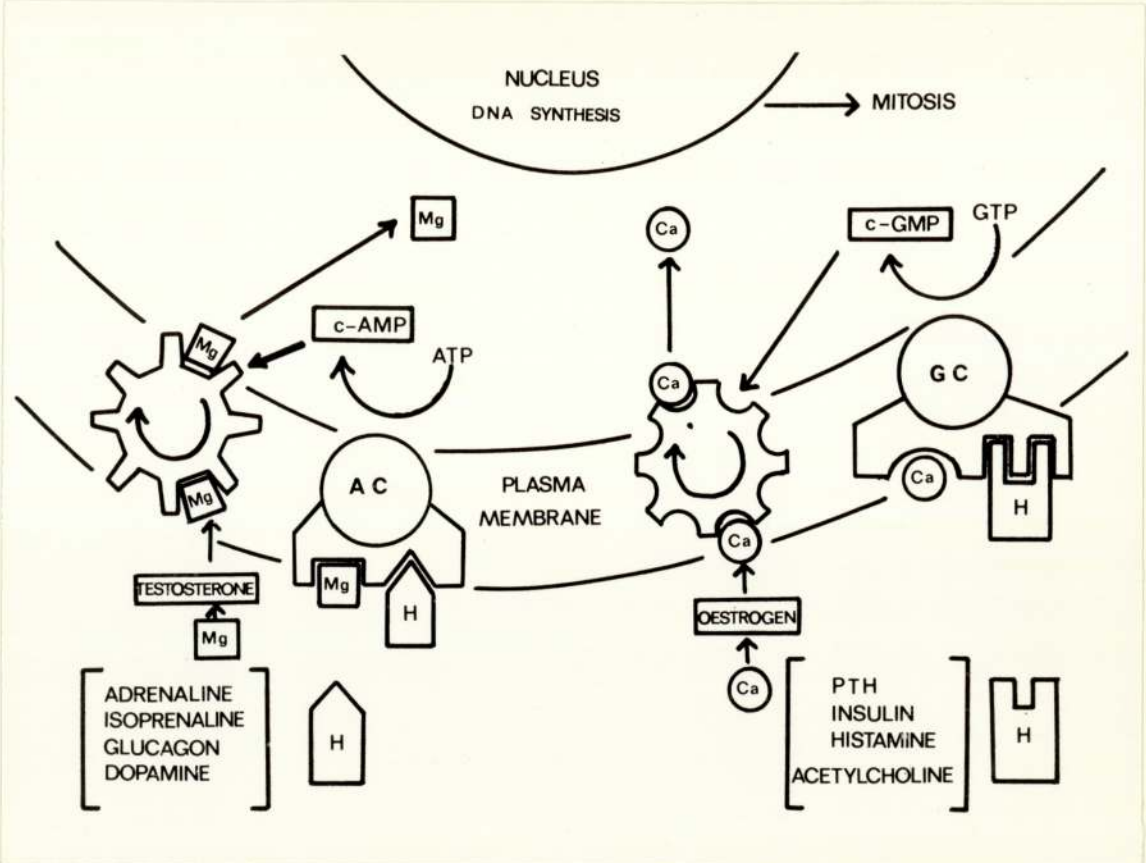
Indeed, as a further consequence of both calcium and magnesium (Morgan & Perris 1974; Smith, Gurson, Riddell & Perris 1975) ions being mitogenic in thymic lymphocytes, it has been shown (Morgan, Hall & Perris 1975a) that several mitogenic hormones have an obligatory requirement for either calcium

or magnesium ions. Thus there have been shown to be two distinct groups of hormones that are known to enhance thymic lymphopoiesis. The first group of hormones, which includes parathyroid hormone, acetylcholine, insulin and histamine (Morgan, Hall & Perris 1975a) and a number of other agents (Perris & Whitfield 1969; Whitfield, Perris & Youdale 1969; Whitfield, Rixon, MacManus & Balk 1973) require calcium ions in order to fulfil their mitogenic role; an action which is inhibited by the presence of oestradiol (Morgan, Hall & Perris 1975a). The second group of hormones which includes glucagon, dopamine, and isoprenaline (Morgan, Hall & Perris 1975a) and probably adrenaline and certain prostaglandins (MacManus, Whitfield & Youdale 1971; Whitfield, MacManus, Braceland & Gillan 1972) require magnesium ions in order to exert their mitogenic potential; an action which is inhibited by the presence of testosterone (Morgan, Hall & Perris 1975a,c).

This clear dichotomy between the calcium-dependent hormones and the magnesium-dependent hormones may be the consequence of the two different cyclic nucleotides utilised by these hormones as 'second messengers', (Posternak 1974). Thus the well known antagonistic actions of adrenaline and acetylcholine or of glucagon and insulin (each hormone having specific and opposing cation dependancy) have been ascribed to the ability of the agonist to increase either intracellular cyclic 3'5' adenosine monophosphate (cyclic-AMP) or cyclic 3'5' guanosine monophosphate (cyclic-GMP) concentrations (Goldberg, Haddox, Enstensen, White, Lopez & Hadden 1973). Since in the presence of divalent cations, both cyclic-AMP (Whitfield, Rixon, MacManus & Balk 1973) and cyclic-GMP (Whitfield, MacManus, Franks, Gillan & Youdale 1971) enhance thymic lymphopoiesis it is feasible that these cyclic nucleotides may be major participants along the two mitogenic axes.

Indeed, it had been shown that both adrenaline (MacManus, Whitfield & Youdale 1971) and glucagon (N.H. Hunt, personal communication) can stimulate adenylate cyclase in plasma membrane preparations from thymic lymphocytes, and both these agents and other magnesium-dependent hormones (Morgan, Hall & Perris 1975a) activate this enzyme in other tissues thus provoking an increase in intracellular cyclic-AMP levels (Robison, Butcher & Sutherland 1971). Although the activation of adenylate cyclase by compounds such as glucagon, dopamine, isoprenaline and epinephrine is itself a magnesium-dependent event (Birnbaumer, Pohl, Michiel, Krans & Rodbell 1970), it is believed that as a consequence of the elevated intracellular cyclic-AMP, this nucleotide may function to regulate magnesium transfer into the cell (Morgan, Hall & Perris 1975b). Testosterone, since it blocks the mitogenic action of high exogenous cyclic-AMP, adenylate cyclase-stimulationg hormonal mitogens and raised extracellular magnesium concentration (Morgan, Hall & Perris 1975a, b, c) must either block this magnesium flux into the cell, or a step subsequent to it (see Figure).

In an analogous fashion, acetylcholine, insulin and histamine might somehow be linked to guanylate cyclase. Certainly in other tissues this appears to be true (Goldberg, O'Dea & Haddox 1973a; Illiano, Tell, Siegel & Cuatrescasas 1973), and indeed PTH fails to activate adenylate cyclase in thymic lymphocytes (Whitfield, MacManus, Youdale & Franks 1971). The obligatory requirements of these group of mitogens for calcium may be a reflection of the necessity for this ion as a cofactor to activate a membranous cyclase enzyme (Hardman, Beavo, Gray, Chrisman, Patterson & Sutherland 1971). Alternatively, calcium may act as an intermediary between the hormone and a soluble guanylate cyclase in the cytoplasm (Schultz, Hardman, Hurwitz & Sutherland 1973). Consequently, these calcium-dependent hormones possibly by stimulating guanylate cyclase and increasing intracellular cyclic-GMP could provoke a calcium influx into the cell. Certainly



Proposed model (after Perris & Morgan, 1975) of the interaction between divalent cations, hormones and cyclic nucleotides in the control of mitosis. Two distinct and totally independent routes to mitosis exist:-

(i) Hormones requiring extracellular calcium ions:

Membranous guanylate cyclase is stimulated by the combined action of calcium and hormone (H). The cyclic-GMP thus formed facilitates calcium influx into the cytosol.

(ii) Hormones requiring extracellular magnesium ions:

Membranous adenylate cyclase is stimulated by the combined action of magnesium and hormone (H). The cyclic-AMP thus formed facilitates magnesium influx into the cytosol.

Oestrogen and testosterone impede the calcium and magnesium ion influx respectively, the necessary prelude to mitosis.

cyclic-GMP is known to enhance calcium influx into splenic T-lymphocytes (Freedman, Raff & Gomperts 1975). The probable mechanism of action of oestradiol can thus be anticipated. Since this steroid blocks the mitogenic action of low exogenous cyclic-GMP, guanylate cyclase-stimulating hormonal mitogens and raised extracellular calcium concentrations (Morgan & Perris 1974; Morgan, Hall & Perris 1975a, b; Smith, Gurson, Riddell & Perris 1975), oestradiol may perhaps impede this calcium movement into the cell (see Figure).

Furthermore, in cation-free media, the high stimulatory dose of cyclic-AMP (10^{-7} - 10^{-8}M) and low stimulatory dose of cyclic-GMP (10^{-10} - 10^{-12}M) which are believed to approximate the normal intracellular concentrations of these nucleotides (Goldberg, O'Dea & Haddox 1973b), are muted (Morgan, Hall & Perris 1975b). From the above evidence it would appear that physiological variations in cyclic nucleotide concentration within the cell might subsequently determine the cationic environment of the cytosol. In turn, therefore, the intracellular calcium or magnesium concentration will function as the ultimate mitogenic messenger, and will dictate whether or not certain quiescent cells will initiate DNA synthesis.

The fundamentals of the model outlined above hinge on the acceptance of the fact that there are two distinct and completely separate routes to mitosis, with each mitogen having no dependence or requirement for the other cation. Thus the model is able to explain clearly why in the presence of physiological concentrations of oestrogen *in vivo* (Smith, Gurson, Riddell & Perris 1975), calcium injections are ineffectual in female animals, but are able to provoke a mitotic response in both bone marrow and thymus tissue of male animals (Fig. 1.). Perhaps not so clear at first sight is the reason why magnesium injections are effective in stimulating cell proliferation in both tissues from male and female animals *in vivo* (Smith, Gurson, Riddell & Perris 1975). Male animals obviously possess circulating

testosterone which would tend to inhibit this response. However, it has been shown (Morgan, Hall & Perris 1975c) that the anti-mitotic action of testosterone was only manifest at pharmacological doses, since no blockade was apparent when concentrations approaching the physiological plasma levels between 2-4ng/ml (Cooper&Waites 1974) were approached. Further support for this specific cation-nucleotide prelude to cell proliferation was shown in the results obtained from the experimentally-induced diabetic animals of Part 2 of this thesis. Insulin has been shown *in vitro* to activate one route leading to mitosis (Morgan, Hall & Perris 1975a) by a calcium-dependent process. Results (Part 2, Table 3.) corroborate this theory since low plasma insulin *in vivo* is accompanied by a reduced mitotic activity in both bone marrow and thymic tissues. Similarly, since evidence would point to insulin leading to elevated cyclic-GMP levels, which would consequently lead to calcium influx into splenic lymphocytes (Freedman, Raff & Gomperts 1975) and presumably elevated cell proliferation, the significant reduction of the splenic weight of the Streptozotocin treated animals (Results, Part 2, Table 3) may be due to diminished splenic lymphopoiesis as a consequence of hypoinsulinaemia.

Despite the well known stimulatory action of oestrogens and progestogens in mammalian mammary (Folley 1955; Smith 1955; Jacobsohn 1958); uterine (Epifanova 1966; Tachi, Tachi & Lindner 1972; Martin, Das & Finn 1973; Lee, Rogers & Trinder 1974) and vaginal (Martin & Claringbold 1960; Thrasher, Clark & Clarke 1967; Lee & Rogers 1972) tissues, the studies outlined in the results section above have shown that in non-target tissues, especially the lymphopoietic and haematopoietic tissues, oestrogen inhibits the cell division induced specifically by the calcium ion.

Indeed, oestrogen is able to exert a much wider influence upon tissue growth generally. Thus female rats are smaller (Jackson 1913) and grow less rapidly than males of the same age (Wade 1972), as a result of oestrogen action (Freudenberger & Clausen 1937a, b; Clausen & Freudenberger 1939; Korenchewsky, Burbank & Hall 1939; Matthews, Schwabe & Emery 1942). Whereas the effect of the female gonadal hormones is exerted continuously during adult life, the effect of testosterone appears to be a persisting consequence of an action exerted prepubertally (Harvey, Harvey & Hutchinson 1973).

There is considerable evidence that the ovarian hormones influence physical activity and food intake patterns which might account for the effects upon growth and body weight. Thus, during dioestrus food intake, level of activity and body weight all increase (Tarttelin & Gorski, 1973; Kenney & Mook 1974). The converse occurs at oestrus. When the ovaries are removed, animals show an increase in the food consumed (Mook, Kenney, Roberts, Nussbaum & Rodier 1972). Indeed it has been shown that intact rats consume 8-12 discrete meals a day (Teitelbaum & Campbell 1958; LeMagnen & Devos 1970); ovariectomy initially causes an increase in the meal size and a reduction in the meal frequency (Kenney & Mook 1974). When oestradiol is administered to the Ox. animal, food intake decreases (Wade & Zucker 1970; Rodier 1971), and body weight returns to a low level. Over the period of several months after ovariectomy, body weight remanins elevated (Figs. 13, 15. & 18.), although hyperphagia is only transitory (Mook, Kenney, Roberts, Nussbaum & Rodier 1972; Tarttelin & Gorski 1973). It is believed that this obesity in chronically Ox. rats is regulated, since they eventually reduce their food intake and regulate their body weight at an elevated but re-set level (Mook, Kenney, Roberts, Nussbaum & Rodier 1972; Tarttelin & Gorski 1973).

A marked contrast was shown in the plasma calcium levels and also the mitotic activity in bone marrow and thymic tissue in the short term (6 days) Ox. as compared to the long term (50 weeks) Ox. female rats. In the former there was no significant difference between total or ionised plasma calcium levels in control or the Ox. animals (Fig. 5.); in the latter, however, total calcium was again unchanged in all of the three groups of animals (Figs. 13, 15. & 18.), but two out of the three groups exhibited significantly elevated plasma ionised calcium (Figs. 15. & 18.). This anomaly may partly be attributed to an altered handling of calcium with age. In order to maintain normocalcaemia, PTH-induced bone resorption occurs continually (Potts, Buckle, Sherwood, Ramberg, Mayer, Kronfeld, Deftos, Care & Aurbach 1968) and in the female this process is inhibited by oestrogen (Lafferty, Spencer & Pearson 1964; Eisenberg 1966; Stott 1968; Young, Jasani, Smith & Nordin 1968; Anderson, Greenfield, Posada & Crackel 1970; Atkins, Zanelli, Peacock & Nordin 1972; Orimo, Fujita & Yoshikawa 1972). In the young Ox. female one might thus expect a transient hypercalcaemic episode which would be rapidly counteracted by an elevated bone accretion directed by calcitonin. However, adult animals are refractory to the action of TC (Care & Duncan 1967; Orimo & Hirsch 1973; Roycroft & Talmage 1973), and even though bone turnover rate is reduced (Copp 1969a), in the absence of oestrogen, PTH-induced bone resorption will predominate. Consequently, one might anticipate hypercalcaemia in these animals, as indeed Gallagher & Nordin (1973) reported in post-menopausal female patients. Why the long term ablation of the ovaries in the rat was manifest by altered plasma ionised but not total calcium levels is unknown.

The enhanced cell proliferation following short term ovariectomy, and indeed subsequent calcium injections to these animals (Table 2.), has been attributed to an influx of calcium ions into the cell; the oestrogen molecule impedes this process. Thus, even though the same sequence of

events presumably operates in adult Ox. females, a reduced cell proliferation was apparent, despite elevated ionised calcium levels (Fig. 18.). It must be stressed that the short term Ox. animals were pair fed, whereas the long term Ox. group were not, and hence the apparent anomolous result could be due to diminished nutrition. Since starvation is known to arrest dividing cells in the G_1 phase of the cell cycle (Cameron & Cleffmann 1964) this could be a plausable explanation.

The dietary changes known to occur throughout the oestrous cycle (Tarttelin & Gorski 1973; Kenney & Mook 1974) are believed to be a consequence of oestrogen acting through the ventromedial nucleus (VMN) (Wade 1974). Since calcium infusion into the cerebral ventricles of rats causes elevated food intake (Myers, Bender, Krstic & Brophy 1972) an antagonism between plasma oestrogens and calcium centrally has been proposed (Bull, Hurley, Kennett, Tamplin & Williams 1974) to bring about these changes.

The VMN has also been accorded a significant role in the control of oestrous rhythmicity (Carrer, Asch & Aron 1973) in female rats. The distinguishing attribute of such rats is that they undergo regular 4-day cycles characterised by fluctuating levels of plasma oestrogens and progestogens (Butcher, Collins & Fugo 1974). The concept that variations in these levels throughout the oestrous cycle of mice and rats may dictate subsequent mitotic rhythm in certain non-target tissues has resulted in conflicting opinions. Ebling (1954) showed that in the sebaceous gland there was no correlation between mitotic activity and the plasma oestrogen level. Bullough (1950) showed that the ear epidermis underwent cyclic growths similar to the target tissues, and hypothesised that oestrogens might be general mitotic stimulants in tissues throughout the body. However, work of Carter (1953) who studied the effect of oestrone injections upon target and non-target tissues does not support this contention.

In contrast, analysis of bone marrow and thymic cell populations revealed a cyclic rhythmicity of mitotic activity showing a close parallelism not between the plasma oestrogen but the plasma total calcium concentration. Even though oestrogen itself is not-mitogenic towards these tissues, it is conceivable that since the food consumption throughout the cycle has been postulated to be a consequence of the intracellular calcium concentration determined by the respective oestrogen level in the VMN, a similar mechanism may operate peripherally.

Plasma oestradiol 17-β levels are characterised by sharply rising concentrations throughout pro-oestrus; peak concentrations are shown at noon of pro-oestrus. Subsequently levels sharply decline throughout oestrus, and remain at these low levels throughout metestrus and dioestrus (Butcher, Collins & Fugo 1974). Thus, since there is a cyclic variation of total plasma calcium (maximal concentrations at oestrus and minimal concentrations at pro-oestrus) it might well be that oestrogens themselves are responsible for these fluctuations in total plasma calcium throughout the 4-day oestrous cycle.

Since bone is considered the major control tissue in calcium homeostasis (see Introduction 3.1), this might in part be explained as a consequence of the well known oestrogen inhibition of PTH-induced bone resorption (Lafferty, Spencer & Pearson 1964; Eisenberg 1966; Stott 1968; Young, Jasani, Smith & Nordin 1968; Anderson, Greenfield, Posada & Crackel 1970; Atkins, Zanelli, Peacock & Nordin 1972; Orimo, Fujita & Yoshikawa 1972). Thus, the rising oestradiol 17-β levels throughout pro-oestrus would lead to maximal inhibition of bone resorption and account for the low plasma calcium at pro-oestrus. Due to the sharp fall in oestradiol levels in late pro-oestrus, there would be a sudden release from this inhibition as oestrus was approached and hence calcium levels might be expected to rise throughout oestrus.

On the other hand, oestrogen might be modulating vitamin D metabolism in some way. It may be anticipated that oestrogen is exerting its effect by an action upon the kidney cell. Thus if oestrogen is able to inhibit calcium influx into the renal cell, as it is known to in skeletal, bone marrow and thymus tissue, the resultant low intracellular calcium would be the stimulus for enhanced production of 1,25 DHCC. The time lag for 1,25 DHCC synthesis and its calcium-promoting action would result in hypercalcaemia at oestrus. Furthermore, Nicholson, Akhtar & Taylor (1975) have shown in the Japanese quail that oestrogen treatment resulted in much greater concentrations of duodenal CaBP. It might be that the female rat shows greater concentrations of CaBP as the plasma oestrogen levels rise. If this is the case, then an elevated CaBP at pro-oestrus would be manifest by hypercalcaemia at oestrus. It is possible, therefore, that one or maybe all of these mechanisms might be in operation at any one time.

Further evidence to substantiate the postulate that oestrogens might control plasma total calcium levels is shown firstly by the fact that following ovariectomy, animals do not cycle, but remain permanently in dioestrus as shown by vaginal smear and plasma total calcium levels (personal observations). Secondly, oestradiol injections into intact female rats resulted in a small but significant reduction in plasma total calcium (Shai & Wallach 1973).

The strong correlation shown between plasma total calcium and mitotic activity throughout the 4-day cycle implies that this long exposure of about a day to an altered calcium environment does influence division despite the presence of oestrogen. In contrast to this relatively long exposure of calcium to these cells throughout the cycle, a short 4h. exposure brought about by injecting $CaCl_2$ into females at each stage of the oestrous cycle was without effect, even though a similar regime to males provoked a further mitotic increment. Hence, despite the presence of oestrogen, these cells eventually responded to the changed calcium environ-

ment, even though oestrogens clearly retard this response.

Plasma total calcium levels are known to be finely controlled within very narrow limits throughout a 24hr. period, such that circadian variability of male rats is only of the order of 0.6mg% (Hunt & Perris 1974). In the female an equally striking degree of control was observed throughout the 4-day oestrous cycle; the change in plasma total calcium between the maximal value at oestrus and the minimal at pro-oestrus was also approximately 0.6mg%. This apparently small change from pro-oestrus to oestrus was however significant ($p < 0.05$). Throughout the year there was a slight variation in calcium levels, which was reflected in the mitotic activity. Since these calcium levels have been assumed to be a reflection of the circulating oestrogen levels, plasma oestrogens might also fluctuate seasonally. Throughout the year the weight of the thyroid gland certainly changes (Manolov & Boyadzhiev 1973) and presumably the parathyroid gland also follows a similar pattern. An antagonism between the two secretory rates of PTH and oestrogens throughout the year may well account for the observed changes.

Variations throughout the oestrous cycle in the haematopoietic tissue have also been reported in other rodents. In the mouse, Fruhman (1966) has shown maximal splenic erythropoiesis at oestrus consistent with the maximal numbers of splenic stem cells at pro-oestrus (Lord & Murphy 1972). Since oestrogens are generally regarded as depressors of both erythropoiesis and circulating red blood cell numbers (Vollmer & Gordon 1941; Finkelstein, Gordon & Charipper 1944) these observation imply that the splenic stem cells and their progeny are still able to undergo maximal proliferation at oestrus, despite maximal levels (in the mouse) of oestrogen.

Several characteristics which in the male depend upon levels of calcium in the extracellular environment, are known to be reduced in the presence of physiological concentrations of oestrogens. Thus female rats regenerate erythrocyte numbers slower than males (Finkelstein, Gordon & Charipper 1944) due to an action of oestrogen (Fisher 1972; Gordon 1973). Even though

erythropoietin (EPO) is known to stimulate erythropoiesis directly (Introduction 2.2) maximal effect of this stimulus is only seen when it can collaborate with the parathyroid gland to produce hypercalcaemia (Hunt & Perris 1973), the necessary prelude to enhanced erythropoiesis (Perris & Whitfield 1971). In the female the retardation of erythropoietic recovery could be due to an inhibition by oestrogen of the action of EPO upon responsive cells in the bone marrow (Jepson & Lowenstein 1966; Peschle, Rappaport, Sasso, Condorelli & Gordon 1973). Alternatively, the work from this thesis would imply that oestrogen could block the influx of calcium into the bone marrow cells, one of the prerequisties for enhanced cell proliferation in this tissue (Perris, Whitfield & Rixon 1967; Rixon 1968; Perris & Whitfield 1971; Hunt & Perris 1973).

Other mitotic changes in the rapidly renewing lymphopoietic and haematopoietic tissue requiring parathyroid dependency to express their mitogenicity show a marked sex difference. Diurnal fluctuations occur in these tissues of the male rat (Hunt & Perris 1974); in the female these are absent but appear after ovariectomy (N.H. Hunt, personal communication), and females have their own daily rhythm (Smith, Edwards & Perris 1975) dependent upon an interaction between calcium and oestrogen.

The rapid growth in overall body weight of male animals (between 125-225g) is known to be correlated with the plasma ionised calcium levels (Perris, Whitfield & Tolg 1968), but in females body growth rate is retarded as a result of oestrogen action (Freudenberger & Clausen 1937a b; Clausen & Freudenberger 1939; Korenchevsky, Burbank & Hall 1939; Matthews, Schwabe & Emery 1942). This thesis has shown that in two calcium-sensitive tissues (the bone marrow and the thymus) oestrogen prevents hyperplasia. It might well be that division and growth of other cell types may also be influenced by the concentration of this ion. Perhaps other factors such as altered concentrations of hormones might affect the sensitivity of individual cells to calcium, or alter the size of a calcium-sensitive subpopulation of cells

in a tissue. Whether or not the general body growth-retarding property of oestrogen is also a consequence of an inhibition of entry of calcium into several cell types in the body remains to be established.

PART TWO

The role of the calcium homeostatic system in normal and pathological cell proliferation in the small intestinal epithelia

Introduction

Several workers (Urban & Schedl 1969; Spielvogel, Farley & Norman 1972; Birge & Alpers 1973) have acclaimed a role for Vitamin D in promoting cell division and growth in the small intestine. With the known links between Vitamin D and calcium homeostasis, it was reasonable to postulate that physiological fluctuations in plasma calcium or changes in the intestinal handling of calcium might also be reflected by paralleled changes in division rate.

Also, the information acquired in Part 1 of Results, in conjunction with all previous work on the mitogenic action of calcium in lymphopoietic and haematopoietic tissue, raises the important question of whether cell division in other rapidly proliferating tissues (e.g. the intestinal epithelium) may also be governed by the extracellular calcium ion concentration.

Further possible indications towards the general theme of a calcium-dependent control of intestinal cell proliferation came from observations on experimentally induced alloxan-diabetic animals. Such animals exhibited a negative calcium balance (Schneider & Schedl 1972; Schneider, Wilson & Schedl 1973; 1974) in conjunction with the marked intestinal hypertrophy (Jervis & Levin 1966; Nakabou, Okita, Takano & Hagihira 1974). Experiments were thus designed to elucidate any such role that calcium might play in the control of normal or pathological cell proliferation in the intestine.

Results

Histological examination of arrested quasi-metaphase figures has been successfully employed for the estimation of mitotic activity in bone marrow and thymic cell populations (see Introduction 3.5 and Results Part 1). The hope that equally rewarding results would be obtained from the intestinal epithelia was met with some disappointment. It was considered essential, in the first instance, to use stained transverse sections of the intestine to establish that it was the crypt epithelial cells that were responding to any of the exogenous factors applied, and not any other mitotically competent cell population such as the lymphocytes of the lamina propria.

The major drawback experienced, apart from the tedious procedure of obtaining 'well-sectioned' crypts, was the uncertainty in identifying precisely the crypt-villus junction. Thus the crypt cell population seemed to vary between 80 and 120 cells (Table 1; Fig.1). This may have been due to a real variation in cellularity or the imprecise identification of the crypt boundary. Whatever the reason, when mitotic activity was expressed as the percentage of the crypt population arrested in metaphase, very variable results were obtained; mitotic figures did not accumulate linearly with time (Fig.1). In view of this crypt population variability results were therefore expressed as arrested mitoses/crypt. In this way minimal counting error was experienced and there was a linear progression of cells into mitosis over a 4h period (Fig.2). By 6h mitotic figures had migrated from the crypt to the villous epithelium (Plate 1). In all subsequent experiments, a 4h accumulation time was therefore employed.

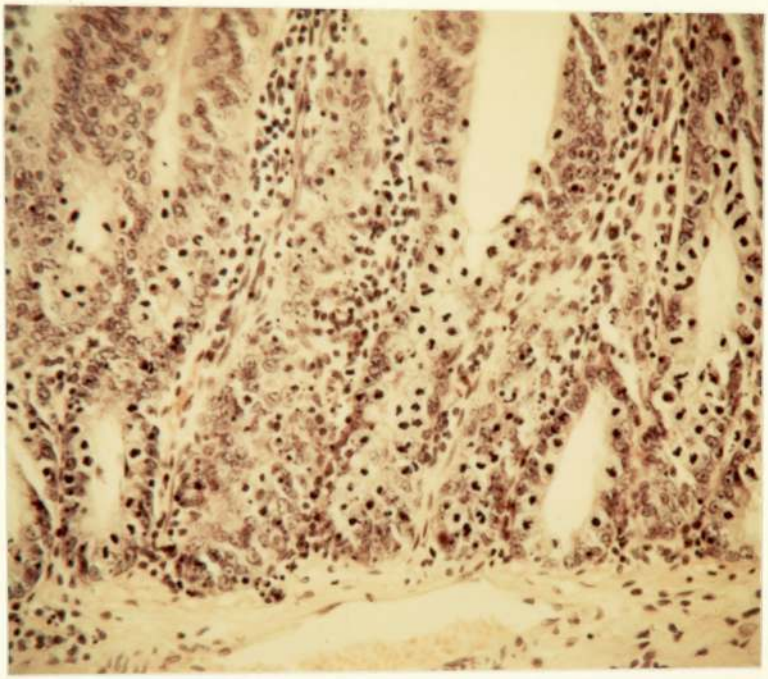


Plate 1: Duodenal crypt showing number of arrested metaphases 6hrs after colcemid. Note the migration of these cells onto the villus.

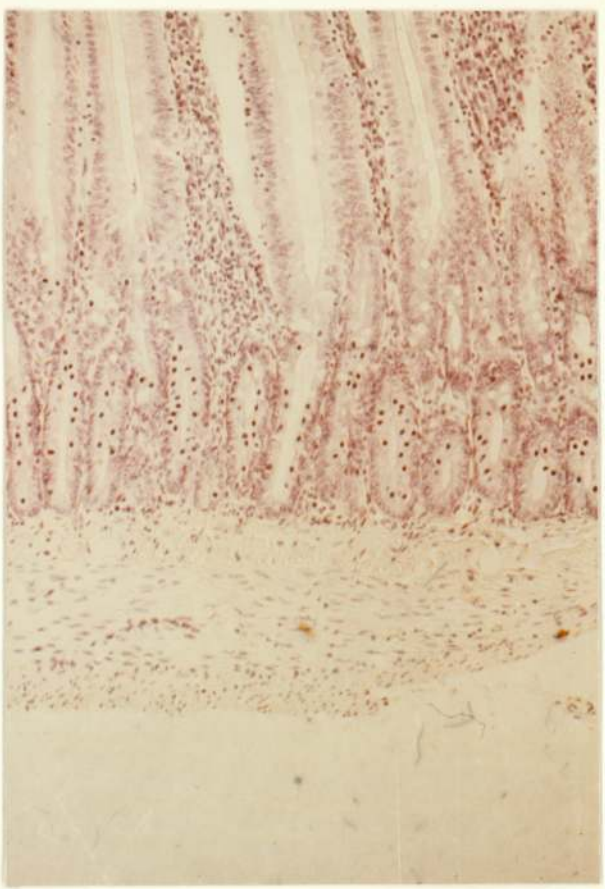


Plate 2: Duodenal crypt showing number of arrested metaphases 2hrs after colcemid.

2 hr		4 hr		6 hr	
	(%)		(%)		(%)
16/99	16.1	31/122	25.4	40/114	35.1
18/100	18.0	25/105	23.8	40/91	44.4
23/91	25.3	30/107	28.0	32/92	34.8
22/86	25.6	26/98	26.5	35/88	39.8
8/88	9.1	33/97	34.0	34/107	31.8
22/111	19.8	35/107	32.7	29/85	34.1
19/93	20.4	22/83	26.5	37/124	29.8
16/96	16.6	22/81	27.2	32/119	26.9
16/87	18.4	34/108	31.5	34/114	29.8
17/107	15.9	22/86	25.6	28/112	25.0
15/85	17.6	28/87	32.2	28/86	32.5
17/91	18.7	33/108	30.6	27/80	33.8

Table 1: Typical results of colcemid accumulation in the duodenum with time, expressed firstly as the number of colcemid metaphases per total crypt cell population showing the variability of the denominator, and secondly as the percentage of colcemid metaphases per crypt.

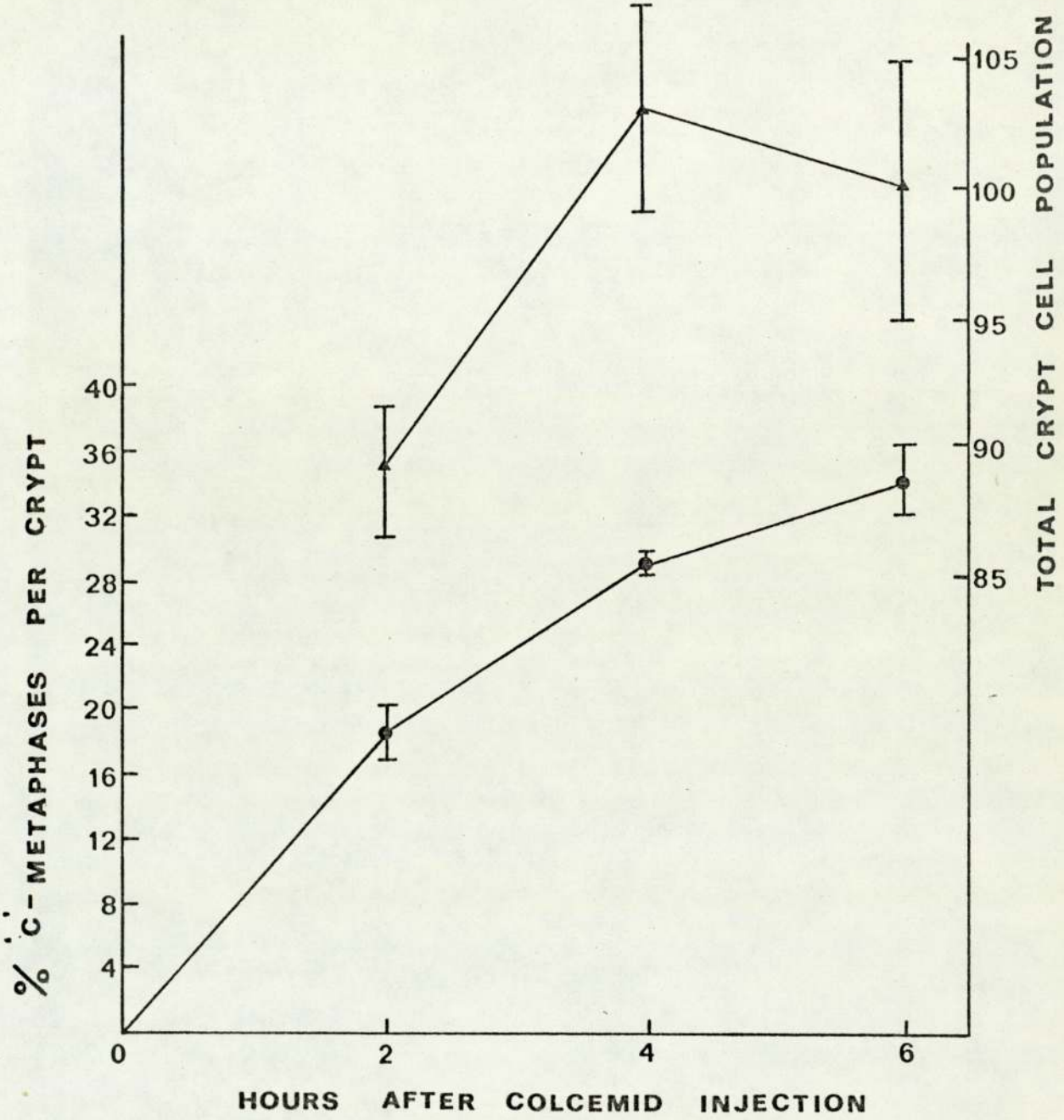


Fig. 1: Non-linearity with time of cell proliferation in the intestine expressed as the % of arrested colcemid ('C') metaphases per duodenal crypt ●——● and the apparent variability of the total crypt cell population ▲——▲. Each point determined from 4 animals; with each animal between 10-20 well sectioned crypts were employed. Values expressed as means ± s.e.m.

