INTRODUCTION

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It has been generally accepted that the micro-environment of the cells - and not their DNA content - plays an appreciable role in cellular kinetics and differentiation. It was first incorrectly thought that the different cell lineages had different chromosomal pattern and hence different DNA content. This view might explain the difference in competence and potentiality of the different cell types. However, methods for performing microsurgery, the development of micropipettes and microneedles and the invention of the dissecting microscope made it possible to carry out the micromanipulation techniques and the investigators began to transplant nuclei from one cell type to another.

Ham and Cormack (1979) demonstrated that the successful transplantation of a nucleus in animal cells and the observation of the genes' interaction in their new locality was only achieved by the beginning of the 1950s. In this context, they described how the investigators had been able to transplant a single nucleus from the intestinal lining cells of tadpoles into an enucleated frog's egg, from which, a normal frog did develop. Later on, Gurdon (1974) showed that the nuclei removed from the skin cells of the frog - grown in tissue culture - when transplanted into enucleated eggs gave the same result. Again, the latter author described how the investigators had been able to rotate the metaphase spindle that developed in the grasshopper neuroblast so that the S-chromosomes (chromatids) that would ordinarily have gone to one pole of the dividing cell would go to the other and vice versa. When mitosis was completed, it was found that the daughter cell that formed at the pole at which - without having the spindle rotated - a

neuroblast was to develop still did so, while the daughter cell at the other pole developed into a nerve cell as it would have happened without this intervention. In addition, Gurdon (1974) explained that what might have otherwise been conceived of as being a differentiation division, involving an unequal distribution of genetic material, was shown to be due to the cytoplasm at the two ends of the cell being different, with that at one end always involving the activation (turning on) of genes that caused the differentiation of the cell into a nerve cell and that at the other pole not exerting this effect.

LYMPHOCYTE KINETICS AND MITOGENICITY.

The "in vitro" system of tissue culture is now widely used to investigate the behaviour of cells, in different cell populations, under varying circumstances. Carrel and Ebeling (1922) were the first to point out the value of culturing blood lymphocytes in media of known composition. The blood leukocytes, having a relatively rapid turnover rate, have been and are being used with great success as an experimental model in this respect. If these cells were kept in culture without any apparent stimulus, a small number of them spontaneously transform into "blast" cells (Chrustschoff and Berlin, 1935; Bloom, 1938; Nowell, 1960 b; Coulson and Chalmers, 1966 and Coulson and Williams, 1974). However, the addition of certain substances known as phytomitogens to the culture medium was found to increase the blastoid transformation of such cells. Examples of these phytomitogens are phytohaemagglutinin (PHA), concanavalin-A (Con-A), lentil mitogen (LM) and pokeweed mitogen (PWM). Hungerford, Donnelly, Nowell and Beck (1959) were the first to describe the "in vitro" mitogenic and blastogenic properties of PHA.

They reported that PHA was capable of inducing transformation of the small lymphocytes in human peripheral blood into large primitive cells. This finding was confirmed by Nowell (1960 b), Carstairs (1962), Marshall and Roberts (1963), Robbins (1964 a,b) and Bach and Hirschhorn (1965). Nevertheless, the mechanism whereby PHA induced blastogenesis and mitosis of normal lymphocytes in culture and the fate of this mitogen in the culture medium was still controversial.

Robbins (1964 a,b) and Bach and Hirschhorn (1965) showed that PHA consisted of a number of biologically active haemagglutinating, leukoagglutinating, protein precipitating and blastogenic constituents. In addition, Elves and Israëls (1963) investigated the fate of PHA during its incubation with human erythrocytes and peripheral leukocytes "in vitro". They incubated leukocytes with varying concentrations of PHA and observed no loss of haemagglutinating activity during the 3-day incubation period. They, therefore, concluded that PHA was not taken up from the medium by the cells during culture. This interpretation of their results was based on the finding that a direct relationship had existed between the ability of PHA to induce blastogenesis of leukocytes and agglutination of erythrocytes since absorption of PHA with agglutinated erythrocytes resulted in a marked diminution in the capacity to induce blastogenesis. However, in view of the large number of studies which had since appeared showing that the absorption of PHA with erythrocytes did not affect its capacity to induce blastogenesis (Barkhan and Ballas, 1963; Punnett and Punnett, 1963; Kolodny and Hirschhorn, 1964; Robbins,1964 a,b; Holland and Holland, 1965 and Weber, Nordman and Gräsbeck, 1967)

it was not feasible to equate blastogenesis with haemagglutination.

The studies of the cell localization of PHA did demonstrate the capacity of this mitogen to penetrate cell membranes. Michalowski, Jasinska, Brzoslo and Nowoslawski (1964) studied PHA cellular localization after its labelling with fluorescein isothiocyanate. During the early period of the culture (4-9 hours), a distinct cellular localization of fluorescent material was found confined to the nuclei of the majority of the lymphocytes but not in the granulocytes. This localization of fluorescein-conjugated PHA did not change during the entire 3-day culture. The authors suggested that the labelled mitogen entered the cell and resided inside the nucleus. On the other hand, Razavi (1966), also using fluorescein-conjugated PHA, observed the fluorescence in leukocytes of all types during the initial period of culture. Subsequently, the fluorescence was localized to the cytoplasm of the blast cells and was not associated with nuclear structures or cytoplasmic membranes. In this context, Rieke (1965) used PHA-P labelled with ^{125}I and added it to cultures of rat thoracic duct lymphocytes. By autoradiography, the author found that the radioactivity was localized to cell membranes and to a lesser extent within the cytoplasm of the lymphocytes. As blast cells developed, radioactivity was commonly noted on nuclear membranes and in apparent Golgi zones. Using the electron microscope, Greenland and Oppenheim (1966) studied the localization of mercury-labelled PHA in lymphocytes. Electron-dense spots were seen in greatest numbers in the cytoplasm of lymphocytes but were also - though more rarely - present in the nucleus. This localization of PHA in the lymphocytes was observed in cells from specimens examined as early as one hour after the addition of