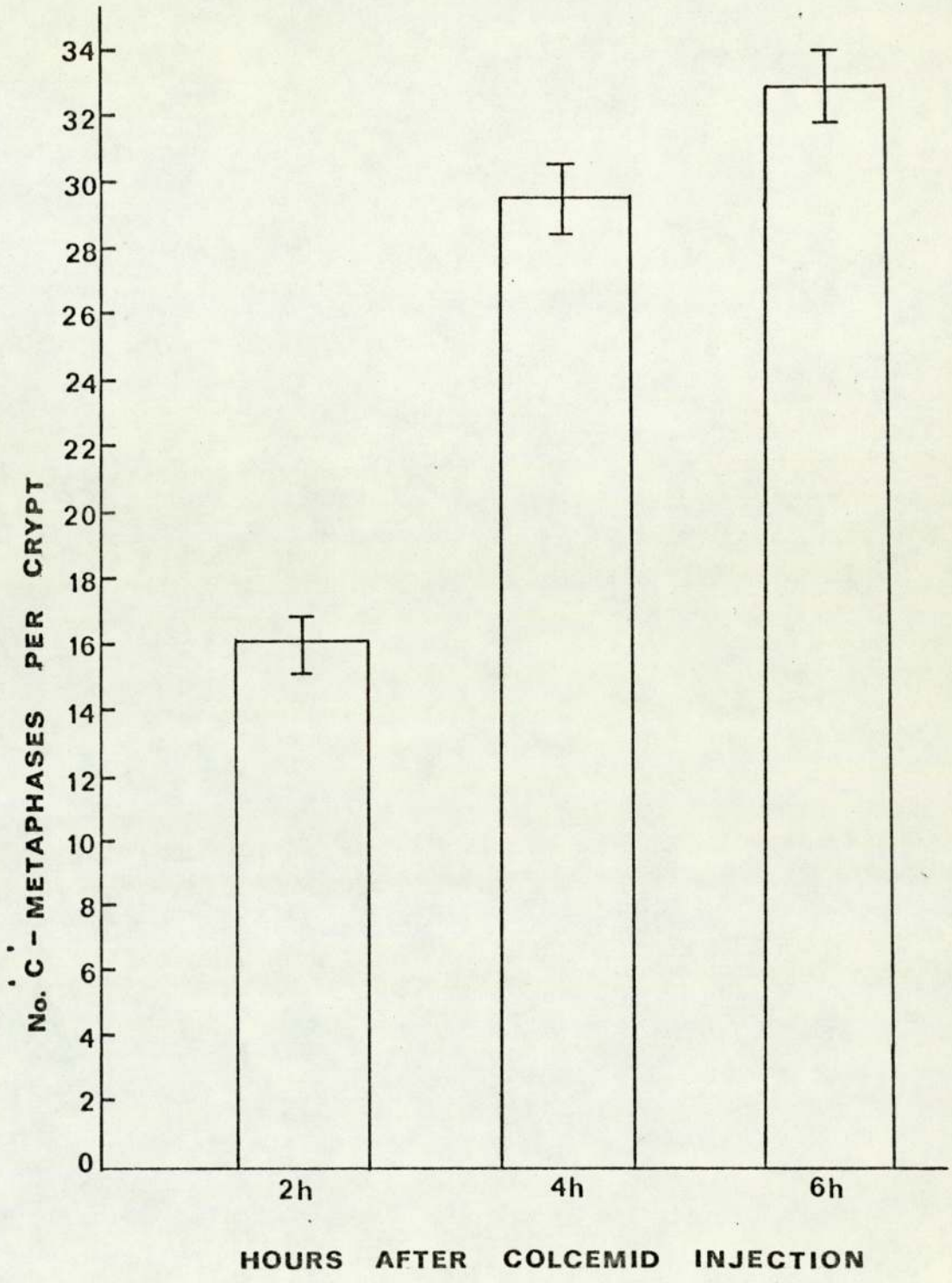


Fig. 2: Linearity with time (up to 4hr) of cell proliferation in the intestine expressed as the number of arrested colcemid ('C') metaphases per duodenal crypt. Values for the time periods indicated following the initial colcemid injection determined from 4 animals, using between 10-20 well-sectioned crypts per animal and expressed as means $\pm$ s.e.m.

To elucidate the possible involvement of the calcium homeostatic hormones in controlling the rate of cell proliferation in the cyrpts of Lieberkuhn, animals were first parathyroidectomised (PTX) by electrocautery and subsequently fed a low calcium diet (Cooper Nutrition Products Ltd, Witham, Essex) following the dietary regime of Hirsch, Gauthier & Munson (1963). This procedure produces a profound hypocalcaemia with plasma calcium concentrations remaining around 5.0 mg% throughout the experimental period (Table 2). Sham-PTX animals were pair-fed on standard diet. When mitotic activity in these groups of animals was assessed, 7 days following ablation of the parathyroid gland, it was found that the PTX group showed a significantly decreased number of mitoses per cyrpt (Fig.3) compared to normal control animals. So in fact did the sham-PTX group. Mitotic activity was not significantly different in the PTX and sham-PTX groups of animals (Plates 5 & 6). Analysis of the post-operative food consumption pattern (Fig.4) showed that by switching to the diet low in calcium, the food intake of the PTX animals dropped drastically. The pair-fed sham-PTX animals also ate considerably less than a similar group of sham-PTX animals allowed food ad libitum (Fig.4). Thus both the PTX and the sham-PTX rats studied 7 days after the operation had severely reduced food intake. Indeed other workers (Hooper & Blair 1958; Brown, Levine & Lipkin 1963; Clarke 1970b; Altmann 1972) have shown that starvation itself reduced cell division in the intestine.

To circumvent this problem imposed by the starvation factor a further group of animals were parathyroidectomised, fed standard diet, and studied 3 days after the operation. Sham-PTX animals

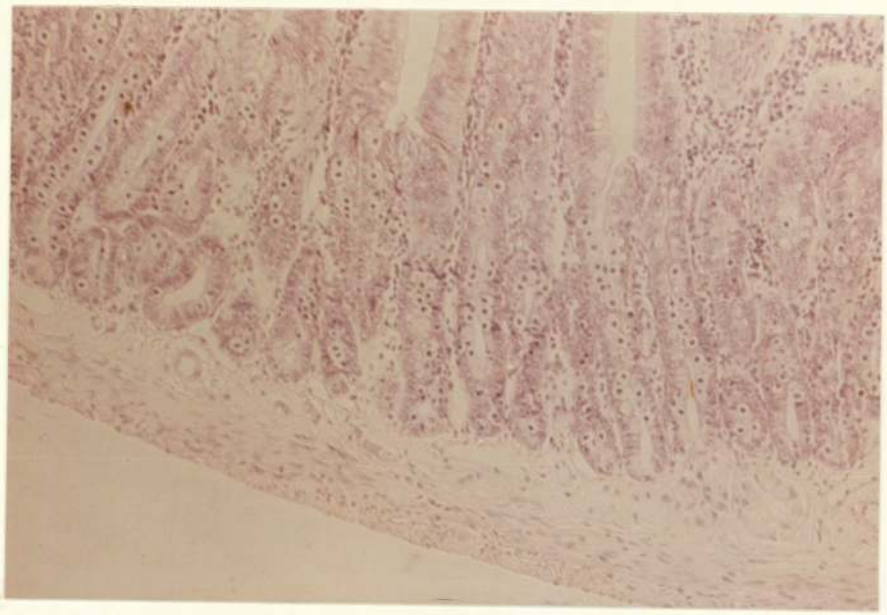


Plate 5: Duodenal crypt showing number of arrested metaphases of sham-PTX animal studied 6 days after cautery.

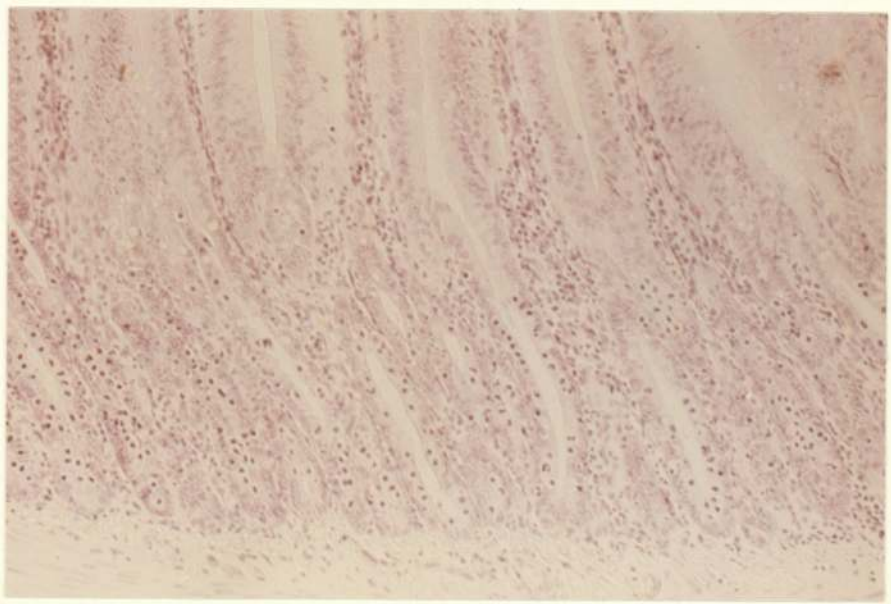


Plate 6: Duodenal crypt showing number of arrested metaphases of PTX animal studied 6 days after cautery.

	Untreated controls	Colcemid Control	$CaCl_2$ injections	Arachis oil control	Vitamin D3(500 I.U.)	Sham PTX (7 day regime)	PTX (7 day regime)	Sham PTX (3 day regime)	PTX (3 day regime)
Plasma Total Calcium(mg%)	9.73±0.13 (10)	9.06±0.19 (4)*	9.66±0.32 (4) NS	9.10±0.18 (4)	10.22±0.24 (4)*	8.73±0.23 (4)	4.75±0.53 (4)**	8.91±0.21 (4)	6.63±0.26 (4) **
Plasma ionised Calcium(mg%)	3.89±0.31 (10)	3.30±0.14 (4) NS	3.97±0.37 (4) NS	3.48±0.11 (4)	4.08±0.19 (4)*	3.58±0.20 (4)	1.60±0.21 (4)**	3.44±0.16 (4)	2.47±0.12 (4) *
Plasma inorganic phosphorus(mg%)	8.00±0.81 (10)	8.60±1.2 (4) NS	9.53±0.97 (4) NS	9.03±0.36 (4)	10.28±0.99 (4) NS	8.04±0.50 (4)	14.17±1.1 (4)*	8.00±0.40 (4)	12.03±0.88 (4) *
Plasma total magnesium (mg%)	2.34±0.1 (10)	2.18±0.2 (4) NS	2.30±0.1 (4) NS	2.35±0.1 (4)	2.27±0.2 (4) NS	2.28±0.1 (4)	2.19±0.3 (4) NS	2.31±0.1 (4)	2.11±0.2 (4) NS

Table 2: Influence of colcemid (for 4 h) and modulation of the calcium homeostatic balance upon various plasma parameters in male rats. Animals received two colcemid injections as specified in Results. Number of animals in each group shown in parentheses. Significance compared to the group immediately preceding the asterisk (*$p<0.05$; **$p<0.01$) or the NS. NS = Not significant.

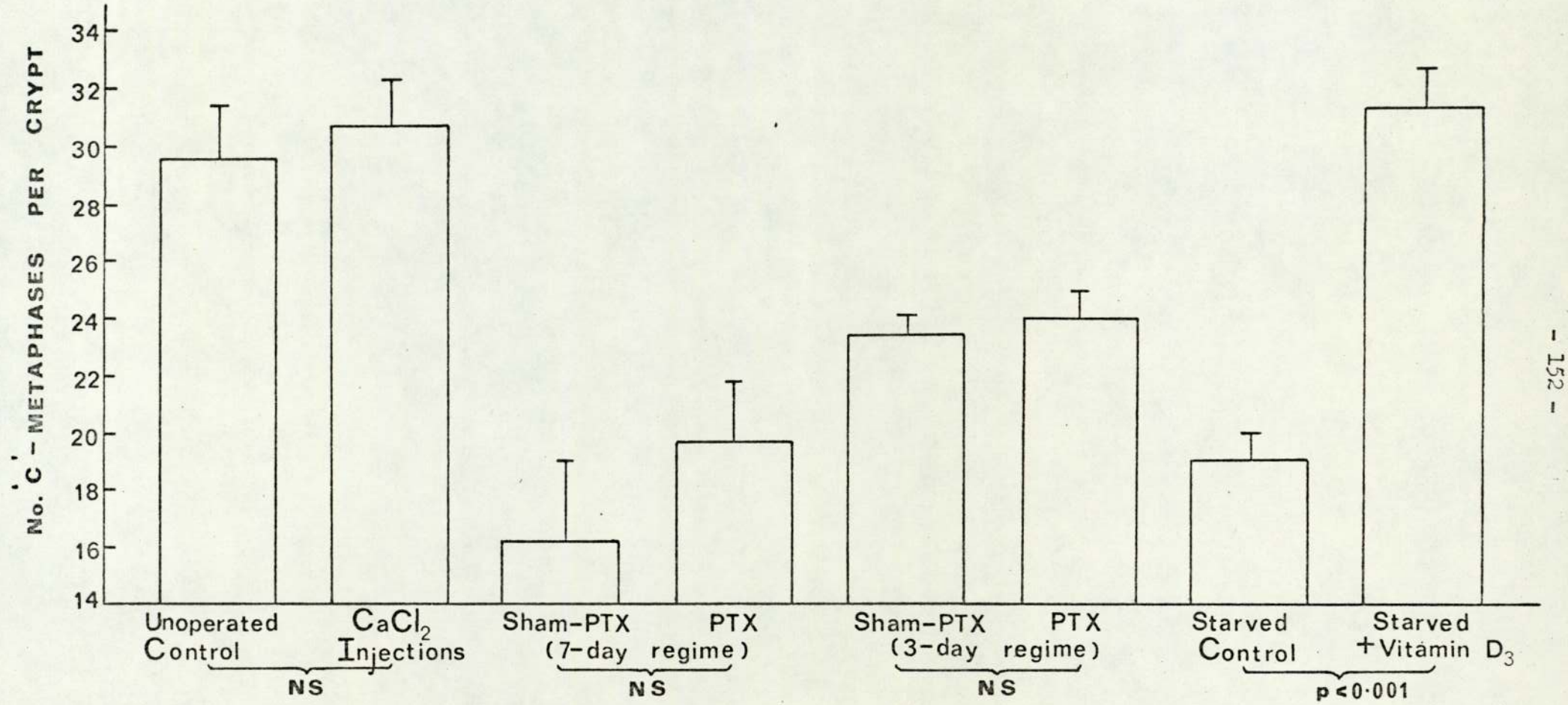


Fig.3: Influence of alterations of the calcium homeostatic balance upon cell proliferation in the duodenum expressed as the number of arrested colcemid ('C')metaphases over a 4h period. Treatments were a series of 62.5mM $CaCl_2$ injections (as detailed in Methods) or parathyroidectomy (PTX). PTX animals (7-day regime) received 41B and Calcium-deficient diet (re Fig.4); those on the 3-day regime received 41B diet for 3 days. Animals receiving Vitamin D or vehicle were starved 48h prior to oral administration. Each value derived from 4 animals, using between 10-20 well-sectioned crypts per animal and expressed as means ± s.e.m. N.S. Not significant.

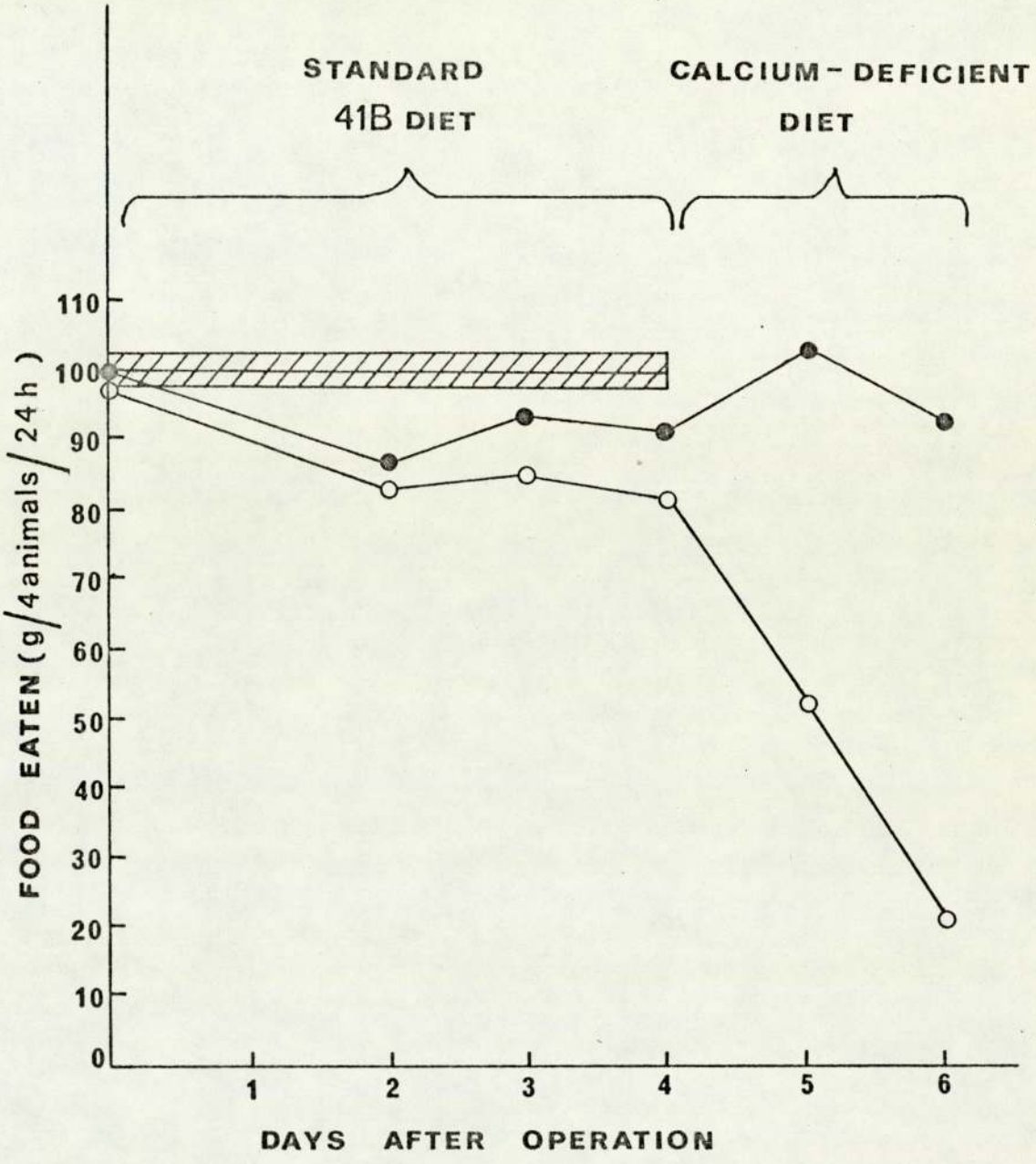


Fig. 4: Influence of parathyroidectomy (PTX) upon daily food consumption of groups of 4 male animals. ▨ Control animals receiving 41B diet ad libitum expressed as mean ± s.e.m. ●—● Sham-PTX animals allowed 41B and calcium deficient diet ad libitum on the days indicated. ○—○ Sham-PTX animals pair-fed with a group of PTX animals according to the regime of Hirsch, Gauthier and Munson (1963).

were again pair-fed with the operated animals. Food consumption up to this time was comparable to unoperated controls (Fig.4) and plasma total calcium in the PTX rats (6.6 mg%) was still significantly lower than the sham-PTX animals (8.9 mg%), as shown in Table 2. Subsequent analysis of the mitotic activity revealed that there was no significant difference between the PTX nor the sham-PTX group (Fig.3; Plates 7 & 8). This indicated that a lowering of the extracellular calcium concentration for a period of 3 days was without effect upon the rate of intestinal cell proliferation.

To bring about a transient elevation of the extracellular calcium, a group of rats were injected with a 62.5 mM solution of $CaCl_2$ as detailed in Methods. Colcemid was administered at 6 & 8½h; control animals also received colcemid and an equivalent volume of 0.9% saline at the appropriate times. Intestinal mitotic activity was found to be the same in both control and calcium-treated rats (Fig.3; Plates 3 & 4). Thus unlike bone marrow and thymus tissue, neither increases nor decreases in plasma calcium concentration affected the rate of cell division in the crypts of Lieberkuhn.

Microtomy has certain inherent disadvantages which have been recognised by Abercrombie (1946); Marrable (1962); & Clarke (1968). Even though the stathmokinetic method appeared to indicate that calcium plays no role in controlling intestinal cell proliferation, it was decided to employ a radioactive label (tritiated thymidine - ^{3}HtdR) to assess intestinal DNA synthesis, the necessary prelude to mitosis. Since the ^{3}H-tdR method does not visualise cells, if these two methods gave comparable results, then it could be reasonably assumed that the ^{3}H-tdR counts truly represented DNA synthesis

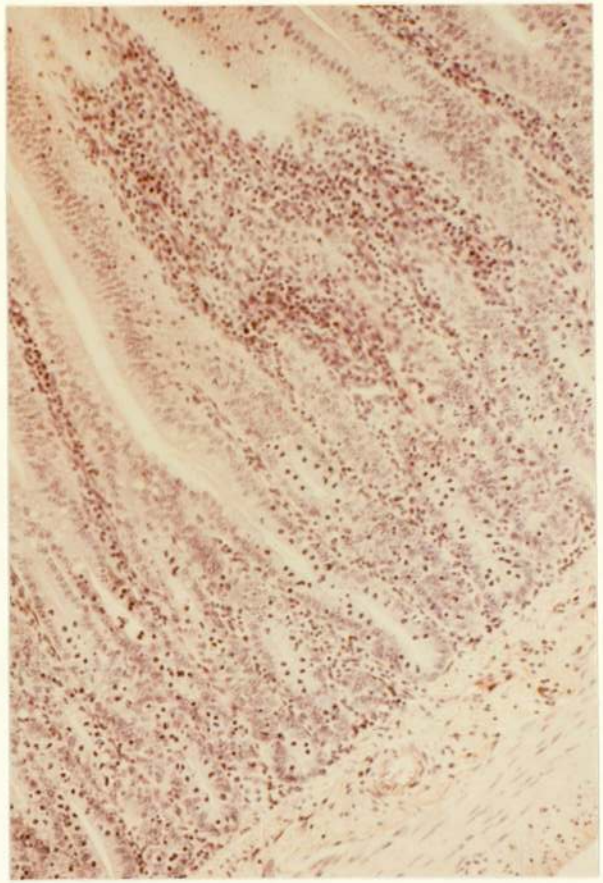


Plate 7: Duodenal crypt showing number of arrested metaphases of sham-PTX animal studied 3 days after cautery.

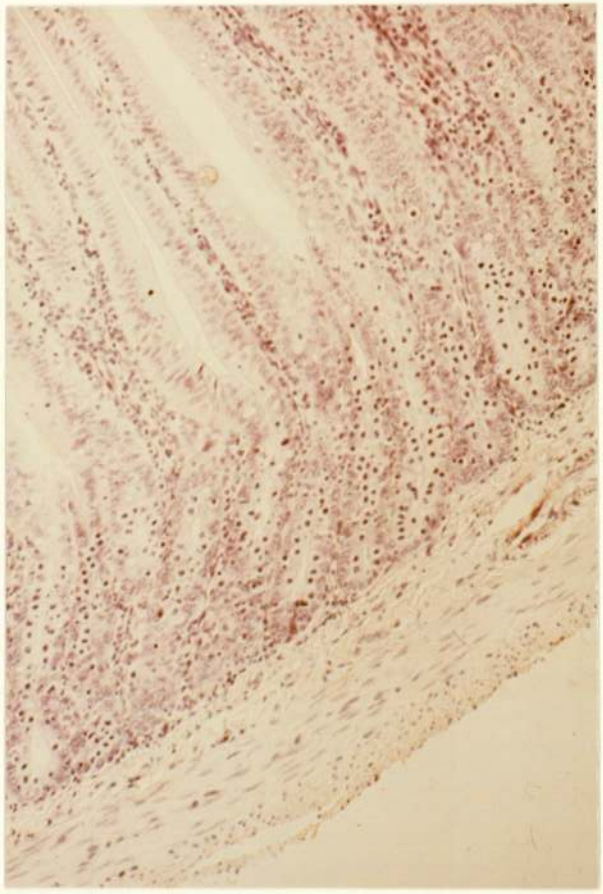


Plate 8: Duodenal crypt showing a number of arrested metaphases of PTX animal studied 3 days after cautery.

Plate 3: Duodenal crypt showing number of arrested metaphases 4hrs after colcemid.

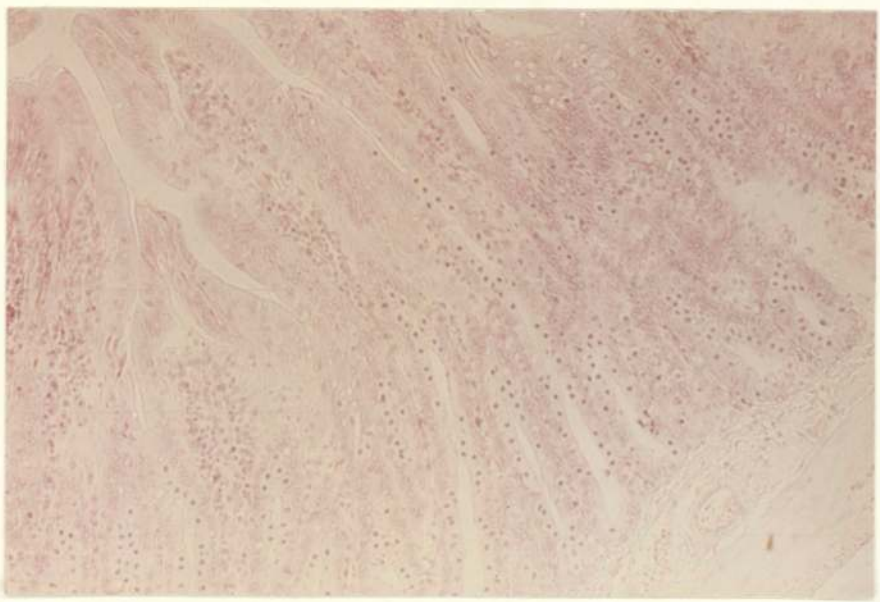


Plate 4: Duodenal crypt showing number of arrested metaphases after a series of $CaCl_2$ injections.

of the same group of mitotically competent crypt epithetial cells observed directly by microscopic examination.

At increasing times after the initial calcium injection, as described in Methods, animals were flash-labelled with a single pulse of ^{3}H-tdR. One hour after the label was introduced animals were sacrificed and the isotope incorporation into the intestine assessed. No significant difference was shown between control and calcium-injected groups up to 4h after the initial calcium injection (Fig.5).

Thus, neither enhanced DNA synthesis nor increased entry of duodenal cells into mitosis was apparent following $CaCl_2$ injections. However, i.p. administration might constrict mesenteric blood vessels, restrict the supply of nutrients to the crypt cells and perhaps prevent expression of the ion's mitogenic potential. To obviate this possibility $CaCl_2$ (12 mg/ml) was infused directly into the jugular vein for 1h to give an elevation of plasma calcium of approximately 1.0 mg%. When ^{3}H-tdR was administered at the start of the infusion, again no significant difference in isotope incorporation was shown compared to 0.9% saline-infused controls (Fig.6), corroborating the previous results suggesting a lack of calcium upon intestinal cells.

Parathyroidectomised animals were studied 3 days after operation. Two groups of sham-PTX animals were compared; firstly by opening the cervical region and closing again, and secondly by electrocautery of the lower thyroid poles. No significant difference in isotope incorporation was shown between either group (Fig.6). These labelling studies in toto provided no evidence for a calcium involvement in determining the rate of cell proliferation in the intestine.

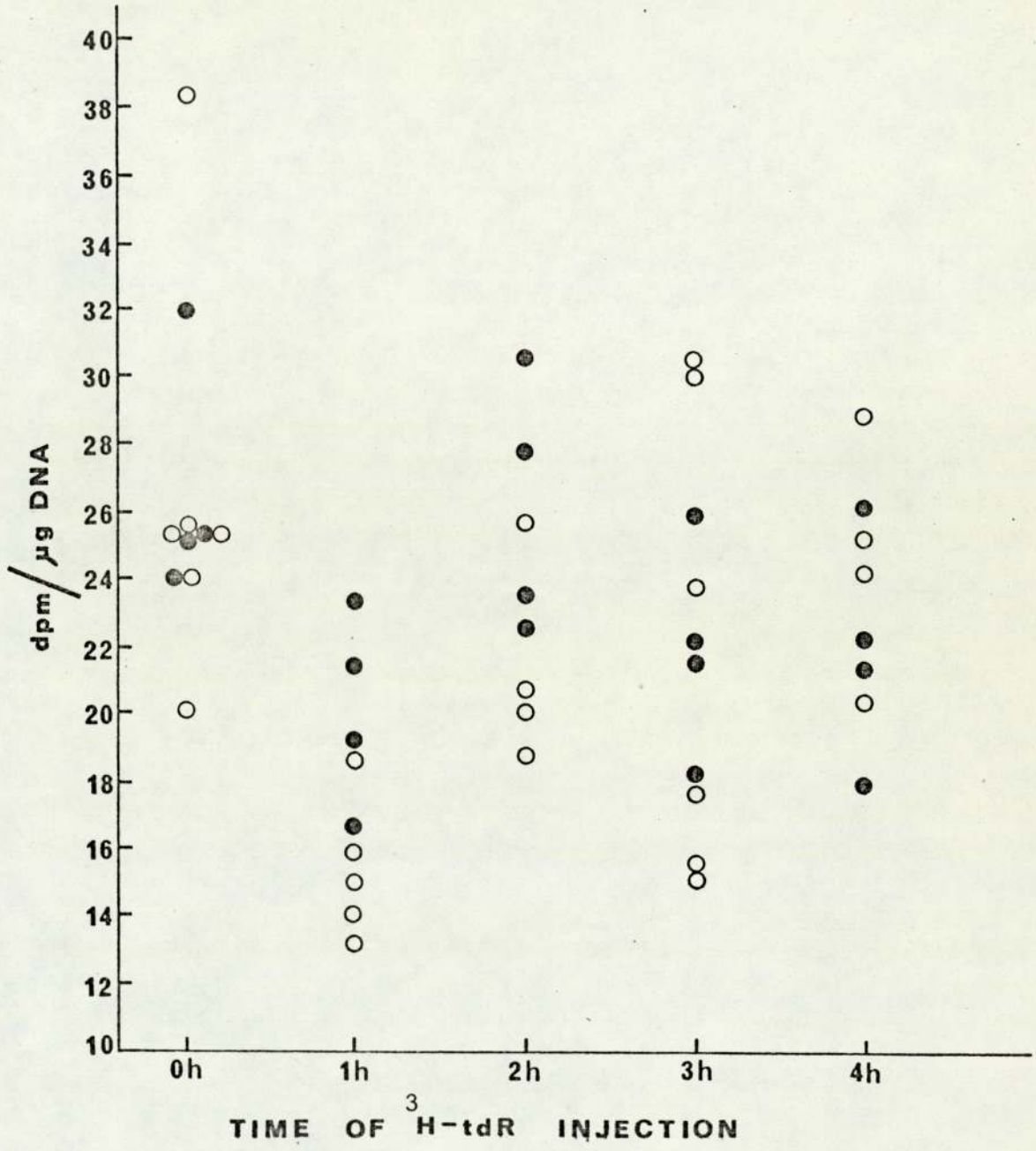


Fig. 5: Influence of $CaCl_2$ (62.5mM) injections upon specific activity in the duodenum expressed as dpm/µg total DNA. Groups of animals received successive doses of calcium at 0. ½, 1, 2, 3 & 4 h; each group received a single dose of tritiated thymidine (^{3}H-tdR) (3µc/100g body weight) at one of the times indicated and sacrified 1 h later. ● Controls. O Calcium-injected.

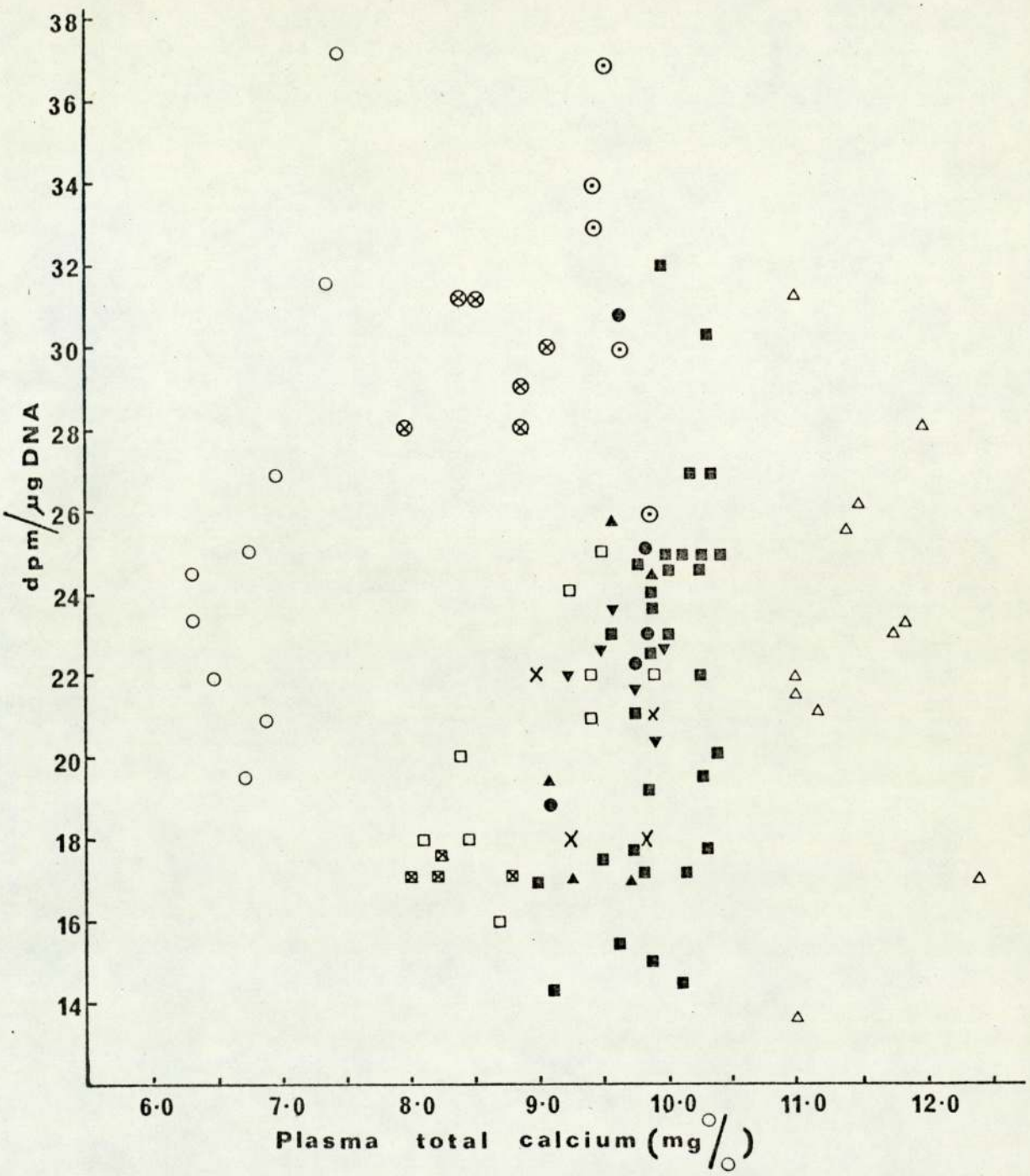


Fig. 6: Influence of alterations of the calcium homeostatic balance upon cell proliferation in the duodenum expressed as specific activity (dpm/µg DNA). Treatments were: ○ Parathyroidectomy (PTX); ● Thyroid-cautery Sham-PTX; ▲ No cautery sham-PTX; ■ Unoperated control; ▼ 0.9% NaCl jugular infusion (1hr); △ 12mg/ml $CaCl_2$ jugular infusion (1hr); ⊗ Calcium-deficient diet (3 weeks); □ Vitamin D deficient diet (3 weeks); ⊠ Vitamin D deficient diet (3 weeks) + 100 U Vitamin D_3 (^{3}H-tdR 4hr later); × Vitamin D deficient diet (3 weeks) + 500 U Vitamin D_3 (^{3}H-tdR 4hrs later); ⊙ Vitamin D deficient diet (3 weeks) + 500 U Vitamin D_3 (^{3}H-tdR 12hrs. later). Animals receiving Vitamin D or vehicle were starved 48hr prior to oral administration.

In contrast, Vitamin D3 proved to be mitogenic. By the histological technique a 4h colcemid metaphase accumulation initiated 4 hrs after 500 I.U. of D3 orally, resulted in a significant increase in the rate of mitosis compared to the starved controls (Fig.3; Plates 9 & 10). To elucidate more clearly the precise location within the cell cycle that the vitamin may be acting, attention was then focused towards the flash labelling technique with ^{3}H-tdR. 4h following either 100 I.U. or 500 I.U. of Vitamin D3 no increase in intestinal nuclear activity was shown (Fig.6); however 12h following 500 I.U. elevated nuclear activity was demonstrated (Fig.6). When endogenous levels of 1,25DHCC (the active metabolite of Vitamin D3) were raised by placing young animals on a low calcium diet (Cooper Nutrition Products Ltd, Witham, Essex) enhanced intestinal DNA synthesis was again observed (Fig.6).

Thus although extracellular calcium doesnot seem to influence intestinal DNA synthesis and mitosis directly, Vitamin D and perhaps its active metabolite which influence the intestinal handling of calcium do seem to have an effect. Since the diabetic intestine also seems to exhibit altered calcium transport characteristics it was predicted that a similar relationship might occur in this pathological situation.

Following injection of the pancreatic β-cytotoxic agent Streptozotocin, 24h food intake measurements were made at intervals over a 10wk period. These animals ate about 2-3 times as much food as their respective age controls (Fig.7), consistent with earlier workers (Jervis & Levin 1966; Nakabou, Okita, Takano & Hagihira 1974) who had employed alloxan.

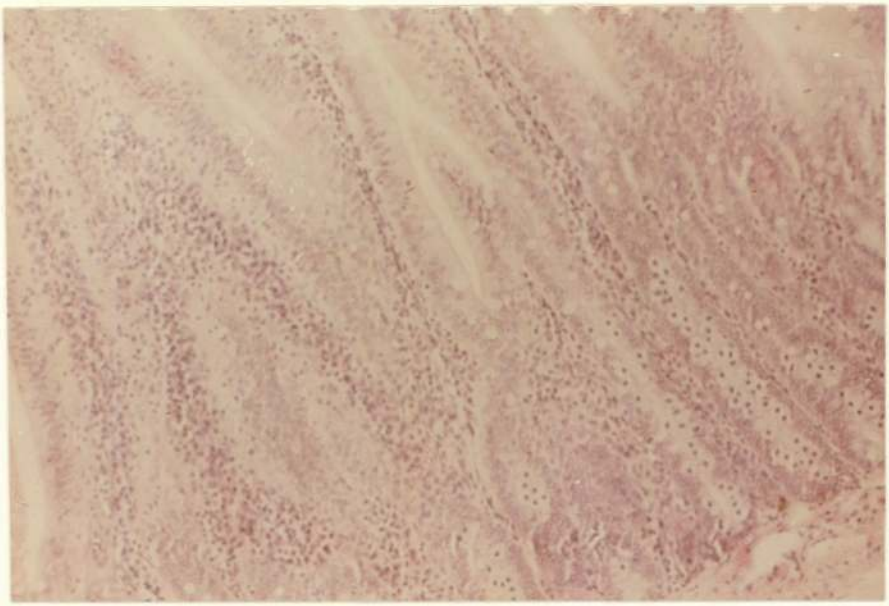


Plate 9: Duodenal crypt showing number of arrested metaphases in an animal starved for 48hrs.

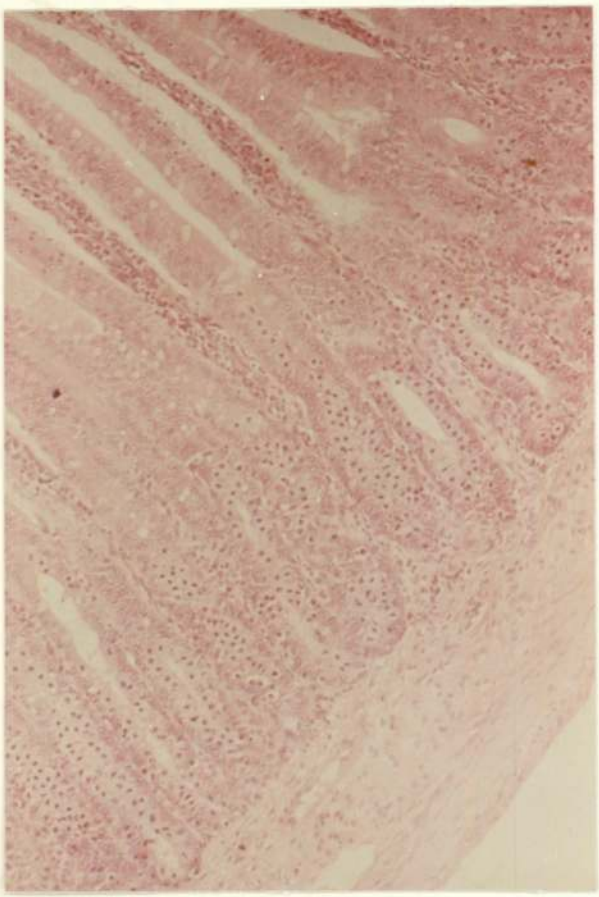


Plate 10: Duodenal crypt showing number of arrested metaphases in an animal starved for 48hrs, then given 500 I.U. of Vitamin D_3.

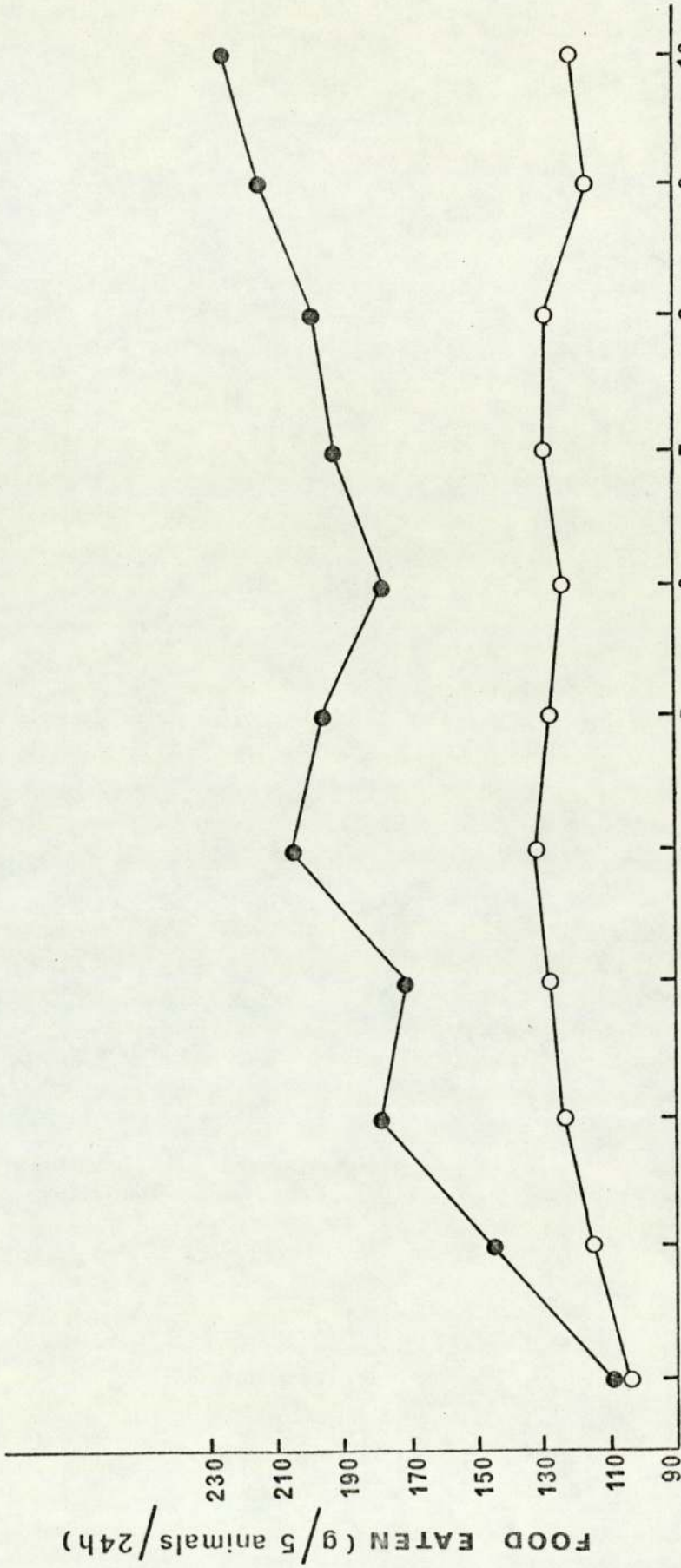


Fig.7: Time course of the hyperphagia of a group of male animals injected with Streptozotocin compared to a group of controls of the same age.

○ Controls; ● Streptozotocin-induced diabetic animals.

Subsequent measurements revealed a very highly significant elevation of the blood glucose and reduction of the plasma insulin (Table 3) confirming that pancreatic β-cell destruction had been successful. Plasma total calcium and plasma total magnesium showed no significant difference between either group (Table 3).

Despite the very highly significant reduction in body weight of the Streptozotocin-treated animals (Table 3; Plate 14), the gastro-intestinal organs showed marked size increases. The stomach, the small intestine and the caecum seemed grossly enlarged in situ. Further analysis of the small intestine revealed a significant increase in the mucosa/muscle ratio, in the thymidine incorporation per unit length of intestine, and of the total DNA per unit length of intestine. The specific activity (dpm per μg of total DNA) however was not significantly altered (Table 3). Histological sectioning showed that these animals had longer villi (Plate 13) compared to their controls (Plates 11 & 12), which was responsible for the increased surface area (E/M) and increased surface/volume ratio (E/L) noted (Table 3).

The lymphoid organs were also affected by this experimentally-induced diabetes. A reduced bone marrow and thymus mitotic index was apparent, although these results were not significantly different. There was also a striking reduction in the size of the spleen in the Streptozotocin-treated animals (Table 3).

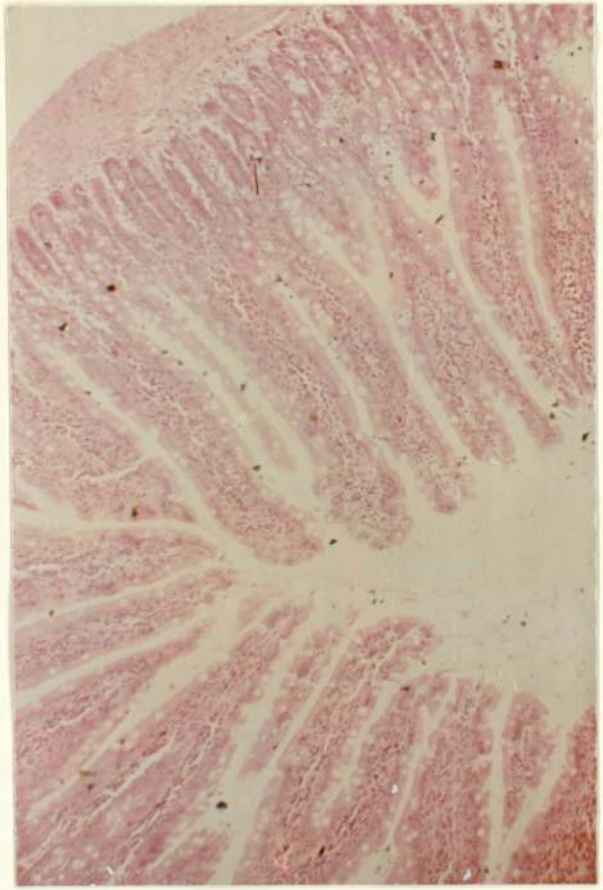


Plate 11:T.S.Duodenum. Control of same weight to Streptozotocin-treated animals.

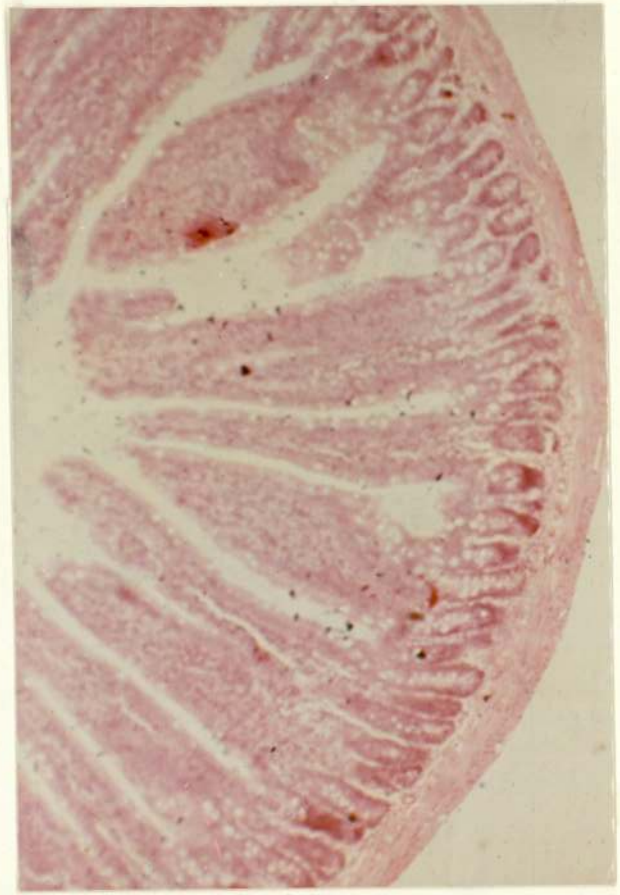


Plate 12: T.S.Duodenum. Control of same age to Streptozotocin-treated animals.

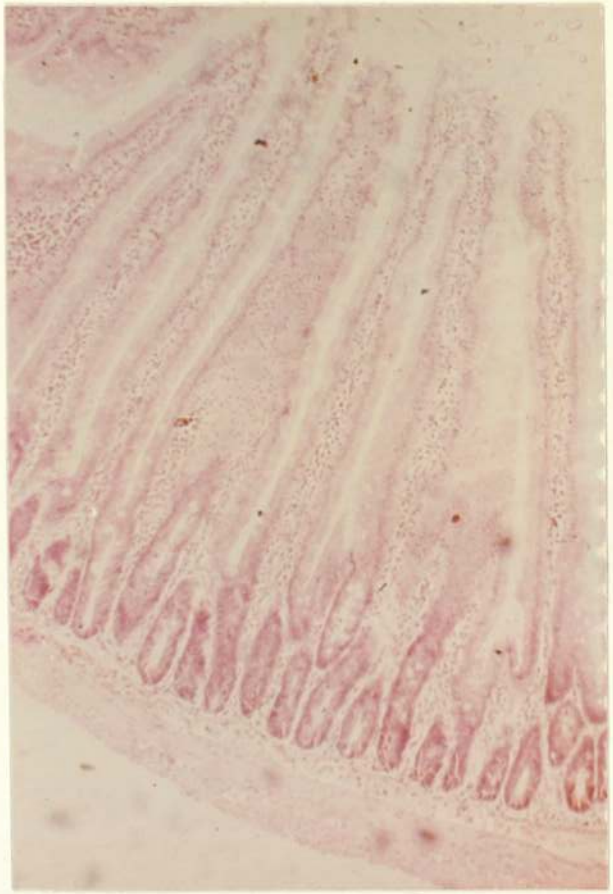


Plate 13: T.S. of Duodenum of animal injected with Streptozotocin 10 weeks previous. Note greatly increased villus length.

Plate 14: Comparison of animal injected with Streptozotocin for 10 weeks with control of the same age. Note the grossly distended abdomen of the former.

	Weight Control	Age Control	Streptozotocin Diabetic
1. Body weight (g)	281 ± 7 (5)	450 ± 3 (3)***	266 ± 17 (4)
2. Duodenum			
a. ^{3}H-tdR incorporation per intestine length (dpm)	5165 ± 364(5)***	6200 ± 950(3)**	12960 ± 761 (4)
b. Total DNA per intestine length (µg/ml)	232 ± 11 (5)***	299 ± 44 (3)**	589 ± 33 (4)
c. Specific activity (dpm/µg) DNA	22.3 ± 1.2 (5) NS	20.8 ± 2.1 (3)NS	22.0 ± 0.4 (4)
d. Mucosa/muscle ratio.	1.20 ± 0.02(5)**	1.31 ± 0.11(3)*	1.86 ± 0.12 (4)
e. E/M	5.8 ± 0.19(5)NS	4.7 ± 0.19(3)*	6.3 ± 0.34 (4)
f. E/L	0.90 ± 0.02(5)NS	0.51 ± 0.05(3)*	0.89 ± 0.09 (4)
3. Spleen weight (g)	0.56 ± 0.04(5)NS	0.81 ± 0.02(3)**	0.55 ± 0.04 (4)
4. Mitotic index			
a. thymus	0.7 ± 0.06(5)NS	0.7 ± 0.03(3)NS	0.5 ± 0.07 (4)
b. bone marrow	1.3 ± 0.05(5)*	1.3 ± 0.09(3)NS	1.1 ± 0.05 (4)
5. Plasma values			
a. total calcium (mg%)	9.71 ± 0.11(5)NS	9.82 ± 0.15(3)NS	9.91 ± 0.10 (4)
b. total magnesium (mg%)	2.26 ± 0.10(5)NS	2.35 ± 0.05(3)NS	2.44 ± 0.22 (4)
c. glucose (mg%)	108 ± 3 (5)***	120 ± 2 (3)***	552 ± 23 (4)
d. insulin (µU/ml)	70 ± 4 (5)***	63 ± 6 (3)***	16 ± 2 (4)

Table 3: Influence of Streptozotocin for a period of 10 weeks upon various parameters. Statistics for controls of the same weight or of the same age are in each case compared to the Diabetic group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) NS, Not significant. Number of animals in each group shown in parentheses.

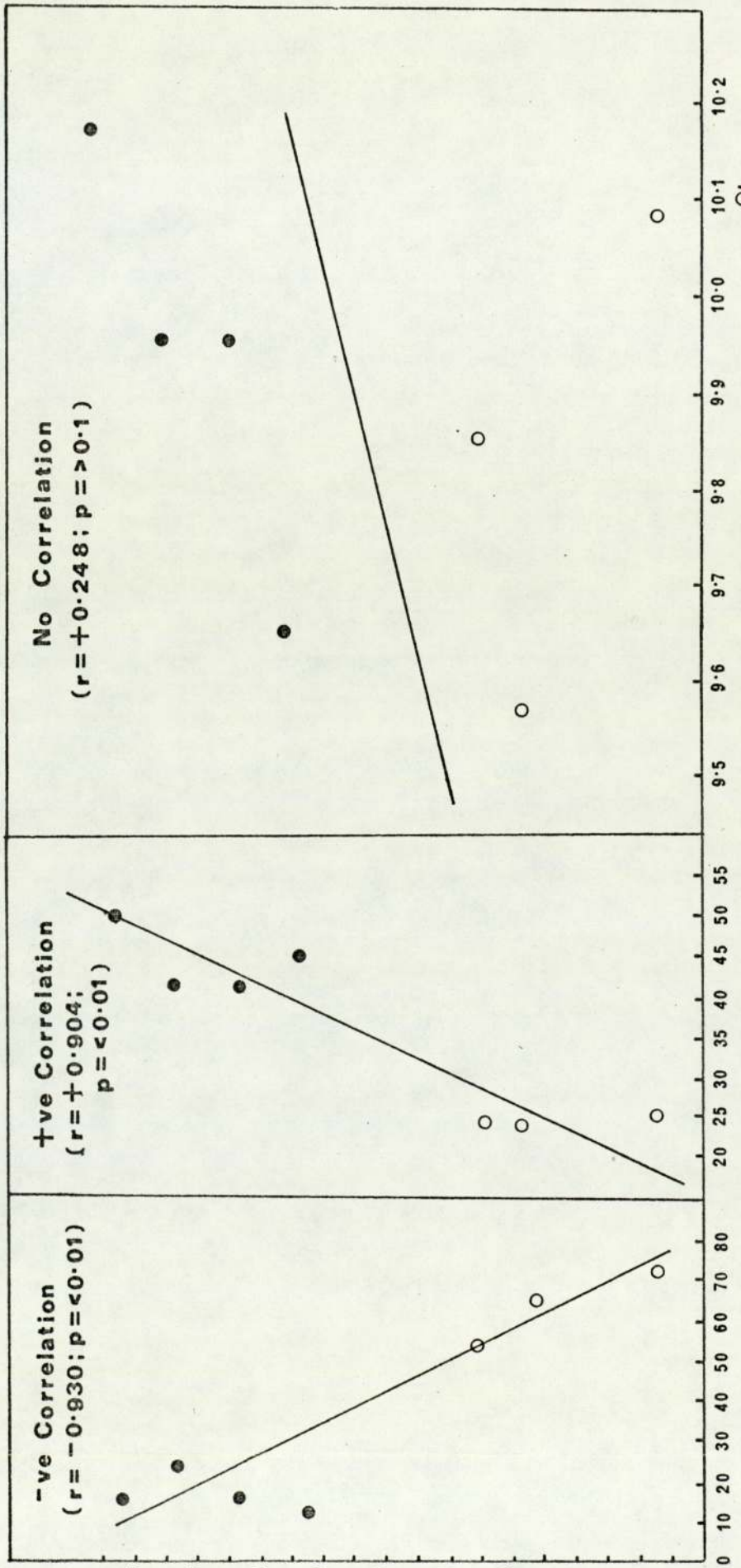


Fig.8: Relationship between the elevated ^{3}H-tdR incorporation of Streptozotocin-treated animals with various parameters. ○ Controls; ● Streptozotocin-induced diabetic animals.

Discussion

It is apparent from this study that physiological fluctuations in extracellular calcium concentration did not influence the progression of cells through the cell cycle in the small intestine. Thus there is neither a reduction in cell cycle time, nor any recruitment from quiescent G^{o} (De Maertelaer & Galand 1975) or G_2^{o} (Pederson & Gelfant 1970) populations, induced by hypercalcaemia.

There is a considerable body of evidence from in vivo systems with variable proliferative rate which suggest that alterations in the rates of entering or leaving G^{o} could play an important part in cell regulation (Bresciani 1968b; Epifanova & Terskikh 1969). This has further been clarified by Izquierdo & Gibbs (1974) who have suggested that if there is a G^{o} phase in a cell-renewing tissue, this is short with respect to the turnover time and that it may be a specific compartment where the control of cell proliferation operates. Such G^{o} or G_2^{o} cell populations were viable candidates for a possible mitogenic action of calcium in the small intestine since physiological increments in cell division in both bone marrow and thymic tissue have been attributed to recruitment of G^{o} cells by the calcium ion (Whitfield, Rixon, Perris & Youdale 1969). These observations are in general agreement with the work of Weber, Bohne & Robe (1970) and Taylor & Jones (1972) who employed various cation-specific chelating agents to study DNA synthesis; they concluded that only the chelating agent that was specific for zinc resulted in any sifnificant decrease in DNA synthesis in the intestine.

In contrast, Vitamin D3 was shown to be mitogenic in the small intestine after a relatively short exposure time. 8h following an oral dose of 500 I.U. of Vitamin D3 a significant elevation of the

number of mitoses/crypt was observed (Fig.3). Long term growth-promoting activity of Vitamin D has been well documented (Urban & Schedl 1969; Spielvogel, Farley & Norman 1972), and recently Birge & Alpers (1973) have recorded maximal incorporation of ^{3}H-tdR into intestinal DNA, 4h after oral administration of 100 I.U. It was thus envisaged that Vitamin D might be promoting the entry of quiescent G^{o} or $G_{2}{}^{o}$ cells into the cell cycle, or possibly causing a reduction in the duration of the G_1 phase. However, since the G_1 phase in the duodenum lasts only 1½h (Cairnie, Lamerton & Steel 1965) a very dramatic reduction in this phase would have to be assumed to account for an elevated mitotic activity. The most likely and feasible explanation therefore resides in a quiescent cell recruitment. Even though hypercalcaemia was noted 8h after an oral dose of 500 I.U., this did not invalidate the results, since previous experiments had ruled out any direct mitogenic action of calcium; secondly 500 I.U. of Vitamin D3 would take between 8-10h to promote intestinal calcium absorption in the rat (Chen & Bosmann 1964; Norman 1966). A resultant hypercalcaemia could not possibly have caused a recruitment of quiescent G^{o} or $G_{2}{}^{o}$ cells to be subsequently arrested at metaphase by 8h. The mitotic action observed must be solely due to the Vitamin D molecule *per se*. This is of particular importance since the increase in weight and dimensions of the intestine (Urban & Schedl 1969) in response to Vitamin D, have been attributed to increased availability of calcium.

In an attempt therefore to characterise which group of quiescent cells were likely candidates for this mitogenic action of Vitamin D, attention was turned towards labelling the dividing cells in the S-phase of the cell cycle with ^{3}H-tdR. Bearing in mind the cell cycle parameters G_1 (1½h); S (6½h); G_2 (1h) and M (1h) of the rat small

intestine (Cairnie, Lamerton & Steel 1965), it was assumed that if the cells observed in metaphase 8h after Vitamin D had originated from a G^{o} population, then these same cells should be passing through the DNA-synthetic phase 4h after the vitamin. However administration of ^{3}H-tdR 4h after an oral 100 I.U. or 500 I.U. dose of Vitamin D3 showed no increase in the incorporation of the radioactive label into DNA (Fig.6). It would appear therefore that G^{o} cells are not those stimulated by Vitamin D, and possibly the G_{2}^{o} population are more likely.

Since dividing cells were arrested in the quasi-metaphase configuration 8h following Vitamin D administration, it was conceivable that this mitotic increment may be the result of an influx of G_{2}^{o} cells into the cycle. By 12h after the vitamin administration these cells would have re-cycled and be in the S-phase. This in fact was shown to be the case, and indicates that Vitamin D per se may be mitogenic by virtue of its ability to recruit quiescent G_{2}^{o} cells. The difficulty in substantiating this G_{2}^{o} effect resides in the lag periods required for the conversion of Vitamin D to more polar active metabolites. Indeed, this effect would well be attributed to metabolites more polar than cholecalciferol, since 25 HCC and 1,25 DHCC may be detected in plasma within 15 and 30 minutes respectively following administration of the parent molecule (Lawson & Emtage 1974).

Intestinal calcium transport is known to be enhanced by Vitamin D_{3}, 25 HCC, and 1,25 DHCC in successively decreasing times (Norman 1974b). Since the principal agent promoting intestinal calcium transport is 1,25 DHCC it is likely that since Vitamin D enhances intestinal cell proliferation, 1,25 DHCC may also be the principal

form of Vitamin D acting on the proliferative axis. Evidence to substantiate this possibility was obtained by feeding young rats a diet deficient in calcium ions, which artificially raises the plasma 1,25 DHCC levels. By so doing an increased incorporation of ^{3}H-tdR in intestinal DNA was observed (Fig.6). These results imply that both Vitamin D_3 and 1,25 DHCC are able to stimulate cell prpliferation in the crypts of Lieberkuhn.

It must be concluded therefore in view of these results that regulation of cell division in the small intestine is not under the direct control of the parathyroid glands, unlike the bone marrow and thymus tissues. (Introduction 3.5). Moreover Vitamin D and its associated metabolites which are closely involved in calcium ion homeostasis do appear to be mitogenic.

Even though fluctuations of extracellular calcium were ineffectual upon mitotic parameters, the stimulatory effect of Vitamin D is perhaps not that surprising since its effect in promoting body growth (Steenbock & Herting 1955) has long been recognised. It might indeed be that this mitogenic action upon intestinal crypt cells is the prerequisite to the well documented Vitamin D - mediated growth properties manifest by increased villus height and increased mucosal weight (Urban & Schedl 1969; Spielvogel, Farley & Norman 1972). The physiological significance of these findings has not been clearly defined but might reflect an increase in the absorptive surface area which in itself may facilitate the absorption of calcium.

It is now well established that the epithelium of the small intestine is replaced rapidly in mammals (Cheng & Leblond 1974a), yet the mechanism of control of this replacement is unknown.

Several hormones (Introduction, (1.3), Table 2) have been shown to influence the intestinal epithelium. However, these workers have paid little attention to cell cycle parameters; they have simply noted gross anatomical changes such as hypertrophy or used the imprecise method of estimating the change in mitotic index. Generally no specific compartment within the cell cycle has been associated with these changes, although Laguchev & Avetisyan (1973) concluded that adrenal corticoids were causing a lengthening of the DNA-synthetic (S) phase of the cell cycle.

The functional compartment of this renewing system is the villous epithelium. This compartment has three related properties (Quastler 1963): size (cell number), transit time (time spent by a cell in the compartment) and flux (rate of entry of cells = rate of loss of cells in the steady state). Presumably the mechanism which controls the replacement acts directly on one or more of these properties.

The hypotheses put forward to explain the control of intestinal epithelial cell replacement may be divided into two groups: 'systemic' and 'local'. The 'systemic' hypotheses assert the existence of a blood-borne control signal, which may be tissue-specific and stimulatory (Loran & Crocker 1963; Loran & Carbone 1968), tissue-specific and inhibitory (i.e. a chalone) (Tutton 1973), or tissue non-specific and stimulatory (Leblond & Carriere 1955; Carriere 1965; Tilson, Philips & Wright 1971). 'Local' hypotheses stress the autonomy of each of the several parts of the small intestine in the regulation of its own epithelial cell replacement (Fell, Smith & Campbell 1963; Jervis & Levin 1966; Dowling & Booth 1967; Gleeson, Cullen & Dowling 1972). In fact there is no overwhelming evidence for any of the possible control mechanisms at present. This thesis has endeavoured

to establish another source of control, namely the calcium homeostatic system, but results have refuted this possibility.

The importance played by the ingesta itself must not be overlooked at this point. The local translocation of food undoubtedly influences division in the cyrpt cells, since it is well known that starvation arrests dividing cells in the G_1 phase; refeeding of starved animals restores the movement of such cells through the cell cycle (Cameron & Cleffmann 1964). It might be anticipated that the quantity of food, directly or indirectly, provides the physiological need for enhanced cell division. On the fundamental assumption that the only fate of crypt cells is migration on to the villus (Loehry, Croft, Singh & Creamer 1969), the ingesta could provide the necessary stimulus for initiation of a new absorptive villous epithelium.

Thus, there have been several reports on the relationship between changes in weight and length of the gastro-intestinal tract and the quantity of food consumed. Addis (1931) found that high-residue diets increased both weight and length of the alimentary canal in rats. He interpreted the observed change as an adaptation to increased work i.e. work hypertrophy. Fell, Smith & Campbell (1963) and Campbell and Fell (1964) showed that the alimentary canal of female lactating rats increased progressively in weight and size and concluded that this hypertrophy was due entirely to increased food intake. Durand, Fauconneau & Penot (1965) reported that in growing rats, which showed progressively increasing food intake, the weight of the intestine per 100g body weight increased, and concluded that the growth was due to hyperplasia.

Thus in the pathological condition of experimentally-induced diabetes the food consumption was about twice that of the respective age controls (Fig.7). The thymidine-incorporation into the dividing crypt cells per unit length of intestine was double that of the controls. So also was the total DNA per unit length of intestine, presumably as a consequence of the greatly increased surface area (E/M) occupied by the villi. The increased number of dividing cells in the crypts may well be a direct result of the elevated food intake. Under physiological conditions, the rate of entry of cells into mitosis is balanced by their loss from the villus tip and transit time is therefore constant (Loehry, Croft, Singh & Creamer 1969). In the Streptozotocin-treated animals villus length was greatly increased; probably this was a compensatory change to accommodate the excess food. In order for the villus dimensions to have increased in this way, there must have been a lengthening of the cell transit time. Indeed, a number of parameters are known to affect the intestinal transit time. These include age, nutritional requirements and disease (Lipkin & Bell 1968).

The underlying factor responsible for the control of the intestinal changes in the diabetic animal have yet to be clearly defined. There are at least two possible causes of the enlargement of the small intestine in the diabetic rat. The first is that the increase in weight is due to hyperplasia associated with polyphagia. The second is that the change is induced by insulin deficiency and not by an increase in food consumption. Jervis & Levin (1966) concluded that although it was possible that the endocrine imbalance of chronic diabetes may affect growth of the small intestine per se, it was likely to be of minor importance compared to the effect of

the changed dietary intake. Nakabou, Okita, Takano & Hagihira (1974) support this idea.

Jervis & Levin (1966) speculated that the increase in diameter and length of the intestines of alloxan-injected rats might be a response to the amount of nutrient ingested. Schedl & Wilson (1971a) carried out similar experiments with alloxan or streptozotocin-induced diabetic rats and obtained the same conclusion. Further evidence in support of the nutrient per se came from diabetic rats on a restricted diet where the small intestine was shown not to enlarge (Nakabou, Okita, Takano & Hagihira 1974).

However, when Jervis & Levin (1966) fed control animals a 'bulked-out' diet by adding an equal amount of talc to the stock diet to augment the mass of the ingested material, there was only a 10% increase in weight of the small intestine, whereas the dry weight of chronic alloxan-diabetic small intestine was doubled. This tends to exclude simple work hypertrophy from increased bulk ingestion as the cause of the changes in experimental diabetes. These observations are supported by Dowling, Riecken, Laws & Booth (1967), who showed that bulk feeding with kaolin did not induce changes in intestinal weight and mucosal thickness in comparison with matched controls. These findings suggest, in accord with other observations (Addis 1931; Wierda 1950) that the degree of intestinal growth found in diabetes is unlikely to result from bulk feeding.

Indeed, another unresolved facet of the diabetic animal is the role played by the calcium homeostatic system. It is known that there is a derangement of calcium metabolism in these animals. Alloxan-diabetic rats exhibit negative magnesium balance (Schneider

& Schedl 1974) as well as negative calcium balance as shown by a marked reduction in duodenal calcium transport (Schneider & Schedl 1972) and greatly reduced concentrations of duodenal CaBP (Schneider, Wilson & Schedl 1973; 1974). The reason for the low levels of CaBP are unknown. It cannot be attributed to poor nutrition, since the animals are hyperphagic (Jervis & Levin 1966) nor can it be the result of any generalised defect in protein synthesis since mucosal growth is greatly enhanced (Schedl & Wilson 1971b).

It may be that the failure of the diabetic rat to produce CaBP is the result of abnormal Vitamin D metabolism, but is certainly not due to parathyroid hormone insufficiency. Diabetic rats maintain normal to slightly elevated serum calcium levels and compensate for the calcium malabsorption by mobilisation of bone through secondary hyperparathyroidism, as evidenced by the elevated serum immunoreactive PTH (Schneider, Hargis, Schedl & Williams 1974). The possible significance of these observations to the intestinal hypertrophy in the diabetic animal remains unknown. Such animals show the paradoxical combination of depressed body growth and enhanced intestinal growth (Schedl & Wilson 1971a) in spite of the depression of calcium transport (Schneider & Schedl 1972), in contrast to the Vitamin D deficient animal where mucosal growth is depressed (Urban & Schedl 1969). Results from this thesis would tend to exclude calcium ions per se as being the underlying cause of the hypertrophy, but could well be due to some altered Vitamin D metabolite, since results show that cholecalciferol and metabolites more polar, enhance cell proliferation in healthy animals.

Evidence favouring some aspect concerned with the food consumption is presented in Fig.8. There is a highly significant positive correlation between the food intake and the thymidine incorporation per unit length of intestine, whereas there is a negative correlation with the plasma insulin and no correlation with the plasma total calcium. One consequence of the much increased food intake in the Streptozotocin-treated animals would be increased gastrin secretion. This process would also occur under physiological conditions of a normal feeding regime, since food is the direct stimulus for gastrin release (McGuigan 1974; Talmage, Doppett & Cooper 1975). Indeed the circulating gastrin concentration could well play a fundamental role in the control of gastrointestinal cell renewal. The reasoning for this is presented below.

The major action of gastrin is considered to be its effect in stimulating gastric acid secretion, but it exhibits a multitude of other actions (McGuigan 1974). It is, for example, known to stimulate cell division of the small intestinal mucosa in vitro (Lichenberger, Miller, Erwin & Johnson 1973; Pansu, Berand, Dechelette & Lambert 1974) which presumably is a direct effect and not one mediated by its ability to enhance intestinal blood flow (Burns & Schenk 1969). The major circulating gastrin in the nonfed state is "big big" gastrin, and in response to feeding, especially protein (Korman, Soveny & Hansky 1971) smaller gastrin species are liberated into the circulation (Yalow & Wu 1973) predominantly from the stomach (Berson & Yalow 1971) but also from the upper small intestine (Berson & Yalow 1971; Stern & Walsh 1972). The lack of gastrointestinal hypertrophy following the 'bulked out

diets' of previous workers (Jervis & Levin 1966; Dowling, Riecken, Laws & Booth 1967) may readily be explained. If the protein content of the food and subsequent stimulation of gastrin is the major stimulus for gastrointestinal proliferation, both in the normal and the diabetic animal, then no cellular changes would be expected from these diets with 'talc' or 'kaolin' added since they would not provide the necessary mitogenic stimulus.

Furthermore, the increased cell proliferation noted following intestinal resection (Loran & Crocker 1963; Loran & Althausen 1970) may well be attributable to an effect of gastrin, since the half life of intravenously injected ^{125}I-gastrin is substantially increased following intestinal resection (Wickbom, Landor, Bushkin & McGuigan, 1975). Gastrin might also provide the explanation for the circadian mitotic activity observed in the rodent intestine (Sigdestad, Bauman & Lesher 1969) since cell proliferation reaches its peak during the hours of darkness when food consumption is known to be maximal (Hunt & Perris 1974).

In view of the fact that the predominant stimulus to the release of secretin by the mucosa of the small intestine is acid, it is believed that acid secreted by the stomach and conveyed to the upper duodenum evokes the release of secretin which then inhibits further release of gastrin (Hansky, Soveny & Korman 1971; Anderson 1973; McGuigan 1974). It would appear likely therefore that since secretin and gastrin have been shown to have opposing effects upon intestinal cell proliferation (gastrin stimulates and secretin inhibits (Pansu, Berand, Dechelette & Lambert 1974) it may well be that this system acts as a self-maintaining feedback control in the regulation of gastrointestinal proliferation.

Since no evidence was obtained to substantiate a role played by the calcium homeostatic hormones, and the relatively long time required to obtain conversion of the renal metabolites of Vitamin D sufficient to constitute the basis of physiological control, the concept of local regulation by means of gastrointestinal hormones would appear a more likely explanation. Villus height decreases along the length of the small intestine (Altmann 1971); the regional variation of stimulatory and inhibitory factors present in the intestinal chyme (Altmann & Leblond 1970) have been acclaimed responsible for this size difference. In a similar manner, therefore, the control of cell renewal in the small intestine might reside in a balance between the actions of secretin and gastrin. The protein content of the diet (and consequent release of gastrin) could well constitute the prime control of regulation since Pederson & Gelfant (1970) have shown that by changing animals from a low to a high protein diet is accompanied by an increase in cell proliferation (by a recruitment of quiescent G_2° cells). Whether or not the mitogenic action of Vitamin D (or its more polar metabolites) is mediated via a secondary release of gastrin has yet to be established, but is conveivable since hypercalcaemia is known to elevate plasma gastrin (Reeder, Jackson, Bon, Clendinnen, Davidson & Thompson 1970; Case 1973; Levant, Walsh & Isenberg 1973).

GENERAL DISCUSSION

Several possible control systems exist that could be responsible for maintaining population dynamics in rapidly renewing tissues, whether these be stimulatory (e.g. hormones) or inhibitory (e.g. chalones) factors (see Introduction 1.3). This thesis has endeavoured to establish the importance of another possible source of control for these tissues (namely the calcium homoestatic system) and the tissue specificity of this control.

The bone marrow, the thymus gland and the mucosa of the small intestine are all rapidly renewing tissues with their mature differentiated cells having extremely short life spans. The accomplishment of this rapid turnover in the bone marrow is provided by the erythrogenin-erythropoietin feedback system. The thymus however is devoid of any such external control; its cell population being maintained by factors secreted from its internal milieu. Nevertheless, both these tissues are also responsive to the mitogenic action of calcium and undergo physiological changes in cell proliferation with a concomitant hypercalcaemia. The small intestine also appears to possess its own inherent control emanating from some influence upon the stem cells in the crypts of Lieberkuhn. This tissue is not influenced by physiological fluctuations in calcium ion concentration, but a substantial body of evidence supports the concept that the ingesta may be the underlying control factor governing rates of cell renewal.

Indeed, apart from the role postulated for the ingesta in controlling cell division in the small intestine (Discussion, Part 2.), results have shown that maintainance of an adequate nutrition to the cells is an important factor that could lead to wrong interpretation of mitotic data. Thus in bone marrow and thymus the increased ionised calcium associated with long term ovariectomy was expected to increase mitosis. The converse in fact was observed, presumbaly as a result of diminished nutrition. Also, in the intestine, the reduced food intake of the PTX and pair fed sham-PTX animals obscured any response attributable to low plasma calcium.

These three tissues all possess quiescent G^{o} cells, but appear to exhibit quite different regulatory mechanisms. An understanding of the mechanisms involved in various tissues of the body in the healthy animal may provide the vital answer to the aetiology of malignant growth. Thus work in this thesis has established the mitogenic action of calcium and magnesium ions *in vivo* in lymphopoietic and haematopoietic tissue of the male rat; more recent work *in vitro* (Morgan, Hall & Perris 1975a,b) has elucidated the role played by these mitogenic cations and cyclic nucleotides in initiating DNA synthesis. It is known that some malignant tissues possess greater calcium content than normal tissue (van Rossum, Galeotti & Morris 1973); certain malignant tumours appear to have an increased requirement for magnesium ions (Heeley, Warner & Mayer 1974; Parsons, Anderson, Clark, Edwards, Ahmad & Hetherington 1974); adenylate cyclase is elevated in certain tumour cells (Brown, Chattopadhyay, Spjut, Spratt & Pennington 1969; Brown, Chattopadhyay, Morris & Pennington 1970) and cyclic-GMP causes proliferation of some tumour cells *in vitro* (J.I. Morgan, personal communication). Although consistent with derangements in cyclic nucleotide metabolism and intracellular ion balance, a direct demonstration of their joint involvement in neoplasia remains to be shown. Indeed, it would be extremely interesting to test the effect of the sex steroids (oestrogen and testosterone) on such transformed cells.

APPENDIX

Composition of standard (41B) and modified animal diets. (major components only).

Ingredient	41B	Calcium-deficient	Vitamin D_3-deficient
Crude oil	2.73%	9.50%	9.50%
Crude protein	16.95%	21.00%	21.00%
Crude fibre	5.87%	5.00%	5.00%
Digestible crude oil	2.26%	8.00%	8.00%
Digestible crude protein	13.68%	18.50%	18.50%
Digestible crude fibre	3.00%	2.00%	2.00%
Digestible carbohydrate	45.07%	44.00%	44.00%
Calcium	1.10%	0.03%	0.80%
Phosphorus	0.85%	0.70%	0.80%
Magnesium	0.35%	0.16%	0.16%
Potassium	0.86%	0.54%	0.54%
Sulphur	0.24%	0.20%	0.20%
Iron	181mg/kg	93mg/kg	93mg/kg
Copper	16mg/kg	20mg/kg	20mg/kg
Manganese	115mg/kg	50mg/kg	50mg/kg
Cobalt	98µg/kg	540µg/kg	540µg/kg
Zinc	45mg/kg	25mg/kg	25mg/kg
Iodine	340µg/kg	540µg/kg	540µg/kg
Vitamin D_3	300i.u./kg	300i.u./kg	-

Together with all other essential amino acids and vitamins.

All diets produced in 3/8" pellets. Pilsbury's Standard 41B diet manufactured by Heygate & Sons Ltd., Northampton; calcium and vitamin D deficient diets manufactured by Cooper Nutrition Products Ltd., Witham, Essex.

ACKNOWLEDGMENTS.

Firstly I must extend my gratitude and thanks to my supervisor Dr. A.D. Perris for his continued guidance throughout this work;

to Mrs. M. Varley and Mr. A. Youngs for their helpful advice on histological procedures;

to the staff of the Visual Aids Section for their excellent photographic work;

to Yvonne Bates for her technical assistance;

and to Elizabeth Perris for the typing of this Thesis.

References.

ABELL C.W. & MONAHAN T.M. (1973).
The role of adenosine 3'5' monophosphate in the regulation of mammalian cell division.
J.Cell Biol. 59 549-558.

ABERCROMBIE M (1946).
Estimation of nuclear populations from microtome sections.
Anat Rec 94 239-247.

ADDIS T. (1931).
Hypertrophy of the gastrointestinal tract and high residue diets.
Am. J. Physiol. 99 417-423.

ADIBI S, PASCHKIS K.E. & CANTAROW A. (1959).
Stimulation of liver mitosis by blood serum from hepatectomised rats.
Expl. Cell. Res. 18, 396-398.

ALCOCK N. & MacINTYRE I. (1962).
Interrelation of calcium and magnesium absorption.
Clin. Sci. 22 , 185-193.

ALCOCK N.W. & SHILS, M.E. (1974).
Comparison of magnesium deficiency in the rat and mouse.
Proc. Soc. exp. Biol Med. 146, 137-141.

ALDRED J.P., KLESZYNSKI R.R. & BASTIAN, J.W., (1970).
Effects of acute administration of porcine and salmon calcitonin on urine electrolyte excretion in rats.
Proc. Soc. exp. Biol Med. 134, 1175-1180.

ALLEN, J.M. (1955).
Influence of hormones on cell division I Estradiol Benzoate.
Anat. Rec. 121, 252-253.

ALLEN, J.M. (1956).
The influence of hormones on cell division.
Expl. Cell Res. 10, 523-532.

ALLEN, J.M. (1958).
The influence of hormones on cell division. II Time response of ear, seminal vesicle, coagulating gland, and ventral prostate of castrate male mice to a single injection of testosterone propionate.
Expl. Cell Res. 14, 142-148.

ALSTON, W.C. & THOMPSON, R.J. (1963).
Humoral and local factors in liver regeneration.
Cancer Res. 23, 901-905.

ALTMANN, G.G. (1971).
Influence of bile and pancreatic secretions on the size of the villi of the small intestine in the rat.
Am. J. Anat. 132, 167-178.

ALTMANN, G.G. (1972).
Influence of starvation and refeeding on mucosal size and epithelial renewal in the rat small intestine.
Am. J. Anat. 133, 391-400.

ALTMANN G.G. & LEBLOND, C.P. (1970).
Factors influencing villus size in the small intestine of adult rats as reveiled by transposition of intestinal segments.
Am. J. Anat. 127, 15-36.

St. AMAND, G.A., ANDERSON, N.G. & GAULDEN, M.E. (1960).
Cell division IV Acceleration of mitotic rate of grasshopper neuroblasts by agmatine.
Expl. Cell Res. 20, 71-76.

ANDERSON, J.J.B., GREENFIELD, J.W., POSADA, J.R. & CRACKEL, W.C., (1970).
Effects of oestrogen on bone mineral turnover in mature female rats measured by ^{85}Strontium.
Proc. Soc. exp. Biol Med. 135, 883-886.

ANDERSON, S. (1973).
Secretion of gastrointestinal hormones.
A.Rev. Physiol. 35, 431-452.

ANDREASEN,E. & CHRISTENSEN, S. (1949).
The rate of mitotic activity in the lymphoid organs of the rat.
Anat. Rec. 103, 401-412.

ANDREASEN, E. & OTTESEN J. (1945).
Studies on the lymphocyte production. Investigations on the nucleic acid turnover in the lymphoid organs.
Acta physiol. Scand. 10, 258-270.

ATKINS, D., ZANELLI, J.M., PEACOCK, M., & NORDIN, B.E.C. (1972).
The effect of oestrogens on the response of bone to parathyroid hormone in vitro.
J. Endocr. 54, 107-117.

AVIOLI, L.V., SCOTT. S., LEE, S.W., & DELUCA, H.F. (1969).
Intestinal calcium absorption: nature of defect in chronic renal disease.
Science 166, 1154-1156.

BAKER, B.L. & BRIDGMAN, R.M. (1954).
The histology of the gastrointestinal mucosa after adrenalectomy or administration of adrenocortical hormones.
Am. J. Anat. 94, 363-397.

BALK, S.D. (1971).
Calcium as a regulator of the proliferation of normal, but not of transformed, chicken fibroblasts in a plasma-containing medium.
Proc. natn. Acad Sci. U.S.A. 68, 271-275.

BALK, S.D., WHITFIELD, J.F., YOUDALE, T., & BRAUN, A.C., (1973).
Roles of calcium, serum, plasma and folic acid in the control of proliferation of normal and Rous sarcoma virus infected chicken fibroblasts.
Proc. natn. Acad. Sci. U.S.A. 70, 675-679.

BAR, A., HURWITZ, S., & COHEN, I., (1972).
Relationship between duodenal calcium binding protein, parathyroid activity, and various parameters of mineral metabolism in the rachitic and vitamin D-treated chick.
Comp. Biochem. Physiol. 43, 519-526.

BAR, A., & WASSERMAN, R.H., (1973).
Control of calcium absorption and intestinal calcium binding protein synthesis.
Biochem. biophys. Res. Commun. 54, 191-196.

BARNICOT, N.A., (1948).
The local action of the parathyroid and other tissues on bone in intracerebral grafts.
J. Anat. 82, 233-248.

BASERGA, R., (1965).
The relationship of the cell cycle to tumor growth and control of cell division; a review.
Cancer Res. 25, 581-595.

BASERGA, R. (1968).
Biochemistry of the cell cycle; a review.
Cell Tissue kinet. 1, 167-191.

BATEMAN, A.E. (1974).
Cell specificity of chalone type inhibitors of DNA synthesis released by blood leucocytes and erythrocytes.
Cell Tissue Kinet. 7, 451-463.

BAYLINK, D.J., (1971).
Bone formation by osteocytes.
Am. J. Physiol. 221, 669-678.

BAYLINK, D.J. & WERGEDAL, J. (1971).
Bone formation and resorption by osteocytes.
In 'Cellular Mechanisms for Calcium Transfer and Homeostasis'.
Eds. Nichols, G., & Wasserman, R.H.
New York, Academic Press.
pp 257-286.

BECKER, F.F. (1973).
Humoral aspects of liver regeneration.
In 'Humoral Control of Growth & Differentiation'.
Eds. Lobue, J., & Gordon, A.S.
Academic Press, New York & London.
pp. 249-256.

BEHAR J., (1974).
Magnesium absorption by rat ileum and colon.
Am. J. Physiol, 227, 334-341.

BELL, G.H., DAVIDSON, J.N. & SCARBOROUGH, H., (1968).
In 'Testbook of Physiology & Biochemistry'.
E.&S. Livingston Ltd., Edinburgh & London.
7th Edition, p.277.

BENDITT E.P. & WONG, R.L., (1957).
On the concentration of 5-hydroxytryptamine in mammalian enterochromaffin cells and its release by reserpine.
J. exp. Med. 105, 509-520.

BENESTAD, H.B., RYTOMAA, T., & KIVINIEMI, K., (1973).
The cell specific effect of the granulocyte chalone demonstrated with the diffusion chamber technique.
Cell Tissue Kinet. 6, 147-154.

BERSON, S.A. & YALOW, R.S. (1971).
Nature of immunoreactive gastrin extracted from tissues of gastro-intestinal tract.
Gastroenterology 60, 215-222.

BERTALANFFY, F.D., (1957).
Mitotic activity and renewal rate of sebaceous gland cells in the rat.
Anat. Rec. 129, 231-241.

BERTALANFFY, F.D. (1960).
Mitotic rates and renewal times of the digestive tract epithelia in the rat.
Acta. anat. 40, 130-148.

BERTALANFFY, F.D. & LAU, C. (1962a).
Cell Renewal.
Int. Rev. Cytol. 13, 357-366.

BERTALANFFY, F.D. & LAU, C., (1962b).
Mitotic rate and renewal time of the corneal epithelium in the rat.
Arch. Ophthalmol. 68, 546-550.

BERTALANFFY, F.D. &LAU, C. (1963).
Mitotic rates, renewal times, and cytodynamics of the female genital tract in the rat.
Acta anat. 54, 39-81.

BHATTACHARYYA, M.H., & DELUCCA, H.F. (1973).
The regulation of rat liver calciferol-25-hydroxylase.
J. biol. Chem. 248, 2969-2973.

BIJVOET, O.L.M., van der SLUYS VEER, J., de VRIES, H.R., & van KOPPEN, A.T.J., (1971).
Natriuretic effect of calcitonin in man.
New Engl. J. Med. 284, 681-688.

BIRGE, S.J. & ALPERS, D.H. (1973).
Stimulation of intestinal mucosal proliferation by vitamin D.
Gastroenterology 64, 977-982.

BIRNBAUMER, L., POHL, S.L., MICHIEL, H., KRANS, J. & RODBELL, M. (1970).
The actions of hormones on the adenyl cyclase system.
Adv. Biochem. Psychopharmacol. 3, 185-208.

BIZZOZERO, G., (1894).
Growth and regeneration of the organism.
Br. med. J. 1, 728-732.

BLENKINSOPP, W.K. (1967).
Proliferation of respiratory tract epithelium in the rat.
Expl. Cell. Res. 46, 144-154.

BLOOM, W. & FAWCETT, D.W. (1962).
The intestines, In A textbook of Histology. Philadelphia: Saunders, p. 443-462.

BLUMENFELD, C.M. & RICE, H.M. (1938).
The volume of the parathyroid glands of the albino rat.
Anat. Rec. 70, 227-233.

BLUMENTHAL, H.T. (1950).
The nature of cyclic variations in mitotic activity: the relation of alimentation and nutrition to this phenomena.
Growth 14, 231-249.

BOLDINGH, W.H. & LAURENCE, E.B., (1968).
Extraction purification and perliminary characterization of the epidermal chalone: a tissue specific mitotic inhibitor obtained from vertebrate skin.
Eur. J. Biochem. 5, 191-198.

BORDIER, P., RASMUSSEN, H. & DORFMANN, H. (1974).
Effectiveness of parathyroid hormone, calcitonin and phosphate on bone cells in Paget's Disease.
Am. J. Med. 56, 850-870.

BORLE, A.B. (1967).
Membrane transfer of calcium.
Clin. Orthop. 52, 267-292.

BORLE, A.B. (1969).
Effect of thyrocalcitonin on calcium transport in kidney cells.
Endocrinology 85, 194-199.

BORLE, A.B. (1974).
Calcium and phosphate metabolism.
A. Rev. Physiol. 36, 361-391.

BOYLE, I.T., GRAY, R.W., & DELUCA, H.F., (1971).
Regulation by calcium of in vivo synthesis of 1,25 dihydroxy cholecalciferol and 21,25 dihydroxycholecalciferol.
Proc. natn. Acad. Sci. U.S.A. 68, 2131-2134.

BOYLE, I.T., MIRAVET, L., GRAY, R.W., HOLICK, M.F.& DELUCA, H.F. (1972).
The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxyvitamin D in nephrectomised rats.
Endocrinology 90, 605-608.

BRAUN, W., ISHIZUKA, M., & SEEMAN, P., (1970).
Suppression and enhancement of antibody formation by alteration of Ca^{2+} levels
Nature 226, 945-946.

BRECHER G., & STOHLMAN, F. Jr., (1959).
In Progress in Hematology, Ed. Tocantis, L.M. Grune & Stratton, N.Y.
Vol. 2, p 110-132.

BRESCIANI, F., (1968a).
Topography of DNA synthesis in the mammary gland of the C3H mouse and its control by ovarian hormones. An auto radiographic study.
Cell Tissue Kinet. 1, 51-63.

BRESCIANI, F. (1968b).
Cell proliferation in cancer.
Eur. J. Cancer 4, 343-366.

BRONNER, F., SAMMON, P.J., NICHOLS, C., STACEY, R.E. & SHAH, B.G. (1968). Thyrocalcitonin and plasma calcium homeostasis in the rat. In Parathyroid Hormone and Thyrocalcitonin. Proceedings of the Third Parathyroid Conference. Eds. TALMAGE, R.V. & BELANGER, L.F. Excerpta Medica Foundation. p 353-369.

BROWN, H.D., CHATTOPADHYAY, S.K., SPJUT, H.J., SPRATT, J.S., & PENNINGTON, S.N. (1969).
Adenylcyclase activity in dimethylamino biphenyl-induced breast carcinoma.
Biochim. biophys. Acta. 192, 372-375.

BROWN, H.D., CHATTOPADHYAY, S.K., MORRIS, H.P. & PENNINGTON, S.N., (1970).
Adenylcyclase activity in Morris Hepatomas 7777, 7794A and 9618A.
Cancer Res. 30, 123-126.

BROWN, H.O., LEVINE, M.L. & LIPKIN, M. (1963).
Inhibition of intestinal epithelial cell renewal and migration induced by starvation.
Am. J. Physiol. 205, 868-872.

BRUES, A.M. (1936).
The effect of colchicine on regenerating liver.
J. Physiol. 86, 63P-64P.

BRUMBAUGH, P.F.& HAUSSLER, M.R., (1973).
Nuclear cytoplasmic receptors for 1,25 dihydroxycholecalciferol in intestinal mucosa.
Biochem. biophys. Res. Commun. 51, 74-81.

BUCHER, N.L.R., (1963).
Regeneration of mammalian liver.
Int. Rev. Cytol. 15, 245-300.

BUCHER, N.L.R. (1967a).
Experimental aspects of hepatic regeneration.
New Engl. J. Med. 277, 686-696.

BUCHER, N.L.R. (1967b).
Experimental aspects of hepatic regeneration.
New Engl. J. Med. 277, 738-746.

BUDY, A.M., URIST, M.R., & McLEAN, F.C. (1952).
The effect of estrogens on the growth apparatus of the bones of immature rats.
Am. J. Path. 28, 1143-1156.

BUETOW,. D.E. (1971).
Cellular content and cellular proliferation changes in the tissues and organs of the aging mammal. In Cellular and Molecular Renewal in the Mammalian Body. Eds. Cameron, I.L. & Thrasher, J.D., Academic Press, New York & London. P. 87-106.

BULL, L.S. HURLEY, W.L. KENNETT, W.S. TAMPLIN, G.B., & WILLIAMS, W.F., (1974).
Effect of sex hormones on food intake in rats.
J. Nutr. 104, 968-975.

BULLOUGH W.S. (1950).
Epidermal mitotic activity in the adult female mouse.
J. Endocr. 6, 340-349.

BULLOUGH, W.S. (1952).
Stress and epidermal mitotic activity I. The effects of the adrenal hormone.
J. Endocr. 8, 265-274.

BULLOUGH, W.S. (1965).
Mitotic and functional homeostasis. A speculative review.
Cancer Res. 25, 1683-1727.

BULLOUGH, W.S. (1967).
In The evolution of differentiation. Academic Press, London & New York.

BULLOUGH, W.S. (1975a).
Chalone control mechanisms.
Life Sci. 16, 323-330.

BULLOUGH, W.S. (1975b).
Mitotic control in adult mammalian tissues.
Biol. Rev. 50, 99-127.

BULLOUGH, W.S. & LAURENCE, E.B. (1961).
Stress and adrenaline in relation to the diurnal cycle of epidermal mitotic activity in adult male mice.
Proc. R. Soc. Series B 154, 540-556.

BULLOUGH, W.S. & LAURENCE, E.B. (1964a).
Mitotic control by internal secretion: the role of the chalone-adrenalin complex.
Expl. Cell. Res. 33, 176-194.

BULLOUGH, W.S. & LAURENCE, E.B. (1964b).
Duration of epidermal mitosis in vitro. Effect of the chalone-adrenalin complex and of energy production.
Expl. Cell. Res. 35, 629-641.

BULLOUGH, W.S. & LAURENCE, E.B. (1966).
Accelerating and decelerating actions of adrenaline on epidermal mitotic activity.
Nature 210, 715-716.

BULLOUGH, W.S. & LAURENCE, E.B. (1967).
Epigenetic Mitotic Control, In Control of Cellular Growth in Adult Organisms. Eds. Teir, H. & Rytomaa, T. Academic Press, New York, P. 28-40.

BULLOUGH, W.S. & LAURENCE, E.B. (1968).
Control of mitosis in mouse and hamster melanomata by means of the melanocyte chalone.
Eur. J. Cancer 4, 587-594.

BULLOUGH, W.S. & LAURENCE, E.B. (1970a).
The lymphocytic chalone and its antimitotic action on mouse lymphoma in vitro.
Eur. J. Cancer, 6, 525-531.

BULLOUGH, W.S. & LAURENCE, E.B. (1970b).
Chalone control of mitotic activity in sebaceous glands.
Cell Tissue Kinet. 3, 291-300.

BULLOUGH, W.S. & VAN OORDT, G.J. (1950).
The mitogenic actions of testosterone propionate and of oestrone on the epidermis of the adult male mouse.
Acta endocr. Copnh. 4, 291-305.

BURKHARDT, E.Z. (1940).
A study of the effects of androgenic substances in the rat by the aid of colchicine.
Ph.D. Thesis, University of Chicago.

BURNS, G.P. & SCHENK, W.G. (1969).
Effect of digestion and excercise on intestinal blood flow and cardiac output. An experimental study in the conscious dog.
Arch. Surg. Chicago 98, 790-794.

BUTCHER, R.L. COLLINS, W.E. & FUGO, N.W. (1974).
Plasma concentration of LH, FSH prolactin, progesterone, and estradiol-17B throughout the 4 day estrous cycle of the rat.
Endocrinology 94, 1704-1708.

CAIRNIE, A.B. & BENTLEY, R.E. (1967).
Cell proliferation studies in the intestinal epithelium of the rat. Hyperplasia during lactation.
Expl. Cell. Res. 46, 428-440.

CAIRNIE, A.B. LAMERTON, L.F. & STEEL, G.G. (1965).
Cell proliferation studies in the intestinal epithelium of the rat II Theoretical aspects.
Expl. Cell. Res. 39, 539-553.

CAMERON, I.L. (1968).
A method for the study of cell proliferation and renewal in the tissues of mammals. In Methods in Cell Physiology. Ed. David M. Prescott. Academic Press, New York. Vol. III, p. 261-276.

CAMERON, I.L. (1971).
Cell proliferation and renewal in the mammalian body. In Cellular and Molecular renewal in the mammalian body. Eds. I.L. Cameron & J.D. Thrasher. Academic Press, New York & London. p. 45-85.

CAMERON, I.L. & CLEFFMANN, G. (1964).
Initiation of mitosis in relation to the cell cycle following feeding of starved chickens.
J. Cell Biol. 21, 169-174.

CAMPBELL, R.M. & FELL, B.F. (1964).
Gastrointestinal hypertrophy in the lactating rat and its relation to food intake.
J. Physiol. 171, 90-97.

CARE, A.D. BRUCE, J.B. BOELKINS, J., KENNY, A.D. CONAWAY, H. & ANAST, C.S. (1971).
The role of pancreozymin-cholecystokinin and structurally related compounds as calcitonin secretogogues.
Endocrinology, 89, 262-271.

CARE, A.D. COOPER, C.W. DUNCAN, T. & ORIMO, H. (1968a).
A study of thyrocalcitonin secretion by direct measurement of in vivo secretion rates in pigs.
Endocrinology 83, 161-169.

CARE, A.D., COOPER, C.W., DUNCAN, T. & ORIMO, H., (1968b).
The direct measurement of thyrocalcitonin secretion rate in vivo.
In Parathyroid hormone and Thyrocalcitonin. Eds. R.V. Talmage & L.F. Belanger. Proceedings of the Third Parathyroid Conference. Excerpta Medica Foundation, 1968, p. 417-427.

CARE, A.D. & DUNCAN, T. (1967).
Age as a factor in the response to thyrocalcitonin secretion.
J. Endocr. 37, 107-108.

CARE, A.D. DUNCAN, T. & WEBSTER, D. (1967).
Thyrocalcitonin and its role in clacium homeostasis.
J. Endocr. 37, 155-167.

CARLSSON, A. & LINDQUIST, B., (1955).
Comparison of intestinal and skeletal effects of vitamin D in relation to dosage.
Acta. physiol. scand. 35, 53-55.

CARRER, H. ASCH, G., & ARON, C. (1973).
New facts concerning the role played by the ventromedial nucleus in the control of estrous cycle duration and sexual receptability in the rat.
Neuroendocr. 13, 129-138.

CARRIERE, R. (1965).
The influence of thyroid and testicular hormones on the epithelium of crypts of Lieberkuhn in the rats' intestine.
Anat. Rec. 156. 423-432.

CARTER, S.B. (1953).
The influence of oestrone on the division of cells.
J. Endocr. 9, 19-29.

CASE, R.M. (1973).
Calcium and gastrointestinal secretion.
Digestion 8, 269-288.

CASPARY, W.F. (1973).
Effect of insulin and experimental diabetes mellitus on the digestive-absorptive function of the small intestine.
Digestion 9, 248-263.

CATER, D. HALMER, B. & MEE, L. (1957).
The effect of growth hormone upon cell division and nucleic acid synthesis in the regenerating liver of the rat.
Biochem. J. 66, 482-486.

CHANG, H.Y. (1951).
Grafts of parathyroid and other tissues to bone.
Anat. Rec. 111, 23-48.

CHEN, P.S. & BOSMANN, H. (1964).
Effects of vitamin D_2 and D_3 on serum calcium and phosphorus in rachitic chicks.
J. Nutr. 83, 133-139.

CHEN, P.S. TORIBARA, T.Y. & WARNER, H., (1956).
Microdetermination of phosphorus.
Analyt. Chem. 28, 1756-1758.

CHENG, H. (1974a).
Origin differentiation and renewal of the four main epithelial cell types in the mouse small intestine. II Mucous Cell.
Am. J. Anat. 141, 481-502.

CHENG, H. (1974b).
Origin differentiation and renewal of the four main epithelial cell types in the mouse small instestine. IV Paneth cells.
Am.J. Anat. 141, 521-536.

CHENG, H. & LEBLOND, C.P. (1974a).
Origin differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I Columnar cell.
Am. J. Anat. 141, 461-480.

CHENG, H., & LEBLOND, C.P. (1974b).
Origin differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III Entero-endocrine cells.
Am. J. Anat. 141, 503-520.

CHENG, H., & LEBLOND, C.P. (1974c).
Origin differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V Unitarian theory of the origin of the four epithelial cell types.
Am. J. Anat. 141, 537-562.

CHUNG, A.C. (1973).
Some in vivo effects of chalone (mitotic inhibitor) obtained from lymphoid tissue.
Natl. Cancer Inst. Monogr. 38, 131-134.

CHURCH, R. B. & McCARTHY, B.J. (1967a).
Ribonucleic acid synthesis in regenerating and embryonic liver. I The synthesis of new species of RNA during regeneration of mouse liver after partial hepatectomy.
J. Molec. Biol. 23, 459-475.

CHURCH, R.B., & McCARTHY, B.J. (1967b).
Ribonucleic acid synthesis in regenerating and embryonic liver II. The synthesis of RNA during embryonic liver development and its relationship to regenerating liver.
J. Molec. Biol. 23, 477-486.

CLAMAN, H.N. CHAPERON, E.A. & TRIPLETT, R.F. (1966).
Immunocompetence of transferred thymus-marrow cell combinations.
J. Immun. 97, 828-832.

CLARK, I. (1968).
Dietary regulation of parathyroid activity. In Parathyroid Hormone and Thyrocalcitonin. Eds. R.V. Talmage & G.F. Belanger. Proceedings of the Third Parathyroid Conference. Excerpta Medica Foundation. p. 428-436.

CLARK, I.& RIVERA-CORDERO, F. (1973).
Effects of endogenous PTH on calcium magnesium and phosphate metabolism in rats.
Endocrinology 92, 62-71.

CLARK, I., & RIVERA-CORDERO, F. (1975).
Effects of endogenous PTH on calcium, magnesium and phosphate metabolism in rats. II Alteration in dietary phosphate.
Endocrinology 95, 360-370.

CLARKE, R.M. (1968).
A comparative analysis of methods of estimating the size of cell populations from microtome sections.
J.R. microsc. Soc. 88, 189-203.

CLARKE, R.M. (1970a).
The duration of mitosis in the crypts of Lieberkuhn of the terminal ileum of the albino rat.
Cell Tissue Kinet. 3, 27-34.

CLARKE, R.M. (1970b).
The effect of starvation upon mucosal architecture and cell production rate in the small intestine of the rat.
J. Anat. 107, 384.

CLARKE, R.M. (1971).
A comparison of metaphase arresting agents and tritiated thymidine autoradiography in measurement of the rate of entry of cells into mitosis in the crypts of Lieberkuhn of the rat.
Cell Tissue Kinet. 4, 263-272.

CLARKE, R.M. (1972).
The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat.
J. Anat. 112, 27-33.

CLARKE, R.M. (1974).
Control of intestinal epithelial replacement: lack of evidence for a tissue specific blood borne factor.
Cell Tissue Kinet. 7, 241-251.

CLAUSEN, F.W. & FREUDENBERGER, C.B. (1939).
A comparison of the effects of male and female sex hormones on immature female rats.
Endocrinology 25, 585-592.

CLEAVER, J.E. (1967).
In Thymidine Metabolism and Cell Kinetics.
Wiley, New York.

CLERMONT, Y. & MAUGER, A. (1974).
Evidence of a spermatogonial chalone in the rat testis.
Cell Tissue Kinet. 7, 165-173.

COFFEY, D.S., SHIMAZAKI, J., & WILLIAMS-ASHMAN, H.G. (1968).
Polymerization of deoxyribonucleotides in relation to androgen-induced prostatic growth.
Archs. Biochem. Biophys. 124, 184-198.

COOPER, C.W., HIRSCH, P.F.& MUNSON, P.L. (1970).
Importance of endogenous thyrocalcitonin for protection against hypercalcaemia in the rat.
Endocrinology 86, 406-415.

COOPER, C.W., SCHWESINGER, W.H., MAHGOUB A.M., ONTJES, D.A., GRAY, T.K., & MUNSON, P.L., (1972).
Regulation of secretion of thyrocalcitonin. In Calcium, Parathyroid hormone and the Calcitonins. Proceedings of the Fourth Parathyroid Conference. Eds. R.V. Talmage & P.L. Munson. Excerpta Medica, Amsterdam, p. 128-139.

COOPER, C.W., SCHWESINGER, W.H., ONTJES, D.A., MAHGOUB, A.M., & MUNSON, P.L. (1972).
Stimulation of secretion of pig thyrocalcitonin by gastrin and related hormonal peptides.
Endocrinology 91, 1079-1089.

COOPER, E.H., BARKHAM, P. & HALE, A.J. (1963).
Observations on the proliferation of human leucocytes cultured with phytohaemagglutinin.
Br. J. Haemat. 9, 101-111.

COOPER, H.L. (1971).
Biochemical Alterations accompanying initiation of growth in resting cells. In The Cell Cycle & Cancer. Ed. R. Baserga. Marcel Dekker Inc., New York. p. 197-226.

COOPER, M.D., PETERSON, R.D.A., SOUTH, M.A., & GOOD, R.A. (1966).
The functions of the thymus system and the bursa system in the chicken.
J. exp. Med. 123, 75-102.

COOPER, T.G. & WAITES, G.M. (1974).
Testosterone in rete testis fluid and blood of rams and rats.
J. Endocr. 62, 619-629.

COPP, D.H. (1969a).
Calcitonin and parathyroid hormone.
A. Rev. Pharmac. 9, 327-344.

COPP, D.H. (1969b).
Endocrine control of calcium homeostasis.
J. Endocr. 43, 137-161

COPP, D.H., CAMERON, E.C., CHENEY, B.A., DAVIDSON, A.G.F., & HENZE, K.G.(1962).
Evidence for calcitonin - a new hormone from the parathyroid that lowers blood calcium.
Endocrinology 70, 638-649.

COPP, D.H., CHENEY, B.A., & STOKOE, N.H., (1963).
Simple and precise micromethod for EDTA titration of calcium.
J. Lab. clin. Invest. 61, 1029-1037.

COPP, D.H., COCKCROFT, D.W., & KUEH, Y. (1967).
Ultimobranchial origin of calcitonin. Hypocalcaemic effect of extracts from chicken glands.
Can. J. Physiol. Pharmac. 45, 1095-1099.

COPP, D.H., & DAVIDSON, A.G.F., (1961).
Direct humoral control of parathyroid function in the dog.
Proc. Soc. exp. Biol. Med. 107, 342-344.

COPP, D.H., & KUCZERPA, A.V. (1970).
A new bioassay for calcitonin and the effect of age and dietary calcium and phosphorus on the response. In Calcitonin 1969. Proceedings Second International Symposium. Ed. S. Taylor. Heinemann Medical Books Ltd., London. p 18.

CRABBE, P.A., CARBONARA, A.O. & HEREMANS, J.F. (1965).
The normal human intestinal mucosa as a major source of plasma cells containing γA-immunoglobulin.
Lab. Inuest. 14, 235-248.

CRADDOCK, C.G., NAKAI, G.S., FUKUTA, H., & VANSLAGER, L.M. (1964).
Proliferative activity of the lymphatic tissues of rats as studied with tritium-labelled thymidine.
J. exp. Med. 120, 389-412.

CRADDOCK, C.G., WINKELSTEIN, A., MATSUYUKI, Y.& LAWRENCE, J.S. (1967).
In Control of Cellular Growth in Adult Organisms. Eds. H. Teir & T. Rytomaa. Academic Press, London & New York. p.153.

CRONKITE, E.P., BOND, V.P., FLIEDNER, T.M., & RUBINI, J.R. (1959).
The use of tritiated thymidine in the study of DNA synthesis and cell turnover in haemopoietic tissues.
Lab. Invest. 8, 263-277.

CURRIE, G.N., & BLACK, D.C. (1972).
Effects of oestrogen on thyrocalcitonin activity in male and female rabbits.
Acta endocr. Copnh. 71, 55-61.

CUTRIGHT, D.E., & BAUER, H., (1967).
Cell renewal in the oral mucosa and skin of the rat. I Turnover time.
Oral Surg. 23, 249-259.

DALLMAN, M.F., & JONES, M.T. (1973).
Corticosteroid feedback control of ACTH secretion: effect of stress-induced corticosterone secretion on subsequent stress responses in the rat.
Endocrinology 92, 1367-1375.

D'AMOUR, F.E., BLOOD, F.R., & BELDEN, D.A. Jr., (1965).
In Manual for laboratory work in mammalian physiology.
Chicago & London: The University of Chicago Press.

DAVID, J.R. (1971).
The elusive humors of the lymphocyte mediators of delayed hypersensitivity.
J. Allergy 47, 237-246.

DAY, H.G. & FOLLIS, R.H. (1941).
Skeletal changes in rats receiving estradiol benzoate as indicated by histological studies and determination of bone ash, serum calcium and phosphatase.
Endocrinology 28, 83-93.

DELORME, M.L., MERCERON, R., RAYMOND, J.P. & KLOTZ, H.P. (1974).
Serum calcitonin, radioimmunological determination in the diagnosis of medullary thyroid carcinoma.
Annls. Endocr. 35, 289-291.

DELUCA, H.F. (1973).
The kidney as a endocrine organ involved in calcium homeostasis.
Kidney Int. 4, 80-88.

DELUCA, H.F. (1974).
Parathyroid hormone, calcitonin, and vitamin D: Clinical considerations (Part II).
Am. J. Med. 57, 1-12.

DEMAERTELAER, V., & GALAND, P. (1975).
Some properties of a G^{0} model of the cell cycle.
Cell Tissue Kinet. 8, 11-22.

DEWEY, D.L. (1973).
The melanocyte chalone,
Natl. Cancer Inst. Monogr. 38, 213-216.

DIAMOND, L.K., JACOBSON, W., & SIDMAN, R.L., (1967).
Testosterone induced DNA synthesis in human bone marrow.
Men. Soc. Endocr. 15, 37-42.

DISCHE, Z., (1955).
In The Nucleic Acids. Eds. E. Chargoff & J.N. Davidson. New York
Academic Press, Vol. I p. 285.

DORRINGTON, K.J., HUI, A., HOFMANN, T., HITCHMAN, A.J.W., & HARRISON, J.E. (1974)
Procine intestinal calcium-binding protein. Molecular properties and
the effect of binding calcium ions.
J. biol. Chem. 249, 199-204.

DOUGLAS, W.W., & POISNER, A.M. (1964).
Stimulus-secretion coupling in a neurosecretory organ: the role of
calcium in the release of vasopressin from the neurohypophysis.
J. Physiol. 172, 1-18.

DOUGLAS, W.W., & RUBIN, R.P. (1963).
The mechanism of catecholamine release from the adrenal medulla and the
role of calcium in stimulus-secretion coupling.
J. Physiol. 167, 288-310.

DOWDLE, E.B., SCHACHTER, D., & SCHENKER, H., (1960).
Requirement for vitamin D for the active transport of calcium by the
intestine.
Am.J.Physiol. 198, 269-274.

DOWLING, R.H. & BOOTH, C.C. (1967).
Structural and functional changes following small intestinal resection
in the rat.
Clin. Sci. 32, 139-149.

DOWLING, R.H., RIECKEN, E.O. LAWS, J.W., & BOOTH, C.C., (1967).
The intestinal response to high bulk feeding in the rat.
Clin. Sci. 32, 1-9.

DUKOR, P., MILLER, J.F.A.P., HOUSE, W., & ALLMAN, V., (1965).
Regeneration of thymus grafts 1. Histological and cytological aspects.
Transplantation 3, 639-668.

DUNN, M.J. (1971).
Magnesium depletion in the rhesus monkey: induction of magnesium-
dependent hypocalcaemia.
Clin. Sci. 41, 333-344.

DUNNILL, M.S. & WHITEHEAD, R., (1972).
A method for the quantitation of small intestinal biopsy specimens.
J. Clin. Path. 25, 243-246.

DURAND, G., FAUCONNEAU, G. & PENOT, E., (1965).
Biochemical study of rat small intestine, liver, and carcass growth: respective roles of proliferation and growth in cell size.
Ann. Biol. Anim. Biochim. Biophys. 5, 163-187.

EARTLY, H., GRAD, B., & LEBLOND, C.P. (1951).
The antagonistic relationship between testosterone and thyroxine in maintaining the epidermis of the male rat.
Endocrinology 49, 677-686.

EBLING, F.J. (1954).
Changes in the sebaceous glands and epidermis during the oestrus cycle of the albino rat.
J. Endocr. 10, 147-154.

EBLING, F.J. (1974).
Hormonal control and methods of measuring sebaceous gland activity.
J. invest. Derm. 62, 161-171.

EIGSTI, O.J. & DUNSTIN, P., Jr., (1955).
In Colchicine in Agriculture, Medicine, Biology & Chemistry. Iowa State College Press, Ames, Iowa.

EISENBERG, E.J. (1966).
Effects of androgens, oestrogens and corticoids on strontium kinetics in man.
J. clin. Endocr. Metab. 26, 566-572.

ELGJO K., (1974).
Evidence for presence of the epidermal G_2-inhibitor (epidermal chalone) in dermis.
Cell Pathology 16, 243-249.

ELIEL, L.P., PALMIERI, G.M.A., THOMPSON, J.S., BIRD, P.C., & HAWRYLKO, J. (1971).
The relationships between adrenal cortical steroids, parathyroid extract and calcitonin.
Pediatrics. 47, 229-237.

EMTAGE, J.S., LAWSON, D.E.M. & KODICEK, E., (1973).
Vitamin D-induced synthesis of mRNA for calcium-binding protein.
Nature 246, 100-101.

EMTAGE, J.S., LAWSON, D.E.M. & KODICEK, E., (1974).
The response of the small intestine to vitamin D. Correlation between calcium-binding protein production and increased calcium absorption.
Biochem. J. 144, 339-346.

EPIFANOVA, O.I. (1966).
Mitotic cycles in oestrogen-treated mice: A radioautographic study.
Expl. Cell. Res. 42, 562-577.

EPIFANOVA, O.I. (1971).
Effects of Hormones on the Cell Cycle. In The Cell Cycle and Cancer.
Ed. R. Baserga. Marcel Dekker Inc., New York.
P. 145-190.

EPIFANOVA, O.I., & TCHOUMAK, M.G. (1963).
On the action of adrenaline upon the mitotic cycle of intestinal epithelium of mice.
Tsitologia 5, 455-458.

EPIFANOVA, O.I. & TERSKIKH, V.V. (1969).
On the resting periods in the cell life cycle.
Cell. Tissue Kinet. 2, 75-93.

ERDHEIM, J. (1906).
Tetania parathyreoprivia.
Mitt. Grenzg. Med. Chir. 16, 632-744.

ERLANDSEN, S.L., & CHASE, D.G. (1972).
Paneth cell function: phogocytosis and intracellular digestion of intestinal micro-organisms.
J. Ultrastruct. Res. 41, 296-318.

ERSPAMER, V., & ASERO, B., (1952).
Identification of enteramine the specific hormone of the enterochromaffin cell system, as a 5-hydroxytryptamine.
Nature 169, 800-801.

EVERETT, N.B., & TYLER, R.W., (1967).
Lymphopoiesis in the thymus and other tissues: Functional implications.
Int. Rev. Cytol. 22, 205-237.

FANTES, P.A., GRANT, W.D., PRITCHARD, R.M., SUDBURY, P.E. & WHEALS, A.E., (1975).
The regulation of cell size and the control of mitosis.
J. theor. Biol. 50, 213-244.

FELDMAN, M., & GLOBERSON, A., (1972).
The inductive phase of antibody production. In Immunogenicity.
Ed. F. Borek. North-Holland Publishing Co., Amsterdam & London.
p. 273-301.

FELL, B.F., SMITH, K.A., & CAMPBELL, R.M. (1963).
Hypertrophic and hyperplastic changes in the alimentary canal of the lactating rat.
J. Path. Bact. 85, 179-188.

FERGUSON, J.H., (1936).
The blood calcium and the calcium factor in blood coagulation.
Physiol. Rev. 16, 640-670.

FINKELSTEIN, G. GORDON, A.S. & CHARIPPER, H.A. (1944).
The effect of sex hormones on the anaemia induced by haemorrhage in the rat.
Endocrinology 35, 267-277.

FINN, C.A., MARTIN, L. & CARTER, J., (1969).
A refractory period following oestrogenic stimulation of cell division in the mouse uterus.
J. Endocr. 44, 121-126.

FISHER, J.W. (1972).
Erythropoietin: Pharmacology, Biogenesis and Control of production.
Pharmac. Rev. 24, 459-508.

FITZGERALD, P.H. & BREHAUT, L.A. (1970).
Depression of DNA synthesis and mitotic index by colchicine in cultured human lymphocytes.
Expl. Cell. Res. 59, 27-31.

FOLLEY, S.J. (1955).
Hormones in mammary growth and function.
Br. Med. Bull. 11, 145-150.

FORD, C.E. (1966).
In The Thymus: Experimental & Clinical Studies. Eds.G.E.W. Wolstenholme & R. Porter. London: Churchill. p. 141.

FORRESTER, J.M. (1972).
The number of villi in rat's jejunum and ileum: effect of normal growth, partial enterectomy and tube feeding.
J. Anat. 111, 283-291.

FRANKFURT. O.S. (1971).
Epidermal chalone. Effect on cell cycle and on development of hyperplasia.
Expl. Cell. Res. 64, 140-144.

FRASER, D.R.& KODICEK, E., (1973).
Regulation of 25-hydroxycholecolciferol-1-hydroxylase activity in kidney by parathyroid hormone.
Nature New Biology 241, 163-166.

FREEDMAN, M.H., RAFF, M.C. & GOMPERTS, B., (1975).
Induction of increased calcium uptake in mouse T lymphocytes by concanavalin A and its modulation by cyclic nucleotides.
Nature 255, 378-382.

FREUDENBERGER, C.B. & CLAUSEN, F.W., (1937a).
The effects of continued theelin injections on the body growth and organ weights of young female rats.
Anat. Rec. 68, 133-144.

FREUDENBERGER, C.B., & CLAUSEN, F.W. (1937b).
Quantitative effects of theelin on body growth and endocrine glands in young albino rats.
Anat. Rec. 69, 171-177.

FRIED, W., TICHLER, T., DEINEBERG, I., BARONE, J. & WANG, F., (1974).
Effects of estrogens on hematropoietic stem cells and on hematopoiesis of mice.
J. Lab. clin. Med. 83, 807-816.

FRIEDMAN, J., AU, W.Y.W. & RAISZ, L.G. (1968).
Responses of fetal rat bone to thyrocalcitonin in tissue culture.
Endocrinology 82, 149-156.

FRUHMAN, G.J. (1966).
The estrous cycle and splenic erythropoiesis in the mouse.
Proc. Soc. exp. Biol. Med. 122, 493-495.

FULLMER, C.S. & WASSERMAN, R.H., (1973).
Bovine intestinal calcium-binding proteins. Purification and some properties,
Biochim. biophys. Acta. 317, 172-186.

GALAND, P., RODESCH, F., LEROY, F., & CHRETIEN, J. (1967).
Radioautographic evaluation of the estrogen-dependent proliferative pool in the stem cell compartment of the mouse uterine and vaginal epithelia.
Expl. Cell. Res. 48, 595-604.

GALANTE, L., COLSTON, K.W., EVANS, I.M.A., BYFIELD, P.G.H., MATHEWS, E.W., & MacINTYRE, I., (1973).
The regulation of vitamin D metabolism.
Nature 244, 438-440.

GALJAARD, H., VAN DER MEER-FIENGGEN, W.& GIESSEN, J., (1972).
Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium.
Expl, Cell. Res. 73, 197-207.

GALLAGHER, J.C., & NORDIN, B.E.C. (1973).
Oestrogens and calcium metabolism.
Frontiers Hormone Res. 2, 98-117.

GARCIA-GIRALT, E., LASALVIA, E., FLORENTIN, I., & MATHE, G., (1970).
Evidence for a lymphocytic chalone.
Eur. J. Clin. Biol. Res. 15, 1012-1015.

GAREL, J.M., & BARLET, J.P. (1974).
The effects of calcitonin and parathormone on plasma magnesium levels before and after birth in the rat.
J. Endocr. 61, 1-13.

GARRY, R., & HALL, R., (1970).
Stimulation of mitosis in rat thyroid by LATS.
Lancet. 2, 693-695.

GELFANT, S., (1959).
A study of mitosis in mouse ear epidermis in vitro.
Expl. Cell. Res. 16, 527-537.

GELFANT, S. (1962).
Initiation of mitosis in relation to the cell division cycle.
Expl. Cell. Res. 26, 395-403.

GELFANT, S., (1963).
Inhibition of cell division: a critical and experimental analysis.
Int. Rev. Cytol. 14, 1-39.

GIBADULIN, R.A.(1962).
On compensatory hypertrophy of the thyroid gland.
Byll. eksp. Biol. Med. 54, 84-87.

GITELMAN, H.J., KUKOLJ, S., & WELT, L.G., (1968a).
The influence of the parathyroid glands on the hypercalcaemia on experimental magnesium depletion in the rat.
J. clin. Invest. 47, 118-126.

GITELMAN, H.J., KUKOLJ, S., & WELT, L.G. (1968b).
Inhibition of parathyroid gland activity by hypermagnesemia.
Am. J. Physiol. 215, 483-485.

GLEESON, M.H., CULLEN, J., & DOWLING, R.H. (1972).
Intestinal structure and function after small bowel bypass in the rat.
Clin. Sci. 43, 731-742.

GLINOS, A.D., (1967).
In Control of Cellular Growth in Adult Organisms. Eds. H. Teir & T. Rytomaa. Academic Press, New York. p. 41-53.

GOLDBERG, N.D., O'DEA,R.F.& HADDOX, M.K. (1973a).
In Advances in Cyclic Nucleotide Research. Eds. P. Greengard & G.A. Robinson. Raven Press, New York, Vol. 3, 155-223.

GOLDBERG, N.D., O'DEA, R.F.& HADDOX, M.K., (1973b).
Cyclic GMP.
Adv. Cyclic Nucleotide Res. 3, 155-223.

GOLDBERG, N.D., HADDOX, M.K. ESTENSEN, R., WHITE, J.G., LOPEZ, C., & HADDEN, J.W., (1973).
In Cyclic-AMP, Cell growth and the Immune response. Eds. W, Braun, L.M. Lichenstein & C.W. Parker. Springer-Verlag, New York. p. 247-262.

GOLDSTEIN, A.L., ASANUMA, Y., BATTISTO, J.R., HARDY, M.A., QUINT, J., & WHITE, A. (1970).
Influence of thymosin on cell-mediated and humoral immune responses in normal and immunologically deficient mice.
J. Immun. 104, 359-366.

GOLDSTEIN, A.L., SLATER, F.D., & WHITE, A., (1966).
Preparation, assay, and partial purification of a thymus lymphocytopoietic factor.
Proc. natn. Acad. Sci. U.S.A., 56, 1010-1017.

GOLTZMAN, D., POTTS, J.T. Jr., CHESTER RIDGWAY, E., & MALOOF, F., (1974).
Calcitonin as a tumour marker in patients with medullary thyroid carcinoma.
New Engl. J. Med. 290, 1035-1040.

GOODMAN, L.S., & GILMAN, A. (Eds.) (1970).
In The Pharmacological Basis of Therapeutics.
The Macmillan Company. 4th Edition, p. 813.

GORDON, A.S. (1957).
Influence of humoral factors on erythropoiesis.
Amer. J. clin. Nutr. 5, 461-472.

GORDON, A.S. (1959).
Hemopoietine.
Physiol. Rev. 39, 1-40.

GORDON, A.S., (1973).
Erythropoietin.
Vit. Horm. 31, 105-174.

GOSS, R.J., (1967).
The Strategy of Growth. In Control of Cellular Growth in Adult Organisms. Eds. H.Teir & T. Rytomaa. Academic Press. London & New York. p. 3-27.

GOWANS, J.L., & McGREGOR, D.D. (1964).
In The Immunologically Competent Cell. Eds. G.E.W. Wolstenholme & J. Knight. Churchill, London. p.20.

GRAY, T.K., & MUNSON, P.L. (1969).
Thyrocalcitonin: evidence for physiological function.
Science 166, 512-513.

GRISHAM, J.W., (1962).
A morphologic study of deoxyribonucleic acid snythesis and cell proliferation in regenerating rat liver: autoradiography with ^{3}H-thymidine.
Cancer Res. 22, 842-849.

GRUTZNER, P. & MENZEL, H., (1879).
Veber bildung und Ausscheidung von Fermenten.
Arch. Ges. Physiol. 20, 395-419.

HALES, C.N. & RANDLE, P.J., (1963).
Immunoassay of insulin with insulin antibody.
Biochem. J. 88, 137-146.

HAM, A.W., & LEESON, T.S., (1961).
In Histology. Pitman Medical Publishing Co. Ltd., London. 5th Edition, p. 692.

HAMILTON, J.W., & COHN, D.V., (1969).
Studies on the biosynthesis in vitro of parathyroid hormone.
J. biol. Chem. 244, 5421-5429.

HAMILTON, T.H. (1968).
Control by estrogen of genetic transcription and translation.
Science 161, 649-661.

HANSKY, J., SOVENY, C. & KORMAN, M.G. (1971).
Effect of secretin on serum gastrin as measured by immunoassay.
Gastroenterology 61, 62-68.

HARDING, C.V., FELDHERR, C., & SRINIVASAN, B.D., (1961).
In The Structure of the Eye. Ed. Smelser, G.K. Academic Press, New York. p.273.

HARDMAN, J.G., BEAVO, J.A., GRAY, J.P., CHRISMAN, T.D. PATTERSON, W.D., & SUTHERLAND, E.W., (1971).
The formation and metabolism of cyclic GMP.
Ann. N.Y. Acad. Sci. 185, 27-35.

HARRISON, H.C., HARRISON, H.E. & PARK, E.A. (1958).
Vitamin D and citrate metabolism effect of vitamin D in rats fed diets adequate in both calcium and phosphorus.
Am. J. Physiol. 192, 432-436.

HARRISON, M.T. (1964).
Interrelationships of vitamin D and parathyroid hormone in calcium homeostasis.
Postgrad. Med. J. 40, 497-505.

HARVEY, J.S., HERVEY, G.R., & HUTCHINSON, I., (1973).
The role of gonadal hormones in determining the sex difference in adult body weight in the rat.
J. Endocr. 57, xxiv

HASHIM, G., & CLARK, I. (1969).
Studies on the effect of vitamin D on calcium absorption and transport.
Biochem. J. 112, 275-283.

HASHIMOTO, I., HENRICKS, D.M., ANDERSON, L.L. & MELAMPY, R.M. (1968).
Progesterone and pregn-4-en-20α-ol-3-one in ovarian venous blood during various reproductive states in the rat.
Endocrinology 82, 333-341.

HATTNER, R.S., JOHNSON, R.W., BERSTEIN, D.S., WACHMAN, A. & BRACKMAN, J. (1970).
Electrochemical determination of apparent ionized serum calcium using a calcium-selective electrode: The method and values in normal humans and a comparison of total serum calcium.
Clinica. chim. Acta. 28, 67-75.

HEATH, D.A., PALMER, J.S., & AURBACH, G.D. (1972).
The hypocalcaemic action of colchicine.
Endocrinology 90, 1589-1593.

HEATON, F.W., (1969).
The kidney and magnesium homeostasis.
Ann. N.Y.Acad. Sci. 162, 775-786.

HEELEY, D.M., WARNER, G.T., MAYER, A.L.R., (1974)
Magnesium in malignant disease.
Lancet 1, 634.

HELL, E. & COX, D.G., (1963).
Effects of colchicine and colcemid on synthesis of desoxyribonucleic acid in the skin of the guinea pig's ear in vitro.
Nature 197, 287-288.

HENDRIX, J.W., ALCOCK, N.W., ARCHIBALD, R.M., (1963).
Competition between calcium, strontium and magnesium for absorption in the isolated rat intestine.
Clin. Chem. 9, 734-744.

HERRMANN, H., MARCHOK, A.C., & BORIL, E.F., (1967).
Growth rate and differentiated function of cells.
Nat. Cancer Inst. Monogr. 26, 303-316.

HIRSCH, P.F., GAUTHIER, G.F., & MUNSON, M.L., (1963).
Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as factors affecting the response to parathyroidectomy in rats.
Endocrinology 73, 244-252.

HITCHMAN, A.J.W., KERR, M.K., & HARRISON, J.E., (1973).
The purification of pig vitamin D-induced intestinal calcium binding protein.
Archs. Biochem. Biophys. 155, 221-222.

HODGSON, G., (1967).
Synthesis of RNA and DNA at various intervals after erythropoietin injection in transfused mice.
Proc. Soc. exp. Biol. Med. 124, 1045-1047.

HOLICK, M.F., & DELUCA, H.F., (1974).
Vitamin D Metabolism.
A. Rev. Med. 25, 349-369.

HOLICK, M.F., KLEINER-BOSSALLER, A., SCHNOES, H.K., KASTEN, P.M., BOYLE, I.T., & DELUCA, H.F., (1973).
1,24,25 trihydroxy vitamin D_3. A metabolite of vitamin D_3 effective on the intestine.
J. biol. Chem. 248, 6691-6696.

HOLICK, M.F., SCHNOES, H.K., & DELUCA, H.F., (1971).
Identification of 1,25 dihydroxycholacalciferol, a form of vitamin D_3 metabolically active in the intestine.
Proc. natn. Acad. Sci. U.S.A. 68, 803-804.

HOLICK, M.F., SCHNOES, H.K., DELUCA, H.F., GRAY, R.W., BOYLE, I.T., & SUDA, T., (1972).
Isolation and identification of 24,25 dihydroxycholecalciferol, a metabolite of vitamin D_3 made in the kidney.
Biochemistry 11, 4251-4255.

HOOPER, C.S., & BLAIR, H., (1958).
The effect of starvation on epithelial renewal in the rat duodenum.
Expl. Cell. Res. 14, 175-181.

HORIUCHI, N., SUDA, T., SASAKI, S., EZAWA, I., SANO, Y., & OGATA, E., (1974).
Direct involvement of vitamin D in the regulation of 25-hydroxycholecalciferol metabolism.
FEBS. Lett. 43, 353-356.

HOUCK, J.C., CHENG, R.F., & SHARMA, V.K., (1973).
Control of fibroblast proliferation.
Natl. Cancer Inst. Monogr. 38, 161-170.

HOUCK, J.C., IRASQUIN, H., & LEIKIN, S., (1971).
Lymphocyte DNA synthesis inhibition.
Science 173, 1139-1141.

HOWARD, A., & PELC, S.R., (1953).
Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage.
Heredity Suppl. 6, 261-273.

HUNT, N.H., (1974).
The role of hormones and ions in the physiological control of cellular proliferation in rat erythropoietic and lymphopoietic tissues.
Ph.D. Thesis. University of Aston in Birmingham.

HUNT, N.H., & PERRIS, A.D., (1973).
Erythropoietin-induced changes in plasma calcium and bone marrow mitosis in the rat.
J. Endocr. 56, 47-57.

HUNT, N.H., & PERRIS, A.D., (1974).
Calcium and the control of circadian mitotic activity in rat bone marrow and thymus.
J. Endocr. 62, 451-462.

ILLIANO, G., TELL, G.P.E., SIEGEL, M.I., & CUATRESCASAS, P., (1973).
Guanosine 3'5' cyclic monophosphate and the action of insulin and acetylcholine.
Proc. natn. Acad. Sci. U.S.A. 70, 2443-2447.

INGLE, D.J., & GRIFFITH, J.P., (1942).
In The rat in laboratory investigation. Eds. J.P. Griffith & E.J. Farris. Philadelphia, J. P. Lippincot Co., Chpt. 16, p.439.

IVERSON, O.H., & ELGJO, K., (1967).
The effect of chalone on the mitotic rate and on the mitotic duration in hairless mouse epidermis. In Control of Cellular Growth in Adult Organisms. Eds. H. Teir & T. Rytomaa. Academic Press, New York. p.83-91.

IZQUIERDO, J.N., & GIBBS, S.J., (1974).
Turnover of cell-renewing populations undergoing circadian rhythms in cell proliferation.
Cell Tissue Kinet. 7, 99-111.

JACKSON, C.M. (1913).
Postnatal growth and variability of body and various organs and systems in the albino rat.
Am. J. Anat. 15, 1-68.

JACKSON, C.M., & P'AN, M.T., (1932).
The effects of dietary deficiency of iodin uptake upon the thyroid and parathyroid glands in the rat.
Endocrinology 16, 146-152.

JACOBSOHN, D., (1958).
Mammary gland growth in relation to hormones with metabolic actions.
Proc. R. Soc. Series B., 149, 325-329.

JEPSON, J.H., & LOWENSTEIN, L. (1966).
Inhibition of the stem cell action of erythropoietin by oestradiol.
Proc. Soc. exp. Biol. Med. 123, 457-460.

JERVIS, E.L., & LEVIN, R.J., (1966).
Anatomic adaptation of the alimentary tract of the rat to the hyperphagia of chronic alloxan-diabetes.
Nature 210, 391-393.

JUNOD, A., LAMBERT, A.E., STAUFFACHER, W., & RENOLD, A.E., (1969).
Diabetogenic action of streptozotocin: Relationship of dose to metabolic response.
J. clin. Invest. 48, 2129-2139.

KALLFELZ, F.A., TAYLOR, A.N.& WASSERMAN, R.H., (1967).
Vitamin D-induced calcium binding factor in rat intestinal mucosa.
Proc. Soc. exp. Biol. Med. 125, 54-58.

KALU, D.N., HADJI-GEOROPOULOS, A., SARR, M.G., SOLOMON, B.A., & FOSTER, G.V., (1974).
The role of parathyroid hormone in the maintainence of plasma calcium levels in rats.
Endocrinology 95, 1156-1166.

KEMM, J.R., (1972).
The effect of previous dietary intake of calcium on calcium absorption in rats.
J. Physiol. 223, 321-332.

KENNEY, N.J., & MOOK, D.G., (1974).
Effects of ovariectomy on meal pattern in the albino rat.
J. Comp. Physiol. Psychol. 87, 302-309.

KENNY, A.D., BOELKINS, J.N., DACKE, C.G., FLEMING, W.R., & HANSON, R.C., (1971).
Plasma calcitonin levels in birds and fish. In Endocrinology 1971, Proceedings of the Third International Symposium. William Heinemann Medical Books, London. p. 39-47.

KIMBERG, D.V., SCHACHTER, D.& SCHENKER, H., (1961).
Active transport of calcium by intestine: effects of dietary calcium.
Am. J. Physiol. 200, 1256-1262.

KINDRED, J.E., (1940).
A quantitative study of the hemopoietic organs of young albino rats.
Am. J. Anat. 67, 99-144.

KIVILAAKSO, E., & RYTOMAA, T., (1971).
Erythrocytic chalone, a tissue specific inhibitor of cell proliferation in the erythron.
Cell Tissue Kinet. 4, 1-9.

KLEIN, D.C., & TALMAGE, R.V., (1968).
Evidence for secretion of thyrocalcitonin at normal and subnormal plasma levels.
Endocrinology 82, 132-136.

KLEIN, J.J., GOLDSTEIN, A.L., & WHITE, A., (1965).
Enhancement of in vivo incomporation of labeled precursors into DNA and total protein of mouse lymph nodes after administration of thymic extracts.
Proc. natn. Acad. Sci. U.S.A. 53, 812-817.

KORENCHEVSKY, V., BURBANK, R., & HALL, K., (1939).
The action of the dipropionate and benzoate butyrate of oestradiol on ovariectomised rats.
Biochem. J. 33, 366-371.

KORMAN, M.G., SOVENY, C., & HANSKY, J., (1971).
Effect of food on serum gastrin evaluated by radioimmoassay.
Gut 12, 619-624.

KRAMER, B., & HOWLAND, J., (1922).
Factors which determine the concentration of calcium and of inorganic phosphorus in the blood serum of rats. I.
Bull. Johns Hopkins Hosp. 33, 313-317.

KRAMER, B., & HOWLAND, J., (1931).
Factors which determine the concentration of calcium and inorganic phosphorus in the blood of rats. II.
J. Nutr. 5, 39-60.

KRAWITT, E.L., (1967).
Effects of thyrocalcitonin on duodenal calcium transport.
Proc. Soc. exp. Biol. Med. 125, 1084-1086.

KUMAR, M.A., & STURTRIDGE, W.C., (1973).
The physiological role of calcitonin assessed through chronic calcitonin deficiency in rats.
J. Physiol. 233, 33-43.

KUNA, S., GORDON, A.S., MORSE, B.S., LANE, F.B., & CHARIPPER, H.A., (1959).
Bone marrow function in perfused isolated hind limbs of rats.
Am. J. Physiol. 196, 769-774.

LAFFERTY, F.S., SPENCER, G.E. Jr., & PEARSON, O.H., (1964).
Effects of androgens, oestrogens and high calcium intakes on bone formation and resorption in osteoporosis.
Am. J. Med. 36, 514-528.

LAGUCHEV, S.S. & AVETISYAN, A.A., (1972).
The action of hydrocortisone on time parameters of the mitotic cycle of epithelial cells in the duodenum.
Byull. eksp. Biol. Med. 73, 86-88.

LAJTHA, L.G., (1962).
Problems in bone marrow cell kinetics.
Postgrad. Med. J. 38, 41-47.

LAJTHA, L.G., (1970).
Stem cell kinetics. In Regulation of Hematopoiesis; I Red Cell Production. Ed. A.S. Gordon. Appleton-Century-Crofts: New York. p. 111-131.

LALA, P.K., MALONEY, M.A., & PATT, H.M., (1964).
A comparison of two markers of cell proliferation in bone marrow.
Acta. haemat. 31, 1-8.

LAMBERT, R., (1965).
In Surgery of the Digestive System in the Rat. Thomas: Springfield Ill. p.9.

LARKINS, R.G., COLSTON, K.W., GALANTE, L.S., MacAULEY, S.J., EVANS, I.M.A., & MacINTYRE, I., (1973).
Regulation of vitamin D metabolism without parathyroid hormone.
Lancet 2, 289-291.

LAWSON, D.E.M., & EASTAGE, J.S., (1974).
Molecular action of vitamin D in the chick intestine.
Vit. Horm. 32, 277-298.

LAWSON, D.E.M., FRASER, D.R., KODICEK, E., MORRIS, H.R., & WILLIAMS, D.H., (1971).
Identification of 1,25 dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism.
Nature 230, 228-230.

LEBLOND, C.P., & CARRIERE, R., (1955).
The effect of growth hormone and thyroxine on the mitotic rate of the intestinal mucosa of the rat.
Endocrinology 56, 261-266.

LEBLOND, C.P., & MESSIER, B., (1955).
Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of ^{3}H-thymidine into mice.
Anat. Rec. 132, 247-259.

LEBLOND, C.P., MESSIER, B.& KOPRIWA, B., (1959).
Thymidine-H^{3} as a tool for the investigation of the renewal of cell populations.
Lab. Invest. 8, 296-308.

LEBLOND, C.P., & STEVEVS, C.E., (1948).
The constant renewal of the intestinal epithelium in the albino rat.
Anat. Rec. 100, 357-378.

LEBLOND, C.P., STEVENS, C.E., & BOGOROCH,R. (1948).
Histological locatization of newly formed desoxyribonucleic acid.
Science 108, 531-533.

LEBLOND, C.P., & WALKER, B.E., (1956).
Renewal of cell populations.
Physiol. Rev. 36, 255-276.

LEDUC, E.H., (1964).
In The Liver. Ed. Ch. Rouiller, Academic Press, New York & London.

LEE, A.E., & ROGERS, L.A., (1972).
Cell division in the mouse vagina during continuous oestrogen stimulation.
J. Endocr. 54, 357-358.

LEE, A.E., ROGERS, L.A., & TRINDER, G., (1974).
Changes in cell proliferation rate in mouse uterine epithelium during continuous oestrogen treatment.
J. Endocr. 61, 117-121.

LEMAGNEN, J., & DEVOS, M., (1970).
Metabolic correlates of the meal onset in the free food intake of rats.
Physiol. Behav. 5, 805-814.

LESHER, S., WALBURG, H.E., & SACHER, G.A., (1964).
Generation cycle in the duodenal crypt cells of germ free and conventional mice.
Nature 202, 884-886.

L'ESTRANGE, J.L., & AXFORD, R.F.E., (1964).
A study of magnesium and calcium metabolism in lactating ewes fed a semi-purified diet low in magnesium.
J. Agric. Sci. 62, 353-368.

LEVANT, J.A., WALSH, J.H., & ISENBERG, J.I., (1973).
Stimulation of gastric secretion and gastrin release by single oral doses of calcium carbonate in man.
New. Engl. J. Med. 289, 555-558.

LEVI, J., MASSRY, S.G., COBURN, J.W., LIACH, F., & KLEEMAN, C.R., (1974).
Hypocalcaemia in magnesium-depleted dogs: evidence of a reduced responsiveness to parathyroid hormone and relative failure of parathyroid gland function.
Metabolism 23, 323-337.

LEWIS, P., RAFFERTY, B., SHELLEY, M., & ROBINSON, C.J., (1971).
A suggested physiological roleof calcitonin: the protection of the skeleton during pregnancy and lactation.
J. Endocr. 49, ix.

LI, T.K., & PIECHOCKI, J.T., (1971).
Determination of serum ionic calcium with an ion-selective electrode: Evaluation of Methodology and normal values.
Clin. Chem. 17, 411-416.

LICHENBERGER, L., MILLER, L.R., ERWIN, D.N., & JOHNSON, L.R., (1973).
Effect of pentagastrin on the adult rat duodenal cells in culture.
Gastroenterology 65, 242-251.

LINDGARDE, F., & ZETTERVALL, O., (1971).
Serum ionized calcium in a normal population studied with a calcium ion-sensitive electrode.
Israel J. Med. Sci. 7, 510-512.

LINDQUIST, B., BUDY, A.M., McLEAN, F.C., & HOWARD, J.L., (1960).
Sketetal metabolism in estrogen-treated rats studied by means of ^{45}Ca.
Endocrinology 66, 100-111

LINMAN, L.W., & BETHELL, F.H., (1960).
In Factors Controlling Erythropoiesis.
Charles C. Thomas, Springfield.

LIPKIN, M., & BELL, B., (1968).
In Cell Proliferation. Ed. C.F. Code. Washington, D.C.
Vol. 5, p. 2861.

LITTLE., J.R., BRECHER, G., BRADLEY, T.R., & ROSE, S., (1962).
Determination of lymphocyte turnover by continuous infusion of ^{3}H thymidine.
Blood 19, 236-242.

LOEHRY, C.A., CROFT, D.N., SINGH, A.K.& CREAMER, B., (1969).
Cell turnover in the rat small intestinal mucosa: an appraisal of cell loss.
Gut 10, 13-18.

LONG, J.A., & EVANS, H.M., (1922).
The oestrus cycle in the rat and its associated phenomena.
Mems. Univ. Calif. 6, 1-148.

LORAN, M.R., ALTHAUSEN, T.L., (1970).
Cell proliferation of intestinal epithelia in the rat two months after partial resection of the ileum.
J. biophys. biochem. Cytol. 7, 667-671.

LORAN, M.R., & CARBONE, J.V., (1968).
The humoral effect of intestinal resection on cellular proliferation and maturation in parabiotic rats. In Gastrointestinal Radiation Injury. Ed. M.F. Sullivan. Excerpta Medica, Amsterdam.
p.127.

LORAN, M.R., & CROCKER, T.T., (1963).
Population dynamics of intestinal epithelia in the rat two months after partial resection of the ileum.
J. Cell Biol. 19, 285-291.

LORD, B.I., & MURPHY, M.J., (1972).
The estrous cycle and the haemopoietic stem cell in the mouse.
Blood 40, 390-393.

LUDFORD, R.J., (1936).
The action of toxic substances upon the division of normal and malignant cells in vitro and in vivo.
Arch. Exp. Zellforsch 18, 411-441.

LUDFORD, R.J., (1945).
Colchicine in the experimental chemotherapy of cancer.
J. Nat. Cancer Inst. 6, 89-101.

LUKERT, B.P., STANBURY, S.W, & MAWER, E.B., (1973).
Vitamin D and intestinal transport of calcium: effects of prednisone.
Endocrinology 93, 718-722.

McCULLOCH, E.A., (1970).
In Regulation of Hematopoiesis. Ed. A.S. Gordon. Appleton, New York,
p. 132-158.

McGUIGAN, J.E., (1974).
Gastrin,
Vit. Horm. 32, 47-88.

McMINN, R.M.H., (1954).
The rate of renewal of intestinal epithelium in the cat.
J. Anat. 88, 527-532.

MACKAY, E.M., CALLAWAY, J.W., & BARNES, R.H., (1940).
Hyperalimentation in normal animals produced by protamine zinc.
J. Nutr. 20, 59-66.

MACKLIN, C.C., & MACKLIN, M.T., (1932).
The intestinal epithelium. In Special Cytology. Ed. E.V. Cowdry.
Hoeber, New York, p. 233-332.

MACMANUS, J.P., PERRIS, A.D., WHITFIELD, J.F., & RIXON, R.H., (1970).
Control of cell division in thymic lymphocytes by parathyroid hormone,
thyrocalcitonin and cyclic 3'5' monophosphate.
In Proceedings V Leukocyte Culture Conference. Ed. J. Harris.
Academic Press Inc., New York, London. p. 125.

MACMANUS, J.P., & WHITFIELD, J.F., (1971).
Cyclic-AMP mediated stimulation by calcium of thymocyte proliferation.
Expl. Cell. Res. 69, 281-288.

MACMANUS, J.P., WHITFIELD, J.F., & YOUDALE, T., (1971).
Stimulation by epinephrine of adenyl cyclase activity, cyclic AMP
formation, DNA synthesis and cell proliferation in populations of
rat thymic lymphocytes.
J. Cell. Physiol. 77, 103-116.

MANUNTA, G., SAROFF, J., & TURNER, C.W., (1957).
Relationship between oestradiol and parathyroid on retention of ^{45}Ca in bone and blood serum of rats.
Proc. Soc. exp. Biol. Med. 94, 785-787.

MANOLOV, K., & BOYADZHIEV, S., (1973).
Seasonal changes in the weight of the thyroid gland in albino rats and its capacity to accumulate ^{131}I.
Byull. eksp. Biol. Med. 76, 98-101.

MARCUS, C.S., & LENGEMANN, F.W., (1962).
Absorption of calcium-45 and strontium-85 from solid and liquid food at various levels of the alimentary tract of the rat.
J. Nutr. 77, 155-160.

MARKS, F., (1971).
Direct evidence of two tissue-specific chalone-like factors regulating mitosis and DNA synthesis in mouse epidermis.
Hoppe-Seylers Z. physiol. Chem. 352, 1273-1274.

MARNAY-GULAT, C., DAM TRUNG TUONG, M., LABITTE, A., & RAOUL, Y., (1972).
Medulla and adrenal cortex and phosphorus and calcium metabolism.
Journal de Physiologie 64, 115-129.

MARRABLE, A.W., (1962).
The counting of cells and nuclei in microtome sections.
Quart. J. microsc. Sci. 103, 331-347.

MARRS, J.M., & VORHEES, J.J., (1971)
Preliminary characterization of an epidermal chalone-like inhibitor.
J. invest. Derm. 56, 353-358.

MARTIN, D.L., & DELUCA, H.F., (1969).
Calcium transport and the role of vitamin D.
Archs. Biochem. Biophys. 134, 139-148.

MARTIN, L., & CLARINGBOLD, P.J., (1960).
The mitogenic action of oestrogens in the vaginal epithelium of ovariectomized mouse.
J. Endocr. 20, 173-186.

MARTIN, L., DAS, R.M., & FINN, C.A., (1973).
The inhibition by progesterone of uterine epithelial proliferation in the mouse.
J. Endocr. 57, 549-554.

MATOTH, Y., & KAUFMANN, L., (1962).
Mitotic activity in vitro of erythroblasts previously exposed to erythropoietin.
Blood 20, 165-172.

MATSUYAMA, M., WIADROWSKI, M.N., & METCALF, D., (1966).
Autoradiographic analysis of lymphocytes and lymphocyte migration in mice bearing multiple thymus grafts.
J. exp. Med. 123, 559-576.

MATTHEWS, C.S., SCHWABE, E.L., & EMERY, F.E., (1942).
The effects of continued oral administration of stilboestrol on body growth and organ weights of adult uncastrated and castrated female rats.
Growth 6, 7-22.

MAURER, W., PILGRIM, C., WEGENER, K., HOLLWEG, S.& LENNARTZ, J., (1965).
Messung der Dauer der DNA Verdopplung und der Generationszeit bei verschiedenen Zellarten von Maus und Ratte durch Doppel-Markierung mit ^{3}H und ^{14}C-Thymidin. In Radioaktive Isotope in Klinik und Forschung.
Eds. K. Fellinger & R. Hofer. Urban und Schwarzenberg, Berlin, p.96.

MAZIA, D., (1974).
The Cell Cycle.
Scient. Am. 230, 54-69.

MEIER, R., SCHAR, B., & NEIPP, L., (1954).
The effect of demecolceinamids on cells in vitro.
Experientia 10, 74-76.

MEINTZER, R.B., & STEENBOCK, H., (1955).
Vitamin D and magnesium absorption.
J. Nutr. 56, 285-294.

MELANCON, Jr., M.J., & DELUCA, H.F., (1969).
Interrelationships between thyrocalcitonin, parathyroid hormone and vitamin D: Control of serum calcium in hypervitaminosis D.
Endocrinology 85, 704-710.

MENDELSOHN, M.L., DOHAN, F.C. Jr., & MOORE, H.A. Jr., (1960).
Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C3H mouse. I Typical cell cycle and time of DNA synthesis.
J. Natl. Cancer Inst. 25, 477-484.

MESSIER, B., & LEBLOND, C.P., (1960).
Cell proliferation and migration as revealed by radioautography after injection of ^{3}H-thymidine into male rats and mice.
Am. J. Anat. 106, 247-285.

METCALF, D., (1964a).
In The Thymus in Immunobiology. Eds. R.A. Good & A.E. Gabrielsen. Haeber Medical Division, New York. p. 150.

METCALF, D., (1964b).
In The Thymus. Eds. V. Defendi & D. Metcalf. Wistar Institute Press, Philadelphia. p. 53.

METCALF, D., (1966).
In The Thymus: Experimental & Clincial Studies. Eds. G.E.W. Wolstenholme & R. Porter. Churchill, London. p. 242.

METCALF, D., SPARROW, N., NAKAMURA, K., & ISHIDATE, M. Jr., (1961).
The behaviour of thymus grafts in high and low leukaemic strains of mice.
Aust. J. exp. Biol. med. Sci. 39, 441-453.

MILHAUD, G., & PERAULT-STAUB, A.M., (1971).
Mechanism of the rapid hypercalcaemic effect of parathormone: inhibition of bone accretion.
Rev. Eur. Etud. Clin. Biol. 16, 451-454.

MILLER, E.R., ULLREY, D.E., ZUTAUT, C.L., BALTZER, B.V., SCHMIDT, D.A., HOEFTER, I.A., & LUECKE, R.W., (1965).
Magnesium requirement of the baby pig.
J. Nutr. 85, 13-20.

MILLER, J.F.A.P. (1965).
The thymus and transplantation immunity.
Br. med. Bull. 21, 111-117.

MINKIN, C., & TALMAGE, R.V., (1968).
A study of secretion and function in normal rats. In Parathyroid hormone and Thyrocalcitonin. Proceedings of the Third Parathyroid Conference. Ed. R.V. Talmage & L.F. Belanger. Excerpta Medica Foundation 1968. p. 339-352.

MITCHISON, N.A., TAYLOR, R.B., & RAJEWSKY, K., (1970).
In Development Aspects of Antibody Formation and Structure. Ed. J. Sterzl. Czechoslovak Academy of Science, Prague. p. 547.

MITTLEMAN, R., CHAUSMER, A., BELLAVIA, J., & WALLACH, S., (1967).
Thyrocalcitonin activity in hypercalcaemia produced by calcium salts, parathyroid hormone and vitamin D.
Endocrinology 81, 599-604.

MOOK, D.G., KENNEY, N.J., ROBERTS, S., NUSSBAUM, A.I., & RODIER, W.I., (1972).
Ovarian-adrenal interactions in regulation of body weight by female rats.
J. Comp. Physiol. Psychol. 81, 198-211.

MOOLTEN, F.L., & BUCHER, N.L.R., (1967).
Regeneration of rat liver, transfer of humoral agent by cross circulation.
Science. 158, 272-274.

MOORE, E.W., (1970).
Ionized calcium in normal serum, ultrafiltrates and whole blood determined by ion-exchange electrodes.
J. clin. Invest. 49, 318-334.

MOORHEAD, J.F., PARASKOVA-TCHERNOZEMSKA, E., PIRRIE, A.J., & HAYES, C., (1969).
Lymphoid inhibitor of human lymphocyte DNA synthesis and mitosis in vitro.
Nature. 224, 1207-1208.

MORGAN, J.I., HALL, A.K., & PERRIS, A.D., (1975a).
Requirements for divalent cations by hormonal mitogens and their interactions with sex steroids.
Biochem. biophys. Res. Commun. 66, 188-194.

MORGAN, J.I., HALL, A.K., & PERRIS, A.D., (1975b).
The ionic dependence and steroid blockade of cyclic nucleotide-induced mitogenesis in isolated rat thymic lymphocytes.
J. Cyclic Nucleotide Res. (in press).

MORGAN, J.I., HALL, A.K., & PERRIS, A.D., (1975c).
Inhibition of magnesium-induced mitogenesis by testosterone.
J. Endocr. 67, 133-134.

MORGAN, J.I., & PERRIS, A.D., (1974).
The influence of sex steroids on calcium- and magnesium-induced mitogenesis in isolated rat thymic lymphocytes.
J. Cell Physiol. 83, 287-296.

MORII, H., & DELUCA, H.F., (1967).
Relationship between vitamin D deficiency, thyrocalcitonin, and parathyroid hormone.
Am. J. Physiol. 213, 358-362.

MORRISON, B., SCOTLAND, C.J., & FLECK, A., (1972).
The determination of plasma glucose in a diabetic clinic.
Clinica chim. Acta. 39, 301-306.

MORTON, H.J., (1968).
Effect of calcium on bone marrow mitosis in vitro.
Proc. Soc. exp. Biol. Med. 128, 112-116.

MOSELEY, J.M., MATTHEWS, E.W., BREED, R.H., GALANTE, L., TSE, A., & MacINTYRE, I., (1968).
Ultimobranchial origin of calcitonin.
Lancet 1, 108-110.

MUNSON, P.L., & HIRSCH, P.F., (1966).
Thyrocalcitonin: newly recognised thyroid hormone concerned with metabolism of bone.
Clin. Orthop. 49, 209-231.

MUNSON, P.L., HIRSCH, P.F., BREWER, H.B., REISFELD, R.A., COOPER, C.W., WASTHED, A.B., ORIMO, H., & POTTS, J.T. Jr., (1968).
Thyrocalcitonin.
Rec. Prog. Horm. Res. 24, 589-650.

MUNSON, P.L., SCHWESINGER, W.H., COOPER, C.W., GRAY, T.K., & PENG, T.C., (1971). Physiological function of Thyrocalcitonin.
In Endocrinology 1971. Proceedings of the 3rd International Symposium. William Heinemann Medical Books, Ltd., London. p. 98-107.

MURDOCH, J.A., & HEATON, F.W., (1968).
Subcellular distribution of metals (sodium, potassium, calcium and magnesium) in rat liver, kidney and intestinal mucosa.
Comp. Biochem. Physiol. 26, 121-128.

MYERS, R.D., BENDER, S.A., KRSTIC, M.K., & BROPHY, P.D., (1972).
Feeding produced in the satiated rat by elevating the concentration of calcium in the brain.
Science 176, 1124-1125.

NAKABOU, Y., OKITA, C., TAKANO, Y., & HAGIHIRA, H., (1974).
Hyperplastic and hypertrophic changes of the small intestine in alloxan diabetic rats.
J. Nutr. Sci. Vitaminol. 20, 227-235.

NEEDHAM, A.E., (1964).
The Control of Growth at the Systemic Level. In The Growth Processes in Animals. Pitman & Sons Ltd., p. 360-384.

NELSON, L., (1971).
Differentiation of the male germ cell. In Developmental aspects of the cell cycle. Eds. I.L. Cameron, G.M. Padilla, & A.M. Zimmerman. Academic Press, New York & London. p. 243-278.

NEUMAN, W.F., (1972).
The bone blood equilibrium: a possible system for its study in vitro. In Calcium, Parathyroid Hormone, and the Calcitonins. Proceedings of the 4th Parathyroid Conference. Eds. R.V. Talmage & P.L. Munson. Amsterdam: Exerpta Medica. P. 389-398.

NEUMAN, W.F., & RAMP, W.K., (1971).
The concept of a bone membrane: some implications. In Cellular Mechanisms for Calcium Transfer and Homeostasis. Eds. G. Nichols Jr., & R. Wasserman, Academic, New York. p. 197-206.

NICHOLSON, R., AKHTAR, M., & TAYLOR, T.G., (1975).
Oestradiol as a regulator of the metabolism of vitamin D_3 in the liver of Japanese quail.
Proceedings of the Society for Endocrinology, Residential Meeting, 1975.

NICOLAYSEN, R., EEG-LARSEN, N., & MALM, O.J., (1953).
Physiology of calcium metabolism.
Physiol. Rev. 33, 424-444.

NIELSEN, S.P., BUCHANAN-LEE, B., MATHEWS, E.W., MOSELEY, J.M., & WILLIAMS, C.C. (1971).
Acute effects of synthetic porcine calcitonins on the renal excretion of magnesium, inorganic phosphate, sodium and potassium.
J. Endocr. 51, 455-464.

NORDIN, B.E.C., & PEACOCK, M., (1969).
Role of kidney in regulation of plasma calcium.
Lancet 2, 1280-1282.

NORMAN, A., SASAKI, M.S., OTTOMAN, R.E., & FINGERHUT, A.G., (1965).
Lymphocyte lifetime in women.
Science 147, 745.

NORMAN, A.W., (1966).
Actinomycin D effect on lag in vitamin D-mediated calcium absorption in the chick.
Am. J. Physiol, 211, 829-834.

NORMAN, A.W., (1968).
The mode of action of vitamin D.
Biol. Rev. 43, 97-137.

NORMAN, A.W., (1974a).
1,25 dihydroxyvitamin D_3: A kidney produced steroid hormone essential to calcium homeostasis.
Am. J. Med. 57, 21-27.

NORMAN, A.W., (1974b).
The hormone-like action of 1,25 $(OH)_2$ cholecalciferol (A metabolite of the fat-soluble vitamin D) in the intestine.
Vit. Horm. 32, 325-384.

OGATA, E., SHIMAZAWA, E., SUZUKI, H., YOSHITOMI, Y., ASANO, H., & ANDO, H., (1970).
Androgens and enhancement of hypocalcaemic response to thyrocalcitonin in rats.
Endocrinology 87, 421-426.

OLSON, E.B.,Jr., DELUCA, H.F., & POTTS, J.T., Jr., (1972).
The effect of calcitonin and parathyroid hormone on calcium transport of isolated intestine. In Calcium, Parathyroid hormone and the Calcitonins. Proceedings of the Fourth Parathyroid Conference.
Eds. R.V. Talmage & P.L. Munson. Excerpta Medica, Amsterdam.
p. 240-246.

OMDAHL, J.L. & DELUCA, H.F., (1973).
Regulation of vitamin D metabolism and function.
Physiol. Rev. 53, 327-365.

ORIMO, H., FUJITA, T., & YOSHIKAWA, M., (1972).
Increased sesnitivity of bone to parathyroid hormone in ovariectomised rats.
Endocrinology 90, 760-763.

ORIMO, H., & HIRSCH, P.F., (1973).
Thyrocalcitonin and Age.
Endocrinology 93, 1206-1211.

PAGE, I.H., (1954).
Sertonin (5 hydroxytryptamine).
Physiol. Rev. 34, 563-588.

PANSU, J.D., BERARD, A., DECHELETTE, M.A., & LAMBERT, R., (1974).
Influence of secretin and pentagastrin on the circadian rhythm of cell proliferation in the intestinal mucosa in rats.
Digestion 11, 266-274.

PAPPENHEIMER, A.M., (1936).
The effect of experimental reduction of kidney substance upon the parathyroid glands and skeletal tissue.
J. exp. Med. 64, 965-980.

PARSONS, F.M., ANDERSON, C.K., CLARK, P.B., EDWARDS, G.F., AHMAD, S., & HETHERINGTON, C., (1974).
Regression of malignant tumours in magnesium and potassium depletion induced by diet and haemodialysis.
Lancet 1, 243-244.

PASCHKIS, K.E., GODDARD, J., CANTAROW, A., & ADIBI, S., (1959).
Stimulation of growth by partial hepatectomy.
Proc. Soc. exp. Biol. Med. 101, 184-186.

PATT, H.M., & LUCKHARDT, A.B., (1942).
Relationship of a low blood calcium to parathyroid secretion.
Endocrinology 31, 384-392.

PATT, H.M., & QUASTLER, H., (1963).
Radiation effects on cell renewal and related systems.
Physiol. Rev. 43, 357-396.

PATZELT, V., (1936).
In Handbuch der mikroskopischen Anatomie des Menschen. Ed. W. von Mollendorff. Berlin: Springer. Vol. 5 part 3.

PAUKOVITS, W.R., (1971).
Control of granulocyte production: separation and chemical identification of specific inhibitor (chalone).
Cell Tissue Kinet. 4, 539-547.

PEACOCK, M., KNOWLES, F., & NORDIN, B.E.C., (1968)
Effect of calcium administration and deprivation on serum and urine calcium in stone-forming and control subjects.
Br. med. J. 2, 729-731.

PEACOCK, M., & NORDIN, B.E.C., (1973).
In Hard Tissue Growth, Repair & Remineralization. Ciba Found. Symp. 11 Elsevier, Amsterdam. p. 409-428.

PEARSE, A.G., & POLAK, J.M., (1971).
Cytochemical evidence for the neural crest origin of mammalian ultimobranchial C cells.
Histochemie. 27, 96-102.

PECKHAM, B., LADINSKY, J., & KIEKHOFER, W., (1963).
Autoradiographic investigation of estrogen response mechanisms in rat vaginal epithelium.
Am. J. Obst. Gynecol. 87, 710-716.

PEDERSON, T., & GELFANT, S., (1970).
G_2-population cells in mouse kidney and duodenum and their behaviour during the cell division cycle.
Expl. Cell. Res. 59, 32-36.

PENG, T.C., & COOPER, C.W., (1971).
The failure of the parathyroid gland to prevent long lasting hypocalcaemia induced by some anaesthetic agents. In Calcium, Parathyroid Hormone and the Calcitonins. Proceedings of the 4th Parathyroid Conference. Ed. R.V. Talmage & P.L. Munson. Excerpta Medica, Amsterdam. p. 207-209.

PENG, T.C., COOPER, C.W., & MUNSON, P.L., (1972).
The hypocalcaemic effect of urethane in rats.
J. Pharmac. exp. Ther. 182, 522-527.

PEPPER, F.J., (1961).
Transplantation of thymic tissue in inbred strains of mice.
J. Endocr. 22, 349-359.

PERAULT-STAUB, A.M., STAUB, J.F., & MILHAUD, G., (1974).
A new concept of plasma calcium homeostasis in the rat.
Endocrinology 95, 480-485.

PERRIS, A.D., (1971).
The calcium homeostatic system as a physiological regulator of cell proliferation in mammalian tissues.
In Cellular Mechanisms for calcium transfer and homeostasis.
Eds. G. Nichols Jr. & R.H. Wasserman, Academic Press, New York.
p. 101-131.

PERRIS, A.D., MACMANUS, J.P., WHITFIELD, J.F., & WEISS, L.A., (1971).
The parathyroid glands and mitotic stimulation in rat bone marrow after haemorrhage.
Am. J. Physiol. 220, 773-778.

PERRIS, A.D., & MORGAN, J.I., (1975).
The interaction of divalent cations, hormones and cyclic nucleotides in the control of mitosis.
Proc. of XI Eur. Symposium on Calcified Tissues. (in press).

PERRIS, A.D., WEISS, L.A., & WHITFIELD, J.F., (1970).
Parathyroidectomy and the induction of thymic atrophy in normal, adrenalectomised and orchidectomised rats.
J. Cell Physiol. 76, 141-150.

PERRIS, A.D., & WHITFIELD, J.F., (1967).
Effect of calcium on mitosis in the thymuses of normal and irradiated rats.
Nature 214, 302-303.

PERRIS, A.D., & WHITFIELD, J.F., (1969).
The mitogenic action of bradykinin on thymic lymphocytes and its dependence upon calcium.
Proc. Soc. exp. Biol. Med. 130, 1198-1201.

PERRIS, A.D., & WHITFIELD, J.F., (1971).
Calcium homeostasis and erythropoietic control in the rat.
Can. J. Physiol. 49, 22-35.

PERRIS, A.D., WHITFIELD, J.F., & RIXON, R.H., (1967).
Stimulation of mitosis in bone marrow and thymus of normal and irradiated rats by divalent cations and parathyroid extract.
Radiat. Res. 32, 550-563.

PERRIS, A.D., WHITFIELD, J.F., & TOIG, P.K. (1968).
The role of calcium in the control of growth and cell division.
Nature 219, 527-529.

PESCHLE, C., RAPPOPORT, I.A., SASSO, G.F., CONDORELLI, M., & GORDON, A.S., (1973).
The role of oestrogen in the regulation of erythropoietin production.
Endocrinology 92, 358-362.

POCHI, P.E., & STRAUSS,J.S., (1974).
Endocrinologic control of the development and activity of the human sebaceous gland.
J. invest. Derm. 62, 191-201.

POINTILLART, A., & GUEGUEN, L., (1973).
Influence du niveau et de la nature de l'apport alimentaire de magnesium sur le metabolism du phosphore du calcium et du magnesium chez le rat en croissance.
Annls. Biol. anim. Biochim. Biophys. 13, 381-397.

PONCHON, G., KENNAN, A.L., & DELUCA, H.F., (1969).
Activation of vitamin D by the liver.
J. clin. Invest. 48, 2032-2037.

POSTERNAK, T., (1974).
Cyclic AMP and cyclic GMP.
A. Rev. Pharmac. 14, 23-35.

POTTS, J.T., Jr., BUCKLE, R.M., SHERWOOD, L.M., RAMBERG, C.F. Jr., MAYER, C.P., KRONFELD, D.S., DEFTOS, L.J., CARD, A.D., & AUBACH, G.D., (1968).
Control of secretion of Parathyroid Hormone. In Parathyroid Hormone and Thyrocalcitonin. Eds. R.V. Talmage & L.F. Belanger. Excerpta Medica Foundation. p. 407-416.

QUASTLER, H., (1963).
The analysis of cell proliferation. In Cell Proliferation. Eds. L.F. Lamerton & R.J.M. Fry. Blackwell Scientific Publications, Oxford. p.18.

QUASTLER, H., & SHERMAN, F.G. (1959).
Cell population kinetics in the intestinal epithelium of the mouse.
Expl. Cell. Res. 17, 420-438.

QUEENER, S.F., & BELL, N.H., (1975).
Calcitonin: A General Survey.
Metabolism 24, 555-567.

RAISZ, L.G. & NIEMANN, I., (1967).
Early effects of parathyroid hormone and thyrocalcitonin on bone in organ culture.
Nature 214, 486-487.

RAISZ, L.G., & TRUMMEL, C.L. (1971).
Induction of bone resorption in tissue culture: interaction of humoral agents and ions. In Cellular Mechanisms for Calcium Transfer and Homeostasis. Eds. G. Nichols Jr., & R. Wasserman. Academic Press, New York, p. 442-451.

RASANEN, T., (1963).
Fluctuations in the mitotic frequency of the glandular stomach, and intestine of rat under the influence of ACTH, stress and heparin.
Acta. physiol. Scand. 58, 201-210.

RASMUSSEN, H., (1970).
Cell communication, calcium ion and cyclic adenosine monophosphate.
Science 170, 404-412.

RASMUSSEN, H., (1971).
Ionic and hormonal control of calcium homeostasis.
Am. J. Med. 50, 567-588.

RASMUSSEN, H., DELUCA, H.F., ARNAUD, C., HAWKER, C., & von STEDINGK, M., (1963).
The relationship between vitamin D and parathyroid hormone.
J. clin. Invest. 42, 1940-1946.

RAUT, S.J., & VISWANATHAN, R., (1972).
Distribution of magnesium in body fluids.
Indian J. Med. Res. 60, 1272-1278.

REDDY, C.R., COBURN, J.W., HARTENBOWER, D.L., FRIEDLER, R.M., BRICKMAN, A.S., MASSRY, S.G., & JOWSEY, J., (1973).
Studies on mechanism of hypocalcaemia of magnesium depletion.
J. clin. Invest. 52, 3000-3010.

REEDER, D.D. JACKSON, B.M., BAN, J., CLENDINNEN, B.G., DAVIDSON, W.D., & THOMPSON, J.C., (1970).
Influence of hypercalcaemia on gastric secretion and serum gastrin concentration in man.
Ann. Surg. 172, 540-546.

REISKIN, A. B.& MENDELSOHN, M.L., (1964).
A comparison ofthe cell cycle in induced carcinomas and their normal counterpart.
Cancer Res. 24, 1131-1136.

REISSMANN, K.R., & SAMORAPOOMPICHIT, S., (1970).
Effect of erythropoietin on proliferation of erythroid stem cells in the absence of transplantable colony forming units.
Blood 36, 287-296.

RENCRICCA, N.J., SOLOMON, J., FINIAN, W.J., HOWARD, D., RIZZOLI, V., & STOHLMAN, F., (1969).
The effect of testosterone on erythropoiesis.
Scand. J. Haemat. 6, 431-436.

REYNELL, P.C., SPRAY, G.H., & TAYLOR, K.B., (1957).
The site of absorption of vitamin B_{12} in the rat.
Clin. Sci. 16, 663-667.

REYNOLDS, J.J., & DINGLE, J.T., (1968).
Time course of action of calcitonin on resorbing mouse bones in vitro.
Nature 218, 1178-1179.

REYNOLDS, J.J., HOLICK, M.F., & DELUCA, H.F., (1973).
The role of vitamin D metabolites in bone resorption.
Calcified Tissue Res. 12, 295-301.

RICHARDSON, J.A., HUFFINES, W.D., & WELT, L.G., (1963).
The effect of coincident hypercalcaemia and potassium depletion on the rat kidney.
Metabolism 12, 560-569.

RICHARDSON, J.A., & WELT, L.G., (1963).
The hypomagnesemia of vitamin D administration.
Clin. Res. 11, 250.

RIJKE, R.P.C., van der MEER-PIENGGEN, W., & GALJAARD, H., (1974).
Effect of villus length on cell proliferation and migration in small intestinal epithelium.
Cell Tissue Kinet. 7, 577-587.

RIXON, R.H., (1968).
Mitotic activity in the bone marrow of rats and its relation to the level of plasma calcium.
Curr. mod. Biol. 2, 68-74.

RIXON, R.H., & WHITFIELD, J.F., (1961).
The radioprotective action of parathyroid extract.
Int. J. Radiat. Biol. 3, 361-367.

RIXON, R.H., & WHITFIELD, J.F., (1963).
Effect of multiple injections of calcium compounds on the survival of X-irradiated rats.
Nature 199, 821-822.

RIXON, R.H., & WHITFIELD, J.F., (1972a).
Hypoplasia of the bone marrow in rats following removal of the parathyroid glands.
J. Cell Physiol. 79, 343-352.

RIXON, R.H., & WHITFIELD, J.F., (1972b).
Parathyroid hormone: a possible initiator of liver regeneration.
Proc. Soc. exp. Biol. Med. 141, 93-97.

RIXON, R.H., WHITFIELD, J.F., & MACMANUS, J.P., (1970).
Stimulation of mitotic activity in rat bone marrow and thymus by exogenous adenosine 3'5' monophosphate (cyclic AMP).
Expl. Cell. Res. 63, 110-116.

RIXON, R.H., WHITFIELD, J.F., & YOUDALE, T., (1958).
Increased survival of rats irradiated with X-rays and treated with parathyroid extract.
Nature 182, 1374.

ROBINSON, C.J., MATTHEWS, E.W., & MACINTYRE, I., (1969).
In Proceedings of the 5th Eur. Symp. of Calcif. Tissue. Ed. G. Milhaud.
S.E.D.E.S. Paris. p.279.

ROBISON, G.A., BUTCHER, R.W., & SUTHERLAND, E.W., (1971).
In Cyclic AMP. Academic Press, New York.

ROCKLIN, R.E., MEYERS, O.M., & DAVID, J.R., (1970).
An in vitro assay for cellular hypersensitivity in man.
J. Immun. 104, 95-102.

RODIER, W.I., (1971).
Progesterone-estrogen interactions in the control of activity-wheel running in the female rat.
J. Comp. Physiol. Psychol. 74, 365-373.

ROSS, D.B., (1962).
In vitro studies on the transport of magnesium across the intestinal wall of the rat.
J. Physiol. 160, 417-428.

ROSS, J.W., (1967).
Calcium selective electrode with liquid ion exchanger.
Science, 156, 1378-1379.

van ROSSUM, G.D.V., GALEOTTI, T., & MORRIS, H.P., (1973).
The mineral content and water compartments of liver and of Morris Hepatoma 5123tc and 3924A and the changes of composition occuring during necrosis in Hepatoma 3924A.
Cancer Res. 33, 1078-1085.

ROWINSKI, J., & KAMINSKI, M., (1973).
Transplanted mucosa of the mouse intestine as model for cell proliferation studies.
Gastroenterology 65, 642-646.

ROWLEY, D.A., GOWANS, J.L., ATKINS, R.C., FORD, W.L. & SMITH, M.E., (1972).
The specific selection of recirculating lymphocytes by antigen in normal and preimmunised rats.
J. exp. Med. 136, 499-513.

ROYCROFT, J., & TALMAGE, R.V., (1973).
Changes due to age in the hypocalcaemic and hypophosphataemic effects of salmon calcitonin in growing rats.
Proc. Soc. exp. Biol. Med. 144, 17-21.

RYTOMAA, T., (1973).
Control of cell division in mammalian cells. In The cell cycle in development and differentiation. Eds. M. Balls, & F.S. Billett. Cambridge University Press. p. 457-472.

RYTOMAA, T., & KIVINIEMI, K., (1968).
Control of granulocyte production. I Chalone and anti chalone, two specific humoral regulators.
Cell Tissue Kinet. 1, 329-340.

SAETREN, H., (1956).
A principle of auto-regulation of growth. Production of organ specific mitose-inhibitors in kidney and liver.
Expl. Cell. Res. 11, 229-232.

SAINTE-MARIE, G., & LEBLOND, C.P., (1965).
Elaboration of a model for the formation of lymphocytes in the thymic cortex of young adult rats.
Blood 26, 765-783.

SAKAI, A., (1970).
Humoral factors triggering DNA synthesis after partial hepatectomy in the rat.
Nature 228, 1186-1187.

SCAIFE, J.F., (1970).
Liver homeostasis: an in vitro evaluation of a possible specific chalone.
Experientia 26, 1071-1072.

SCHACHTER, D., FINKELSTEIN, J.D., & KOWARSKI, S., (1964).
Metabolism of vitamin D I. Preparation of radioactive vitamin D and its intestinal absorption in rat.
J. clin. Invest. 43, 787-796.

SCHACHTER, D., KIMBERG, D.V., & SCHENKER, H., (1961).
Active transport of calcium by intestine: Action and bioassay of vitamin D.
Am. J. Physiol. 200, 1263-1271.

SCHACHTER, D., KOWARSKI, S., & REID, P., (1967).
Molecular basis for vitamin D action in the small intestine.
J. clin. Invest. 46, 1113-1114.

SCHACHTER, D., & ROSEN, S.M., (1959).
Active transport of 45Ca by the small intestine and its dependence on vitamin D.
Am. J. Physiol. 196, 357-362.

SCHAR, B., LOUSTALOT, P., & GROSS, F., (1954).
The effect of demecolceinamids on cells in vitro.
Klin. Wochschr. 32, 49.

SCHEDL, H.P., & WILSON, H.D., (1971a).
Effect of diabetes on intestinal growth in the rat.
J.exp. Zool. 176, 487-496.

SCHEDL, H.P., & WILSON, H.D., (1971b).
Effects of diabetes on intestinal growth and hexose transport in the rat.
Am. J. Physiol. 220, 1739-1745.

SCHEVING, L.E., BURNS, E.R., & PAULY, J.E., (1972).
Circadian rhythms in mitotic activity and ^{3}H-thymidine uptake in the duodenum: effect of isoproterenal on the mitotic rhythm.
Am. J. Anat. 135, 311-318.

SCHLUETER, R.J., & CALDWELL, A.L., (1967).
Thyrocalcitonin: Parameters of bio-assay.
Endocrinology 81, 854-860.

SCHNEIDER, L.E., HARGIS, G.K., SCHEDL, H.P., & WILLIAMS, G.A., (1974).
Parathyroid function in the alloxan diabetic rat.
Endocrinology 95, 749-753.

SCHNEIDER, L.E., & SCHEDL, H.P., (1972).
Diabetes and intestinal calcium absorption in the rat.
Am. J. Physiol. 223, 1319-1321.

SCHNEIDER, L.E., & SCHEDL, H.P., (1974).
Alloxan diabetes, and magnesium metabolism in the rat.
Proc. Soc. exp. Biol. Med. 147, 494-497.

SCHNEIDER, L.E., WILSON, H.D., & SCHEDL, H.P., (1973).
Intestinal calcium binding protein in the Diabetic Rat.
Nature 245, 327-328.

SCHNEIDER, L.E., WILSON, H.D., & SCHEDL, H.P., (1974).
Effects of alloxan diabetes on duodenal calcium binding protein in the rat.
Am. J. Physiol. 227, 832-839.

SCHULTZ, G., HARDMAN, J.G., HURWITZ, L., & SUTHERLAND, E.W., (1973).
Importance of calcium for the control of cyclic GMP levels.
Fedn. Proc. 32, 773.

SCHWARTZ, H.D., McCONVILLE, B.C., & CHRISTOPHERSON, E.F., (1971).
Serum ionized calcium by specific ion electrode.
Clinica chim. Acta. 31, 97-107.

SELYE, H., (1971).
In Hormones and Resistance, Part I.
Springer-Verlag, Berlin, Heidelberg, New York.

SHAI, F., & WALLACH, S., (1973).
Interactions of oestradiol and calcitonin on the rat skeleton.
Endocrinology 93, 1044-1051.

SHAIKH, A.A., (1971).
Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy.
Biol. Reprod. 5, 297-307.

SHANES, A.M., (1958).
Electrochemical aspects of physiological and pharmacological action in excitable cells; I & II.
Pharmac. Rev. 10, 59-273.

SHANES, A.M., (1963).
Movement of calcium in muscle and nerve. In The Transfer of Calcium and Strontium across Biological Membranes. Ed. R.H. Wasserman.
Academic Press Inc., New York.

SHEPPARD, H., TSIEN, W.H., MAYER, P., & HOWIE, N., (1965).
Metabolism of the accessary sex organs of the immature male rat: changes in nucleic acid composition and uptake of ^{3}H-thymidine induced by castration and methandrostenolone.

SHERMAN, F.G., QUASTLER, H., WIMBER, D.R., (1961).
Cell population kinetics in the ear epidermis of mice.
Expl. Cell. Res. 25, 114-119.

SHERWOOD, L.M., ABE, M., RODMAN, J.S., LUNDBERG, W.B., Jr., TARGOVNIK, J.H., (1972).
Parathyroid hormone: synthesis, storage and secretion. In Calcium, Parathyroid Hormone and the Calcitonins. Proceedings of the 4th Parathyroid Conference. Eds. R.V. Talmage & P.L. Munson. Excerpta Medica, Amsterdam. p. 183-196.

SHERWOOD, L.M., HERRMANN, I., & BASSETT, C.A., (1969).
In vitro studies of normal and abnormal parathyroid tissue.
Archs. intern. Med. 124, 426-430.

SHERWOOD, L.M., HERRMANN, I., & BASSETT, C.A., (1970).
Parathyroid hormone secretion in vitro: regulation by calcium and magnesium ions.
Nature 225, 1056-1058.

SHERWOOD, L.M., RODMAN, J.S., & LUNDBERG, W.B. Jr., (1970).
Evidence for a precursor to circulating parathyroid hormone.
Proc. natn. Acad. Sci. U.S.A., 67, 1631-1638.

SHILS, M.E., (1969).
Experimental human magnesium depletion.
Medicine 48, 61-85.

SIGDESTAD, C.P., BAUMAN, T., & LESHER, S.W., (1969).
Diurnal fluctuations in the number of cells in mitosis and DNA synthesis in the jejenum of the mouse.
Expl. Cell. Res. 58, 159-162.

SIMNETT, J.D., FISHER, J.M., & HEPPLESTON, A.G., (1969).
Tissue-specific inhibition of lung alveolar cell mitosis in organ culture.
Nature 223, 944-946.

SMITH, G.R., EDWARDS, D.J., PERRIS, A.D., (1975).
Fluctuations in plasma calcium concentration and mitotic activity in bone marrow and thymus during the oestrous cycle of the rat.
J. Endocr. Proceedings (in press).

SMITH, G.R., GURSON, M.L., RIDDELL, A.J.& PERRIS, A.D., (1975).
Inhibitory action of oestrogens on calcium-induced mitosis in rat bone marrow and thymus.
J. Endocr. 65 45-53.

SMITH, R.H., (1959).
Calcium and magnesium metabolism in calves.
Biochem. J. 71, 609-614.

SMITH, T.C., (1955).
The effect of oestrogen and progesterone on mammary gland growth in the rat.
Endocrinology 57, 33-43.

SORENSEN, O.H., & HINDBERG, I., (1971).
Influence of gonadal hormones on the action of calcitonin in the rat.
Acta. endocr. Copnh. 68, 585-596.

SPIELVOGEL, A.M., FARLEY, R.D., & NORMAN, A.W., (1972).
Studies on the mechanism of action of calciferol V. Turnover time of chick intestinal epithelial cells in relation to the intestinal action of vitamin D.
Expl. Cell. Res. 74, 359-366.

STARLING, E.H., & LOVATT EVANS, C., (1968).
In Principles of Human Physiology. Eds. H. Davidson & M.G. Eggleton. J.A. Churchill, 1968. Chapter 64, p. 1469.

STEENBOCK, H., & HERTING, D.C., (1955).
Vitamin D and growth.
J. Nutr. 57, 449-468.

STERN, D.H., WALSH, J.H. (1972).
Release of gastrin in postoperative duodenal ulcer patients.
Gastroenterology 62, 817.

STEVENS, J.F., (1971).
Determination of glucose by an automatic analyser.
Clinica chim. Acta. 32, 199-201.

STEVENS HOOPER, C.E., (1961).
Use of colchicine for the measurement of mitotic rate in the intestinal epithelium.
Am. J. Anat. 108, 231-244.

STOHLMAN, F.Jr., EBBE, S., NORSE, B.S., HOWARD, D., & DONOVAN, J., (1968).
Regulation of erythropoiesis XX Kinetics of red cell production.
Ann. N.Y. Acad. Sci. 149, 156-172.

STOHS, S.J., & DELUCA, H.F., (1967).
Subcellular localization of vitamin D and its metabolites in intestinal mucosa after 10 IU dose.
Biochemistry 6, 3338-3349.

STOTT, G.H., (1968).
Dietary influence on the incidence of parturient paresis.
Fedn. Proc. 27, 156-161.

SUDA, T., DELUCA, H.F., SCHNOES, H.K., TANAKA, Y., & HOLICK, M.F., (1970).
25, 26 dihydroxycholecalciferol, a metabolite of vitamin D3 with intestinal calcium transport activity.
Biochemistry 9, 4776-4780.

SWAMINATHAN, R., BATES, R.F.L., BLOOM, S.R., GANGULI, P.C., & CARE, A.D., (1973).
The relationship between food, gastrointestinal hormones and calcitonin secretion.
J. Endocr. 59, 217-230.

SWAMINATHAN, R., BATES, R.F.L., & CARE, A.D., (1972).
Fresh evidence for a physiological role of calcitonin in calcium homeostasis.
J. Endocr. 54, 525-526.

SWAMINATHAN, R., KER, J., & CARE, A.D., (1974).
Calcitonin and intestinal calcium absorption.
J. Endocr. 61, 83-94.

SWANN. M.M., (1958).
The control of cell division: A review II Special Mechanisms.
Cancer. Res. 18, 1117-1160.

TACHI, C., TACHI, S., & LINDNER, H.R., (1972).
Modification by progesterone of oestradiol induced cell proliferation RNA synthesis and oestradiol distribution in the rat uterus.
J. Reprod. Fert. 31, 59-77.

TALMAGE, R.V., (1956).
Studies on the maintenance of serum calcium levels by parathyroid action on bone and kidney.
Ann. N.Y. Acad. Sci. 64, 326-335.

TALMAGE, R.V., COOPER, C.W., & PARK, H.Z., (1970).
Regulation of calcium transport in bone by parathyroid hormone.
Vit. Horm. 28, 103-140.

TALMAGE, R.V., DOPPELT, S.H., & COOPER, C.W., (1975).
Relationship of blood concentration of calcium, phosphate, gastrin and calcitonin on the onset of feeding in the rat.
Proc.Soc. exp. Biol. Med. 149, 855-859.

TALMAGE, R.V., NEUENSCHWANDER, J., & KRAINTZ, L., (1964).
Presence of thyrocalcitonin secretion in the rat.
Fedn. Proc. 23, 204.

TANAKA, Y., & DELUCA, H.F., (1973).
The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus.
Archs. Biochem. Biophys. 154, 566-574.

TANNOCK, I.F., (1967).
A comparison of the relative efficiencies of various metaphase arrest agents.
Expl. Cell. Res. 47, 345-356.

TARGOVNIK, J.H., RODMAN, J.S., & SHERWOOD, L.M., (1971).
Regulation of parathyroid hormone secretion in vitro. Quantitative aspects of calcium and magnesium ion control.
Endocrinology 88, 1477-1482.

TARTTELIN, M.F., & GORSKI, R.A., (1973).
The effects of ovarian steroids on food and water intake and body weight in the female rat.
Acta. endocr. Copnh. 72, 551-568.

TAYLOR, A.N., & WASSERMAN, R.H., (1967).
Vitamin D_3-induced calcium-binding protein: partial purification electrophoretic visualization, and tissue distribution.
Arch. Biochem. Biophys. 119, 536-540.

TAYLOR, A.N., & WASSERMAN, R.H., (1969).
Correlations between the vitamin D induced calcium binding protein and intestinal absorption of calcium.
Fedn. Proc. 28, 1834-1838.

TAYLOR, A.N., WASSERMAN, R.H., & JOWSEY, J., (1968).
A vitamin D-dependent calcium-binding protein in canine intestinal mucosa.
Redn. Proc. 27, 675.

TAYLOR, D.M., & JONES, J.D., (1972).
Effects of ethylenediaminetetraacetate,- diethylenetriaminepentaacetate on DNA synthesis in kidney and intestinal mucosa of folate treated rats.
Biochem. Pharmac. 21, 3313-3315.

TEITELBAUM, P., & CAMPBELL, B.A., (1958).
Ingestion patterns in hyperphagic and normal rats.
J. Comp. Physiol & Psychol. 51, 134-141.

THOMSON, D.L., & COLLIP, J.B., (1932).
The parathyroid glands.
Physiol. Rev. 12, 309-383.

THRASHER, J.D., CLARK, F.I., & CLARKE, D.R., (1967).
Changes in the vaginal epithelial cell cycle in relation to events of the estrous cycle.
Expl. Cell. Res. 45, 232-236.

TIBBETTS, D.M., & AUB, J.C., (1937).
Magnesium metabolism in health and disease I. The magnesium and calcium excretion of normal individuals, also the effects of magnesium, chloride, and phosphate ions.
J. clin. Invest. 16, 491-501.

TILSON, M.D., PHILIPS, S., & WRIGHT, H.K., (1971).
An effect of deoxycorticosterone upon the ileum stimulating compensatory hypertrophy of the gut.
Surgery 69, 730-735.

TORIBARA, T. Y., TEREPKA, A.R., & DEWEY, P.A., (1957).
The ultrafiltrable calcium of human serum I. Ultrafiltration methods and normal values.
J. clin. Invest. 36, 738-748.

TRAININ, N., SMALL, M., & KIMMI, Y., (1973).
In Thymic Hormones. Ed. T.D. Luckey, University Park Press, Baltimore, London, Tokyo. p. 135

TRIER, J.S. (1963).
Studies on small intestinal crypt epithelium I. The fine structure of the crypt epithelium of the proximal small intestine of fasting humans.
J. Cell. Biol. 18, 599-620.

TRIGGS, S.M., HESCH, R.D., WOODHEAD, J.S., & WILLIAMS, E.D., (1975).
Calcitonin production by rat thyroid tumours.
J. Endocr. 66, 37-43.

TRUESDELL, A.H., & CHRISTE, C.L., (1967).
In Glass Electrodes for hydrogen and other cations: principles and practice. Ed. G. Eisenman. Marcell Dekker: New York. p. 293.

TUCKER, G., GAGNON, R.E., & HAUSSLER, M.R., (1973).
Vitamin D3-25-hydroxylase: tissue occurrence and apparent lack of regulation.
Archs. Biochem. Biophys. 155, 47-57.

TUOHIMAA, P., & NIEMI, M., (1968).
The effect of testosterone on cell renewal and mitotic cycles in sex accessory glands of castrated mice.
Acta. endocr. Copenh. 58, 696-704.

TUTTON, P.J.M., (1973).
Control of epithelial cell proliferation in the small intestinal crypt.
Cell Tissue Kinet. 6, 211-216.

TUTTON, P.J.M., (1974).
The influence of serotonin on crypt cell proliferation in the jejenum of rat.
Cell Pathology 16, 79-89.

TUTTON, P.J.M., & HELME, R.D., (1974).
The influence of adrenoreceptor activity on crypt cell proliferation in the rat jejenum.
Cell Tissue Kinet. 7, 125-136.

URBAN, E., & SCHEDL, H.P., (1969).
Mucosal growth effect of vitamin D on the duodenum.
Experientia 25, 1270-1271.

VASAMA, R., & VASAMA, R., (1958).
On the diurnal cycle of mitotic activity in the corneal epithelium of mice.
Acta. anat. 33, 230-237.

VERLEY, W.G., DESCHAMPS, Y., PUSHPATHADAM, J., & DESROSIERS, M., (1971).
The hepatic chalone I. Assay method for the hormone and purification of the rabbit liver chalone.
Can. J. Biochem. 12, 1376-1383.

VINCENT, P.C., BORNER, G., CHANANA, A.D., CRONKITE, E.P., GREENBERG, M.L., JOEL, D.D., SHIFFER, L.M., & STRYCKMANS, P.A., (1969).
Studies on lymphocytes. XIV Measurement of DNA synthesis time in bovine thoracic duct lymphocytes by analysis of labelled mitoses and by double labelling, before and after extra-corporeal irradiation of the lymph.
Cell Tissue Kinet. 2, 235-247.

VIROLAINEN, M., (1967).
Humoral factors in liver cell proliferation.
In Control of Cellular Growth in Adult Organisms. Eds. H. Teir & T. Rytomaa, Academic Press, London & New York. p. 232-249.

VOADEN, M.J.A., (1968).
A chalone in the rabbit lens?
Expl. Eye Res. 7, 326-331.

VOLKMAN, A., & GOWANS, J.L., (1965a).
The production of macrophages in the rat.
Br. J. exp. Path. 46, 50-61.

VOLKMAN, A., & GOWANS, J.L., (1965b).
The origin of macrophages from the bone marrow in the rat.
Br. J. exp. Path. 46, 62-70.

VOLLMER, E.P., & GORDON, A.S., (1941).
Effect of sex and gonadotrophic hormones upon the blood picture of the rat.
Endocrinology 29, 828-837.

VOLM, M., WAYSS, K., & HINDERER, H., (1969).
A new model of growth regulation.
Naturwissenschaften 56, 566-567.

WACKER, W.E.C., (1969).
The biochemistry of magnesium.
Ann. N.Y. Acad. Sci. 162, 717-726.

WADE, G.N., (1972).
Gonadal hormones and behavioural regulation of body weight.
Physiol. Behav. 8, 523-534.

WADE, G.N., (1974).
Interaction between estradiol-17β and growth hormone in control of food intake in weaning rats.
J. Comp. Physiol. Psychol. 86, 359-362.

WADE, G.N., & ZUCKER, I., (1970).
Modulation of food intake and locomotor activity in female rats by diencephalic hormone implants.
J. Comp. Physiol. Psychol. 72, 328-336.

WALKER, B.E., (1959).
Cell production and migration in the female mouse genital tract.
Anat. Rec. 133, 459.

WALL, A.J., MIDDLETON, W.R.T., PEARSE, A.G.E., & BOOTH, C.C. (1970).
Intestinal mucosal hyperplasia following induced hyperthyroidism in the rat.
Virchows. Arch. Abt. B. Zellpath. 6, 79-87.

WANG, J., ROGLER, J.C.& DOOLITTLE, D.P., (1973).
Effect of alloxan on calcium metabolism in mice and rats.
Horm. Metab. Res. 5, 51-52.

WASE, A.W., PETERSON, A., RICKES, E., & SOLEWSKI, J., (1966).
Some effects of thyrocalcitonin on the calcium metabolism of the rat.
Endocrinology 79, 687-691.

WASSERMAN, R.H., CORRADINO, R.A.; & TAYLOR, A.N., (1969).
Binding proteins from animals with possible transport function.
J. Gen. Physiol. 54, 114s-137s.

WASSERMAN, R.H., & TAYLOR, A.N., (1966).
Vitamin D3-induced calcium binding protein in chick intestinal mucosa.
Science 152, 791-793.

WASSERMAN, R.H., & TAYLOR, A.N., (1968).
Vitamin D-dependent calcium-binding protein. Response to some physiological and nutritional variables.
J. Biol. Chem. 243, 3987-3993.

WEBER, K., BOHNE, F., & ROBE, V., (1970).
Decrease of DNA synthesis in duodenal crypt cells of rats treated with Na3 (Ca-DTPA).
Eur. J. Pharmac. 11, 117-118.

WEISS, P.A., (1968).
In Dynamics of Development: Experiments and Inferences.
Academic Press, New York.

WEISS, P., & KAVANAU, J.L., (1957).
A model of growth and growth control in mathematical terms.
J. Gen. Physiol. 41, 1-47.

WHEELER, E.J., & WHEELER, J.K., (1964).
Comparative study of Paneth cell in vertebrates.
Anat. Rec. 148, 350.

WHITFIELD, J.F., BROHEE, H., & YOUDALE, T., (1964a).
Reduction of mitotic delay in irradiated suspension cultures of rat thymocytes by an elevated salt concentration.
Expl. Cell Res. 35, 207-210

WHITFIELD, J.F., BROHEE, H., & YOUDALE, T., (1964b).
The effects of X-irradiation on the chromatin structure and histone component of rat thymocyte nuclei.
Expl. Cell. Res. 36, 341-353.

WHITFIELD, J.F., MACMANUS, J.P., BRACELAND, B.M., & GILLAN, D.J., (1972).
The influence of calcium on the cyclic AMP-mediated stimulation of DNA synthesis and cell proliferation by prostaglandin E_1.
J. Cell Physiol. 79, 353-362.

WHITFIELD, J.F., MACMANUS, J.P., FRANKS, D.J., GILLAN, D.J., & YOUDALE, T., (1971).
The possible mediation by cyclic AMP of the stimulation of thymocyte proliferation by cyclic GMP.
Proc. Soc. exp. Biol. Med. 137, 453-457.

WHITFIELD, J.F., MACMANUS, J.P. & GILLAN, D.J., (1970).
The possible mediation by cyclic AMP of the stimulation of thymocyte proliferation by vasopressin and the inhibition of this mitogenic action by thyrocalcitonin.
J. Cell. Physiol. 76, 65-76.

WHITFIELD, J.F., MACMANUS, J.P., & GILLAN, D.J., (1973a).
The calcium-dependent stimulation of thymic lymphoblast DNA synthesis and proliferation by a low concentration of cortisol.
Horm. Metab. Res. 5, 200-203.

WHITFIELD, J.F., MACMANUS, J.P., & GILLAN, D.J., (1973b).
The ability of calcium to change cyclic AMP from a stimulator to an inhibitor of thymic lymphoblast proliferation.
J. Cell. Physiol. 81, 241-250.

WHITFIELD, J.F., MACMANUS, J.P., & RIXON, R.H., (1970a).
Cyclic-AMP-mediated stimulation of thymocyte proliferation by low concentrations of cortisol.
Proc. Soc. exp. Biol. Med. 114, 1170-1174.

WHITFIELD, J.F., MACMANUS, J.P., & RIXON, R.H., (1970b).
The possible mediation by cyclic AMP of parathyroid hormone-induced stimulation of mitotic activity of deoxyribonucleic acid synthesis in rat thymic lymphocytes.
J. Cell. Physiol. 75, 213-224.

WHITFIELD, J.F., MACMANUS, J.P., YOUDALE, T., & FRANKS, D.J., (1971).
The roles of calcium and cyclic AMP in the stimulatory action of parathyroid hormone on thymic lymphocyte proliferation.
J. Cell Physiol. 78, 355-368.

WHITFIELD, J.F., PERRIS, A.D., & YOUDALE, T., (1968).
The role of calcium in the mitotic stimulation of rat thymocytes by detergents, agmatine, and poly-L-lysine.
Expl. Cell. Res. 53, 155-165.

WHITFIELD, J.F., PERRIS, A.D., & YOUDALE, T., (1969).
The calcium-mediated promotion of mitotic activity in rat thymocyte populations by growth hormone, neurohormones, parathyroid hormone, and prolactin.
J. Cell Physiol. 73, 203-212.

WHITFIELD, J.F., RIXON, R.H., MACMANUS, J.P., & BALK, S.D., (1973).
Calcium, cyclic adenosine 3'5' monophosphate, and the control of cell proliferation: a review.
In Vitro 8, 257-259.

WHITFIELD, J.F., RIXON, R.H., PERRIS, A.D., & YOUDALE, T., (1969).
Stimulation by calcium of the entry of thymocytes into the deoxyribonucleic acid-synthetic (S) phase of the cell cycle.
Expl. Cell. Res. 57, 8-12.

WHITFIELD, J.F., RIXON, R.H., & YOUDALE, T., (1969).
The calcium-dependent stimulation of mitotic activity in normal and irradiated rat thymocyte populations by somatotrophic hormone.
Int. J. Radiat. Biol. 15, 385-388.

WICKBOM, G., LANDOR, J.H., BUSHKIN, F.L., & McGUIGAN, J.E., (1975).
Changes in canine gastric acid output and serum gastrin levels following massive small intestinal resection.
Gastroenterology 69, 448-452.

WIERDA, J.L., (1950).
A comparison of the weight of the intestine with the body and kidney weights in rats which were fed artificial unbalanced diets.
Anat. Rec. 107, 221-233.

WILEY, C.L., WILLIAMS, W.W., & McDONALD, C.J., (1973).
The effects of the epidermal chalone on DNA synthesis in mammalian epidermal cell.
J. invest. Derm. 60, 160-165.

WILLIAMS, G.A., PETERSON, W.I., BOWSER, E.N., HENDERSON, W.J., HARGIS, G.K., & MARTINEZ, N.J., (1974).
Interrelationship of parathyroid and adrenocortical function in calcium homeostasis in the rat.
Endocrinology 95, 707-713.

WILLS, M.R., (1973).
Intestinal absorption of calcium.
Lancet 1, 820-822.

WINTER, W., MORAVA, E., SIMON, G., & SOS, J., (1970).
The role of the parathyroid glands in the absorption of calcium from the small intestine.
J. Endocr. 47, 65-72.

WOODHOUSE, N.J.Y., BORDIER, P., FISHER, M., JOPLIN, G.F., REINER, M., KALU, D.N., FOSTER, G.V., & MacINTYRE, I., (1971).
Human calcitonin in the treatment of Paget's bone disease.
Lancet 1, 1139-1143.

WRBA, H., & RABES, H., (1967).
Humoral regulation of liver regeneration. In Control of Cellular Growth in Adult Organisms. Eds. H. Teir & T. Rytomaa. Academic Press, London & New York. p. 221-231.

WRIGHT, N.A., MORLEY, A.R., & APPLETON, D., (1972).
The effect of testosterone on cell proliferation and differentiation in the small intestine.
J. Endocr. 52, 161-175.

YALOW, R.S., & WU, N., (1973).
Additional studies on the nature of big big gastrin.
Gastroenterology 65, 19-27.

YOFFEY, J.M., (1960).
In Quantitative Cellular Haematology. Springfield Ill. Charles C. Thomas.

YOUNG, M.M., JASANI, C., SMITH, D.A., & NORDIN, B., (1968).
Some effects of ethinyl oestradiol on calcium and phosphorus metabolism in osteoporosis.
Clin. Sci. 34, 411-417.

ZARROW, M.X., YOCHIM, J.M., & McCARTHY, J.L., (1964).
In Experimental endocrinology: a sourcebook of basic techniques.
Academic Press, New York & London.

J. Endocr. (1975), **65**, 45–53 *Printed in Great Britain*

INHIBITORY ACTION OF OESTROGEN ON CALCIUM-INDUCED MITOSIS IN RAT BONE MARROW AND THYMUS

G. R. SMITH, MOLLY L. GURSON, A. J. RIDDELL AND A. D. PERRIS

Department of Biological Sciences, The University of Aston in Birmingham, Gosta Green, Birmingham, B4 7ET

(*Received* 2 *July* 1974)

SUMMARY

In the male rat injections of $CaCl_2$ and $MgCl_2$ stimulated mitosis in bone marrow and thymus tissue. The magnesium salt was also mitogenic in the normal female, but calcium only exerted its mitogenic effect after ovariectomy. Oestradiol, but not progesterone replacement therapy abolished calcium-induced mitosis in the ovariectomized rat. The inability of calcium to stimulate cell division was also apparent in the thyroparathyroidectomized female rat, suggesting the oestradiol blockade did not operate via some indirect action on the calcium homeostatic hormones calcitonin or parathyroid hormone.

When thymic lymphocytes derived from male or female rats were isolated and maintained in suspension, increased calcium or magnesium concentrations in the culture medium stimulated the entry of cells into mitosis. Addition of oestradiol to the culture medium abolished the mitogenic effect of increased calcium levels, but had no effect on magnesium-induced proliferation. These experiments suggested that oestradiol might act at the cell surface to prevent the influx of calcium but not magnesium ions into the interior of the cell and thus to block the sequence of biochemical events which lead to the initiation of DNA synthesis and culminate in mitosis.

INTRODUCTION

It has long been recognized that oestrogens and progestogens powerfully stimulate cell division in mammalian uterine, vaginal and mammary tissue (Folley, 1955; Jacobsohn, 1958; Martin & Claringbold, 1960; Martin & Finn, 1968, 1969, 1971; Baulieu, Wira, Milgrom & Raynaud-Jammet, 1972; Das, 1972*a*, *b*; Lee & Rogers, 1972; Martin, Das & Finn, 1973; Martin, Finn & Trinder, 1973*a*, *b*; Lee, Rogers & Trinder, 1974). Despite the stimulatory actions in these target tissues, an inhibitory effect of oestrogen on cell proliferation and growth in other tissues may exist. Thus female rats and mice are smaller, and grow less rapidly than males of the same age (Jackson, 1913; Santisteban, 1960) as a result of oestrogen action (Freudenberger & Clausen, 1937*a*, *b*; Clausen & Freudenberger, 1939; Korenchevsky, Burbank & Hall, 1939; Matthews, Schwabe & Emery, 1942). The arrest of growth at puberty (Harvey, Hervey & Hutchinson, 1973) and the inhibition in the restoration of normal erythrocyte numbers after haemorrhage can similarly be attributable to oestrogen action in female animals (Finkelstein, Gordon & Charipper, 1944). In addition, the marked diurnal fluctuations in mitotic activity in bone marrow and thymus tissues of the male rat are absent in the female rat, but appear after ovariectomy (N. H. Hunt, 1973, personal communication).

In thymus and bone marrow tissue of the male rat, physiological changes in cell division seem to be determined by parallel alterations in plasma calcium concentration (Perris,

Whitfield & Tolg, 1968; Perris, MacManus, Whitfield & Weiss, 1971; Hunt & Perris, 1974). Since these mitotic changes are muted or absent in the female rat as outlined above, it is possible that the female sex steroids may be able to block calcium-induced mitotic stimulation. The present studies were designed to test this possibility.

MATERIALS AND METHODS

Male and female Wistar rats, weighing 190–210 g, were used. Food and water were supplied *ad libitum* except where indicated to the contrary. To assess proliferation in the bone marrow and thymus tissue, animals received two i.p. injections of a solution (0·4 mg/ml 0·9 % NaCl soln) of the metaphase-arresting agent, des-acetyl-*N*-methyl-colchicine (Colcemid, CIBA Ltd) at 0 and 2·5 h. At each injection, 0·2 mg/100 g body weight was administered. Six hours after the first injection the animals were anaesthetized with ether and the thymus gland and right femur rapidly removed. Cell suspensions of thymic lymphocytes and bone marrow cells were then prepared, smeared on slides, fixed and stained as described by Whitfield, Brohée & Youdale (1964*b*). The percentage of nucleated cells which had entered mitosis and reached metaphase during the 6 h period was then evaluated by scoring the number of arrested metaphase figures in a sample of at least 1000 cells. Two independent workers counted each slide.

To study the effect of divalent cations on the rate of entry of cells into mitosis, groups of male or female rats received a series of 1 ml i.p. injections of either $CaCl_2$ or $MgCl_2$ solutions (62·5 mmol/l) at 0, 0·5, 1, 2, 3, 4 and 5 h after the start of the Colcemid treatment. Control animals did not routinely receive a comparable series of 0·9 % saline injections since preliminary experiments had shown that such injections did not significantly alter mitotic activity in either bone marrow or thymus tissue.

In experiments designed to remove the potential effect of the female sex hormones on mitotic events, a group of rats were bilaterally ovariectomized under ether anaesthesia (D'Amour, Blood & Belden, 1965). The food intake of these rats was recorded daily. A second group of female rats was sham-ovariectomized on the day after ovariectomy of the animals in the first group, and their diet was restricted to the food intake of the ovariectomized animals. Mitotic activity in these two groups of rats, with or without calcium injections, was subsequently determined as described above on day 6 after the operation. From days 2 to 5 after operation some rats received a daily injection of either oestradiol benzoate or progesterone in an attempt to analyse the relative contribution of these steroids to mitotic events. Oestradiol benzoate dissolved in 0·1 ml arachis oil was given s.c. at a dose of 1 μg/100 g body weight/day and progesterone dissolved in 0·1 ml arachis oil was given s.c. at a dose of 1 mg/100 g body weight/day. These dosages were chosen on the basis of the normal daily secretion rates of these steroids and adequately compensate for lost ovarian function (Hashimoto, Henricks, Anderson & Melampy, 1968; Shaikh, 1971). Preliminary experiments had shown that the vehicle alone had no significant effect on mitotic activity. Mitotic activity in bone marrow and thymus tissue of treated animals, with or without calcium injections, was measured 6 days after ovariectomy (4 days after replacement therapy was begun) as previously described.

Since any inhibition that female sex hormones might exert on calcium-induced mitosis could operate either directly on the bone marrow or thymus cells or indirectly by some effect on the calcium homeostatic hormones, some experiments to eliminate the latter possibility were carried out in thyroparathyroidectomized (TPTX) female rats. Animals were TPTX under ether anaesthesia according to the method of Ingle & Griffith (1942). In order to bring about a permanent lowering of the plasma calcium levels after TPTX, animals were fed a calcium-deficient diet (Cooper Nutrition Products Ltd, Witham, Essex)

following the feeding régime of Hirsch, Gauthier & Munson (1963). Mitotic activity in bone marrow and thymus tissue of animals, with or without the régime of calcium injections previously described, was measured 6 days after TPTX.

To investigate more precisely the role of oestradiol on cell proliferation stimulated by calcium, some observations were made *in vitro* using isolated rat thymic lymphocytes suspended in a balanced glucose salts (BGS) medium containing 0·062 mM-colchicine (Whitfield, Perris & Youdale, 1968, 1969). The basic composition of the medium was 5·5 mM-glucose, 5·0 mM-KCl, 0·6 mM-$CaCl_2$, 1·0 mM-$MgSO_4$, 5·0 mM-Na_2HPO_4, 120 mM-NaCl and 5·0 mM-Tris (hydroxy methyl) amino methane buffer. The pH was adjusted to 7·2 with HCl. Calcium and magnesium concentrations could be altered by appropriate additions of concentrated stock solutions. In the presence of colchicine, cells continue to enter into mitosis during a 6 h incubation period, and to accumulate at metaphase as they do *in vivo* (Whitfield, Brohée & Youdale, 1964*a*; Perris, Whitfield & Rixon, 1967; Whitfield *et al.* 1969). Thymocytes from both male and female rats were suspended and incubated in BGS media containing different concentrations of calcium or magnesium ions. In some experiments oestradiol benzoate dissolved in a minimum quantity of ethyl alcohol was also added to the cultures, usually to give a final concentration of 0·1 μg/ml. Control cultures were treated with the same amount of ethyl alcohol. At the end of a 6 h incubation period at 37 °C, samples were taken from the suspensions, mixed with a small volume of calf serum on a slide, dried, fixed, stained and scored as described above.

RESULTS

Injections of $CaCl_2$ significantly increased the division of both bone marrow and thymus cells in the male rat. However, in the female rat, although the basal level of mitotic activity in both tissues was not significantly different from that in the male, cells were completely unresponsive mitotically to the calcium challenge (Fig. 1). Magnesium chloride injections provoked a highly significant mitotic stimulation in bone marrow and thymus cells in both sexes (Fig. 2).

The hypothesis that some ovarian secretion may have a restraining rôle and be responsible for the inhibition of calcium-induced mitogenesis in the female rat was strengthened by the observation that the basal level of mitotic activity in bone marrow and thymus tissue was increased after ovariectomy (Table 1). Furthermore, in the ovariectomized rat, in contrast to the intact females, calcium injections markedly stimulated proliferation (Table 1). When replacement therapy with 1 μg oestradiol benzoate 100 g body weight/day was given to restore oestrogen levels in the blood (Shaikh, 1971), the mitogenic capacity of calcium was again abolished (Table 1). Injections of progesterone at a dose of 1 mg/100 g body weight/day were without effect (Table 1). Thus the presence of oestrogen, but not progesterone, prevents the mitogenic action of calcium, but not magnesium, on the bone marrow and thymus of the female rat.

This inhibitory action of oestrogen on mitotically competent cells could be either direct or indirect. Thus if oestrogen were able to interact with the calcium homeostatic mechanism, the response to calcium challenge in the female rat might be modified so that the usual mitotic response could not be elicited (Currie & Black, 1972; Klotz, Delorme & Ochoa, 1972; Swaroop & Krant, 1973). For example, if oestrogen facilitated either the release of calcitonin or the inhibition of parathyroid hormone output in response to calcium chloride injections, the calcium load would rapidly be eliminated and mitogenic levels in the plasma never attained. However, when rats of both sexes were thyroparathyroidectomized, the basal level of mitotic activity in both tissues was reduced below that of the control values in accordance with previous observations (Perris, Weiss & Whitfield, 1970; Rixon & Whitfield,

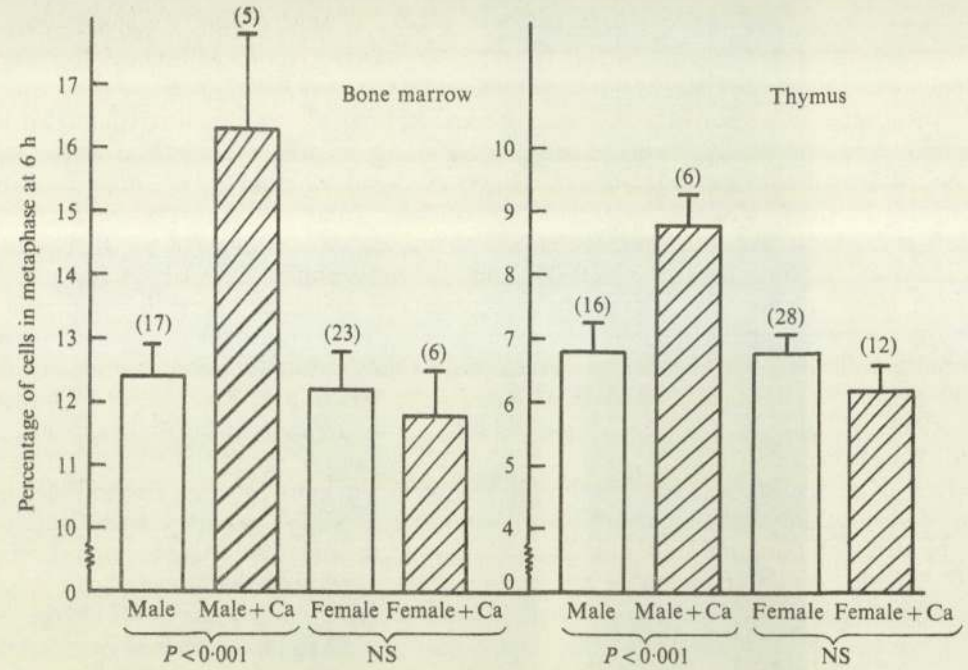


Fig. 1. Effect of a series of i.p. injections of 62·5 mM-$CaCl_2$ (Ca) on mitotic activity in bone marrow and thymus tissue of male and female rats over a 6 h period. Number of animals in each group shown in parentheses. Vertical bars indicate ±S.E.M. NS, not significant.

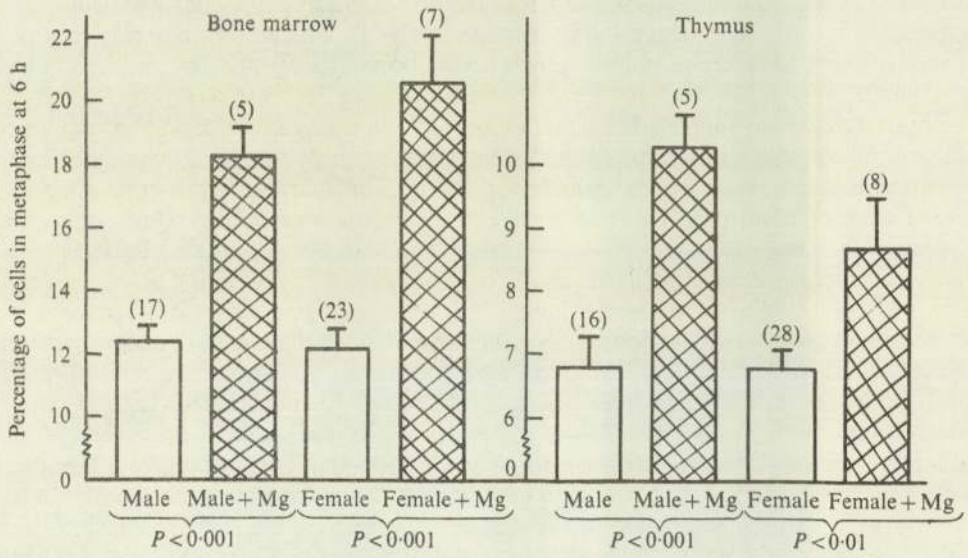


Fig. 2. Effect of a series of i.p. injections of 62·5 mM-$MgCl_2$ (Mg) on mitotic activity in bone marrow and thymus tissue of male and female rats over a 6 h period. Number of animals in each group shown in parentheses. Vertical bars indicate ±S.E.M.

Table 1. *Effect of $CaCl_2$ injections on mitotic activity in ovariectomized female rats, expressed as the percentage of cells in metaphase 6 h after the first injection (means ± S.E.M.)*

Treatment	Bone marrow	*P*	Thymus	*P*
Normal female	12·2 ± 0·6 (23)	NS	6·8 ± 0·3 (28)	NS
Sham-Ox	12·4 ± 0·9 (4)	< 0·05	6·8 ± 0·8 (4)	< 0·05
Ox	15·5 ± 0·6 (18)	< 0·001	8·9 ± 0·4 (17)	< 0·001
Ox + Ca	22·2 ± 0·9 (17)		11·5 ± 0·4 (17)	
Ox + OB	15·2 ± 1·7 (5)	NS	7·7 ± 0·4 (6)	NS
Ox + OB + Ca	14·5 ± 0·7 (6)		8·1 ± 0·3 (6)	
Ox + Prog	14·6 ± 1·1 (5)	< 0·01	8·4 ± 0·7 (5)	< 0·001
Ox + Prog + Ca	21·1 ± 0·9 (5)		13·0 ± 0·3 (4)	

Number of animals in each group is shown in parentheses. Treatments were: ovariectomy (Ox); series of i.p. injections of 62·5 mM-$CaCl_2$ (Ca); replacement therapy with either oestradiol benzoate (OB) or progesterone (Prog) as described in Methods. NS, not significant.

Table 2. *Effect of $CaCl_2$ injections on mitotic activity in normal and thyroparathyroidectomized rats, expressed as the percentage of cells in metaphase 6 h after the first injection (means ± S.E.M.)*

	Male				Female			
Treatment	Bone marrow	*P*	Thymus	*P*	Bone marrow	*P*	Thymus	*P*
Normal	12·4 ± 0·5 (17)	< 0·001	6·8 ± 0·5 (16)	< 0·001	12·2 ± 0·6 (23)	NS	6·8 ± 0·3 (28)	NS
Normal + Ca	16·3 ± 1·5 (5)		8·8 ± 0·5 (6)		11·8 ± 0·7 (6)		6·2 ± 0·4 (12)	
TPTX	6·6 ± 0·5 (7)	< 0·001	4·9 ± 0·5 (7)	< 0·05	9·6 ± 0·7 (5)	NS	5·0 ± 0·5 (6)	NS
TPTX + Ca	14·1 ± 1·2 (6)		7·1 ± 0·7 (6)		8·6 ± 0·8 (5)		5·1 ± 0·6 (5)	

Number of animals in each group is shown in parentheses. Treatments were: a series of i.p. injections of 62·5 mM-$CaCl_2$ (Ca) and thyroparathyroidectomy (TPTX). NS, not significant.

1972) and calcium injections again stimulated cell division in the male rat, but had no effect on female bone marrow and thymus tissue (Table 2). A more direct inhibitory action of oestrogen on the mitogenic capacity of calcium was therefore considered.

In an attempt to clarify the mechanism of this direct action of oestrogen, we carried out experiments on cell division *in vitro*. Isolated thymic lymphocytes maintained in a BGS medium containing colchicine continued to enter mitosis, and accumulate at metaphase as they did *in vivo*. Similarly, when the calcium concentration in the medium was increased above 0·6 mmol/l, there was a significant stimulation of cell division (Fig. 3). This occurred whether the thymic lymphocytes were of male or female origin. Thus cells taken from the oestrogen-rich environment of the female organism possessed no inherent resistance to the mitogenic action of calcium. However, when oestradiol benzoate was added to the cultures of thymocytes taken from either male or female animals, the steroid again blocked calcium-stimulated cell division, even when calcium concentrations were as high as 3·0 mmol/l (Fig. 3). The highest oestradiol benzoate concentration in the culture medium was 0·1 μg/ml but a concentration of 0·001 μg/ml was equally effective (not illustrated). If the calcium concentration was kept constant at 0·6 mmol/l and the magnesium concentration increased above 1·0 mmol/l in cultures of thymocytes from male and female animals, stimulation of

division also resulted. The level of mitosis was raised from a mean value of 3·7 % to 5·5 % when the magnesium concentration in the media was increased from 1·0 to 2·5 mmol/l. No significant difference was found between values in the absence or presence of a final oestradiol benzoate concentration of 0·1 μg/ml. These findings also suggest a direct action of oestrogen on calcium-induced mitogenesis in bone marrow and thymus.

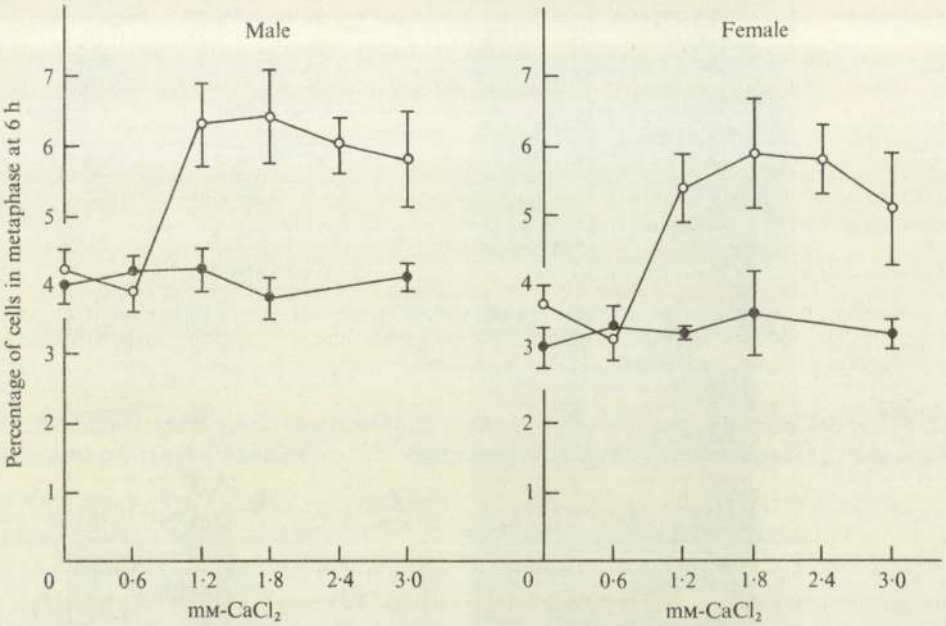


Fig. 3. Influence of oestradiol benzoate (OB) on mitotic activity of isolated thymocytes from male and female rats exposed to varying concentrations of $CaCl_2$ in culture. Control media (○); media containing 0·1 μg OB/ml (●). The magnesium ion concentration was maintained throughout at 1·0 mmol/l. Each value is a mean derived from at least five separate experiments. Vertical lines represent ±1 S.E.M.

DISCUSSION

The present study clearly demonstrates that although the calcium ion is a potent mitogen for bone marrow and thymus cells in normal and aparathyroid male rats, it is ineffectual in the female (Table 2). Ovarian factors are responsible for this blockade (Table 1), as they are for thymic hypoplasia (Golding & Ramirez, 1928; Dougherty, 1952), and since oestradiol prevented calcium-induced mitogenesis in isolated suspensions of thymic lymphocytes (Fig. 3), a direct effect of oestrogens on the mitotically competent cells of the bone marrow and thymus gland must be postulated.

The mechanism by which calcium stimulates proliferation of thymic lymphocytes (and by inference bone marrow cells) has been amply reviewed by Whitfield, Rixon, MacManus & Balk (1972). Briefly, an influx of calcium into the cell in some way inhibits the enzyme phosphodiesterase which is responsible for the degradation of cyclic AMP. The ensuing increase in the intracellular concentration of this cyclic nucleotide activates a chain of biochemical events which culminates in heightened DNA synthesis and ultimately cell division. The ability of oestrogen to block this sequence of events could occur in a number of ways.

If oestrogen were to induce new RNA and protein synthesis in these cells as it does in uterine tissue (Tachi, Tachi & Lindner, 1972), such a protein might inhibit one or more of the biochemical stages which links the calcium-stimulus to mitosis. If such an inhibitor

exists it must have an exceedingly short lifespan; thymocytes derived from the relatively oestrogen-rich environment of the female rat responded strongly to the mitogenic challenge of increased ambient calcium levels. In the whole animal and *in vitro*, oestrogens had to be present at the time the mitotic stimulus was applied in order to exert their inhibitory effect. Perhaps the simplest explanation therefore would be to ascribe to oestrogen the ability to prevent the entry of the calcium ion into sites on or in the cell where the initiation of the co-ordinated chain of events which lead to mitosis occurs. The low ambient plasma calcium concentration in the TPTX female rat, coupled with the presence of oestrogen which would further inhibit diffusional entry, could account for the diminished mitotic activity in the bone marrow and thymus of the aparathyroid female (Table 2). Clearly, the access of the divalent magnesium ion to initiator sites was not impeded by oestrogen. This might suggest that magnesium enters the cell by a completely different route from calcium and indeed it has been suggested it may stimulate cell division via a mechanism which does not depend upon cyclic AMP (Morgan & Perris, 1974).

The consequences for the female mammal of this oestrogen blockade of calcium-induced cell division may be the lesser growth rates (Harvey *et al.* 1973), poorer post-haemorrhagic recovery (Steinglass, Gordon & Charipper, 1941; Finkelstein *et al.* 1944; Jepson & Lowenstein, 1966) and the absence of calcium-linked circadian mitotic variations (Hunt & Perris, 1974) found in females of many species. In male animals altered rates of growth, erythropoiesis and diurnal cell proliferation require the presence of the parathyroid gland and are paralleled by concomitant changes in plasma calcium concentration (Perris *et al.* 1968, 1971; Perris, 1972; Hunt & Perris, 1973, 1974). The inhibition by oestrogen of the action of erythropoietin upon responsive stem cells in the bone marrow (Jepson & Lowenstein, 1966; Peschle, Rappaport, Sasso, Condorelli & Gordon, 1973) may also have a similar basis; it has recently been shown that the maximum effect of this erythropoietic stimulant is only observed when it can collaborate with the parathyroid gland to produce hypercalcaemia (Hunt & Perris, 1973). This hypercalcaemia is thought to stimulate bone marrow cell division, the prelude of enhanced erythrocyte production. The present study suggests oestrogen would inhibit this calcium-mediated response.

The well-known oestrogen inhibition of parathyroid hormone-stimulated bone resorption (Lafferty, Spencer & Pearson, 1964; Eisenberg, 1966; Kronfeld, 1968; Stott, 1968; Young, Jasani, Smith & Nordin, 1968; Anderson, Greenfield, Posada & Crackel, 1970), and postmenopausal osteoporosis (Gallagher & Nordin, 1972; Gallagher, Young & Nordin, 1972) might also all be explained by an effect of oestrogen (or the lack of it) on calcium influx to bone cells which may be a prerequisite for bone resorption (Talmage, Cooper & Park, 1970). Whether these examples constitute general or special cases is not certain, but clearly where a calcium influx to a cell dictates subsequent metabolic events, the possible interplay of oestrogen and this ion at the cell surface may be of considerable interest and significance.

REFERENCES

Anderson, J. J. B., Greenfield, J. W., Posada, J. R. & Crackel, W. C. (1970). Effects of oestrogen on bone mineral turnover in mature female rats measured by 85 Strontium. *Proceedings of the Society for Experimental Biology and Medicine* **135**, 883–886.

Baulieu, E. E., Wira, C. R., Milgrom, E. & Raynaud-Jammet, C. (1972). RNA synthesis and oestradiol action in the uterus. *Acta endocrinologica* Suppl. **168**, 396–419.

Clausen, F. W. & Freudenberger, C. B. (1939). A comparison of the effects of male and female sex hormones on immature female rats. *Endocrinology* **25**, 585–592.

Currie, G. N. & Black, D. C. (1972). Effects of oestrogen on thyrocalcitonin activity in male and female rabbits. *Acta endocrinologica* **71**, 55–61.

D'Amour, F. E., Blood, F. R. & Belden, D. A., Jr. (1965). *Manual for laboratory work in mammalian physiology*. Chicago and London: The University of Chicago Press.

Das, R. M. (1972*a*). The effects of oestrogen on the cell cycle in epithelial and connective tissues of the mouse uterus. *Journal of Endocrinology* **55**, 21–30.

Das, R. M. (1972*b*). The time course of the mitotic response to oestrogen in the epithelia and stroma of the mouse uterus. *Journal of Endocrinology* **55**, 203–204.

Dougherty, T. F. (1952). Effect of hormones on lymphatic tissue. *Physiological Reviews* **32**, 379–401.

Eisenberg, E. J. (1966). Effects of androgens, oestrogens and corticoids on strontium kinetics in man. *Journal of Clinical Endocrinology and Metabolism* **26**, 566–572.

Finkelstein, G., Gordon, A. S. & Charipper, H. A. (1944). The effect of sex hormones on the anaemia induced by haemorrhage in the rat. *Endocrinology* **35**, 267–277.

Folley, S. J. (1955). Hormones in mammary growth and function. *British Medical Bulletin* **11**, 145–150.

Freudenberger, C. B. & Clausen, F. W. (1937*a*). The effects of continued theelin injections on the body growth and organ weights of young female rats. *Anatomical Record* **68**, 133–144.

Freudenberger, C. B. & Clausen, F. W. (1937*b*). Quantitative effects of theelin on body growth and endocrine glands in young albino rats. *Anatomical Record* **69**, 171–177.

Gallagher, J. C. & Nordin, B. E. C. (1972). Treatment with oestrogens of primary hyperparathyroidism in post-menopausal women. *Lancet* i, 503–507.

Gallagher, J. C., Young, M. M. & Nordin, B. E. C. (1972). Effects of artificial menopause on plasma and urine calcium and phosphate. *Clinical Endocrinology* **1**, 57–64.

Golding, G. T. & Ramirez, F. T. (1928). Ovarian and placental hormone effects in normal immature albino rats. *Endocrinology* **12**, 804–812.

Harvey, J. S., Hervey, G. R. & Hutchinson, I. (1973). The role of gonadal hormones in determining the sex difference in adult body weight in the rat. *Journal of Endocrinology* **57**, xxiv.

Hashimoto, I., Henricks, D. M., Anderson, L. L. & Melampy, R. M. (1968). Progesterone and pregn-4-en-20α-ol-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology* **82**, 333–341.

Hirsch, P. F., Gauthier, G. F. & Munson, P. L. (1963). Thyroid hypocalcaemic principle and recurrent laryngeal nerve injury as factors affecting the response to parathyroidectomy in rats. *Endocrinology* **73**, 244–252.

Hunt, N. H. & Perris, A. D. (1973). Erythropoietin-induced changes in plasma calcium and bone marrow mitosis in the rat. *Journal of Endocrinology* **56**, 47–57.

Hunt, N. H. & Perris, A. D. (1974). Calcium and the control of circadian mitotic activity in rat bone marrow and thymus. *Journal of Endocrinology* **62**, 451–462.

Ingle, D. J. & Griffith, J. P. (1942). In *The rat in laboratory investigation*, Chap. 16, p. 439. Eds J. P. Griffith & E. J. Farris. Philadelphia: J. B. Lippincot Co.

Jackson, C. M. (1913). Postnatal growth and variability of body and various organs and systems in the albino rat. *American Journal of Anatomy* **15**, 1–68.

Jacobsohn, D. (1958). Mammary gland growth in relation to hormones with metabolic actions. *Proceedings of the Royal Society*, Series B **149**, 325–329.

Jepson, J. H. & Lowenstein, L. (1966). Inhibition of the stem cell action of erythropoietin by oestradiol. *Proceedings of the Society for Experimental Biology and Medicine* **123**, 457–460.

Klotz, H. P., Delorme, M. L. & Ochoa, F. (1972). Oestrogènes et calcitonine. *La Nouvelle Presse Médicale* **1**, 1845–1846.

Korenchevsky, V., Burbank, R. & Hall, K. (1939). The action of the dipropionate and benzoate butyrate of oestradiol on ovariectomised rats. *Biochemical Journal* **33**, 366–371.

Kronfeld, D. S. (1968). Eighth Annual Ruminant Nutrition Conference. Introduction and hypothesis. *Federation Proceedings. Federation of American Societies for Experimental Biology* **27**, 137–138.

Lafferty, F. S., Spencer, G. E., Jr & Pearson, O. H. (1964). Effects of androgens, oestrogens and high calcium intakes on bone formation and resorption in osteoporosis. *American Journal of Medicine* **36**, 514–528.

Lee, A. E. & Rogers, L. A. (1972). Cell division in the mouse vagina during continuous oestrogen stimulation. *Journal of Endocrinology* **54**, 357–358.

Lee, A. E., Rogers, L. A. & Trinder, G. (1974). Changes in cell proliferation rate in mouse uterine epithelium during continuous oestrogen treatment. *Journal of Endocrinology* **61**, 117–121.

Martin, L. & Claringbold, P. J. (1960). The mitogenic action of oestrogens in the vaginal epithelium of ovariectomized mouse. *Journal of Endocrinology* **20**, 173–186.

Martin, L., Das, R. M. & Finn, C. A. (1973). The inhibition by progesterone of uterine epithelial proliferation in the mouse. *Journal of Endocrinology* **57**, 549–554.

Martin, L. & Finn, C. A. (1968). Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. *Journal of Endocrinology* **41**, 363–371.

Martin, L. & Finn, C. A. (1969). Duration of progesterone treatment required for a stromal response to oestradiol-17β in the uterus of the mouse. *Journal of Endocrinology* **44**, 279–280.

Martin, L. & Finn, C. A. (1971). Oestrogen–gestation interactions on mitosis in target tissues. In *Basic actions of sex steroids on target organs*, pp. 172–188. Eds P. O. Hubinont, F. LeRoy & P. Galand. Basal: S. Karger.

Martin, L., Finn, C. A. & Trinder, G. (1973*a*). Hypertrophy and hyperplasia in the mouse uterus after oestrogen treatment: an autoradiographic study. *Journal of Endocrinology* **56**, 133–145.

Martin, L., Finn, C. A. & Trinder, G. (1973*b*). DNA synthesis in the endometrium of progesterone-treated mice. *Journal of Endocrinology* **56**, 303–309.

Matthews, C. S., Schwabe, E. L. & Emery, F. E. (1942). The effects of continued oral administration of stilboestrol on body growth and organ weights of adult uncastrated and castrated female rats. *Growth* **6**, 7–22.

Morgan, J. I. & Perris, A. D. (1974). The influence of sex steroids on calcium- and magnesium-induced mitogenesis in isolated rat thymic lymphocytes. *Journal of Cellular Physiology* **831**, 287–296.

Perris, A. D. (1972). Calcium dependent changes in rat bone marrow mitosis induced by erythropoietin. *Journal of Endocrinology* **53**, xlix.

Perris, A. D., MacManus, J. P., Whitfield, J. F. & Weiss, L. A. (1971). The parathyroid glands and mitotic stimulation in rat bone marrow after haemorrhage. *American Journal of Physiology* **220**, 773–778.

Perris, A. D., Weiss, L. A. & Whitfield, J. F. (1970). Parathyroidectomy and the induction of thymic atrophy in normal, adrenalectomised and orchidectomised rats. *Journal of Cellular Physiology* **76**, 141–150.

Perris, A. D., Whitfield, J. F. & Rixon, R. H. (1967). Stimulation of mitosis in bone marrow and thymus of normal and irradiated rats by divalent cations and parathyroid extract. *Radiation Research* **32**, 550–563.

Perris, A. D., Whitfield, J. F. & Tolg, P. K. (1968). Role of calcium in the control of growth and cell division. *Nature* **219**, 527–529.

Peschle, C., Rappaport, I. A., Sasso, G. F., Condorelli, M. & Gordon, A. S. (1973). The role of oestrogen in the regulation of erythropoietin production. *Endocrinology* **92**, 358–362.

Rixon, R. H. & Whitfield, J. F. (1972). Hypoplasia of the bone marrow in rats following removal of the parathyroid glands. *Journal of Cellular Physiology* **79**, 343–352.

Santisteban, G. A. (1960). The growth and involution of lymphatic tissue and its interrelationships to aging and to the growth of the adrenal glands and sex organs in CBA mice. *Anatomical Record* **136**, 117–126.

Shaikh, A. A. (1971). Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. *Biology of Reproduction* **5**, 297–307.

Steinglass, P., Gordon, A. S. & Charipper, H. A. (1941). Effect of castration and sex hormones on the blood of the rat. *Proceedings of the Society for Experimental Biology and Medicine* **48**, 169–177.

Stott, G. H. (1968). Dietary influence on the incidence of parturient paresis. *Federation Proceedings. Federation of American Societies for Experimental Biology* **27**, 156–161.

Swaroop, S. & Krant, M. J. (1973). Oestrogen induced hypercalcaemia. *Journal of the American Medical Association* **223**, 913–914.

Tachi, C., Tachi, S. & Lindner, H. R. (1972). Modification by progesterone of oestradiol induced cell proliferation, RNA synthesis and oestradiol distribution in the rat uterus. *Journal of Reproduction and Fertility* **31**, 59–77.

Talmage, R. V., Cooper, C. W. & Park, H. W. (1970). The regulation of calcium transport in bone by parathyroid hormone. *Vitamins and Hormones* **28**, 103–140.

Whitfield, J. F., Brohée, H. & Youdale, T. (1964*a*). Reduction of mitotic delay in irradiated suspension cultures of rat thymocytes by an elevated salt concentration. *Experimental Cell Research* **35**, 207–210.

Whitfield, J. F., Brohée, H. & Youdale, T. (1964*b*). The effects of X-irradiation on the chromatin structure and histone component of rat thymocyte nuclei. *Experimental Cell Research* **36**, 341–353.

Whitfield, J. F., Perris, A. D. & Youdale, T. (1968). The role of calcium in the mitotic stimulation of rat thymocytes by detergents, agmatine, and poly-L-lysine. *Experimental Cell Research* **53**, 155–165.

Whitfield, J. F., Perris, A. D. & Youdale, T. (1969). The calcium mediated promotion of mitotic activity in rat thymocyte populations by growth hormone, neurohormones, parathyroid hormone and prolactin. *Journal of Cellular Physiology* **73**, 203–212.

Whitfield, J. F., Rixon, R. H., MacManus, J. P. & Balk, S. D. (1972). Calcium, cyclic-AMP and the control of cell proliferation *in vitro*. *In Vitro, Journal of the American Tissue Culture Association* **8**, 257–278.

Young, M. M., Jasani, C., Smith, D. A. & Nordin, B. (1968). Some effects of ethinyl oestradiol on calcium and phosphorus metabolism in osteoporosis. *Clinical Science* **34**, 411–417.