PHA and its localization did not alter up to 72 hours. Moreover, Conard (1967) obtained tritium-labelled PHA by growing red kidney bean plants (Phaseolus Vulgaris) in nutrient solution containing tritiated water. This PHA was added to cultures of normal human blood leukocytes and the results were evaluated by autoradiography. Most of the leukocytes were labelled after 4-6 hours of culture. At first, the label appeared mainly in the cytoplasm but after 2-3 days, labelling was predominant in large blast cells and was mainly localized in the nuclei. Naspitz and Richter (1968) emphasized that it was not justifiable to equate the localization of the label with the localization of the blastogenic factor since any or all of the constituents of PHA preparation could have been labelled. In their initial experiments, the latter authors demonstrated that incubation of lymphocytes with PHA for as short a period of time as 5 minutes followed by culture for 72 hours in the absence of PHA was sufficient to initiate a low degree of blastogenesis as determined by the incorporation of radioactive thymidine. An initial 3-hour incubation with PHA was sufficient to induce blastogenesis to a degree of 50 percent of that observed with cultures incubated with PHA for either 6 hours or 72 hours, the latter two incubation periods both gave similar results.

The initial interaction of phytomitogens with lymphocytes was studied by many investigators. Borberg, Yesner, Gesner and Silber (1968); Landy and Chessin (1969); Powell and Leon (1970) and Allan, Auger and Crumpton (1971) suggested that such an interaction was likely to occur via plasma membrane binding sites. Janossy and Greaves (1971) emphasized that these binding sites were present on the cell surface of lymphocytes. Based on microscopic

agglutination and immunofluorescence studies, Greaves, Bauminger and Janossy (1972) have shown that virtually 100% of T and B lymphocytes had binding sites for those phytomitogens. Their quantitative absorption experiments have demonstrated that there was no difference in the relative average density of binding sites on T and B lymphocytes. They also found that PHA, Con-A and LM activated only T cells while PWM stimulated both T and B cells. In contrast, Greaves and Bauminger (1972) found that B cells did proliferate in response to insoluble PHA covalently linked onto Sepharose particles. This result demonstrated that both T and B cells had the capacity to respond to PHA but that the physical configurational form of the mitogen played a critical role in the triggering event.

Kornfeld and Kornfeld (1969) and Allan et al. (1971) found that the chemical nature of the receptor sites for PHA was a glycopeptide. Based on immunofluorescence observations, Osunkoya, Williams, Adler and Smith (1970) and Smith and Hollers (1970) suggested that the binding sites for Con-A were restricted to a crescent-shaped area of the lymphocyte cell surface. On the contrary, Greaves et al. (1972), using the same techniques, stated that the binding sites were uniformly distributed over the entire cell surface. However, they added that when lymphocytes were metabolically active, an iteresting altered localization of bound Con-A molecules to one pole of the cell (capping) took place.

Concerning the responsiveness of lymphocytes and their ability to transform into blast cells in vitro, many workers have stated that there were substances, other than phytomitogens, which could induce such blastoid transformation. These substances were collectively

termed lymphocyte activating factors (LAF) or mitogenic factors (MF). Jacobsson and Blomgren (1975) showed that the lymph node cells of the mouse had the capacity to release a mitogenic factor when exposed to phytomitogens. They proposed that the relative capacity of T lymphocytes to produce mitogenic factors seemed to parallel their phytomitogen reactivities. They also assumed that there existed one subpopulation of cells in the thymus more responsive to phytomitogens than to mitogenic factors and another more responsive to mitogenic factors than to phytomitogens. In addition, they proposed that the cells, more responsive to phytomitogens, were those that could produce the mitogenic factor. Adorini, Ruco, Uccini, De Franceschi, Baroni and Doria (1976) and Blyden and Handschumacher (1977) showed that the lymphocyte activating factors were produced by lipopolysaccharide stimulated human adherent cells (monocytes) and peripheral leukocytes. They added that other macrophage activators, antigen-antibody complexes and barium sulfate induced the production of LAF. Adorini et al. (1976) studied the effect of lipopolysaccharide injection on the response of mouse thymocytes to PHA and Con-A. They found that the thymocytes from lipopolysaccharide-treated mice were more reactive to PHA and Con-A than normal thymocytes. They added that the increase of responsiveness was dependent upon the dose of the injected lipopolysaccharide.

Studies on the mitogenic stimulation of lymphocytes by certain specific antigens aroused the assumption that the action of phytomitogens was of immunological significance. Elves and Israëls (1963) suggested that the lymphocyte transformation in response to PHA might be the result of antigenic stimulation and presumably accompanied by the production of antibody. Again,

Elves, Roath and Israëls (1963) stated that if this was true, a similar process should result from the exposure of lymphocytes "in culture" to any antigen to which the cell donor had previously been sensitized. The validity of such proposal had been already emphasized by Gowans, Gesner and McGregor (1961) and Porter and Cooper (1962) in their "in vivo" studies on antigenically-challenged laboratory animals. Pearmain, Lycette and Fitzgerald (1963) tested this hypothesis by substituting tuberculin for PHA in peripheral blood leukocyte cultures made from persons sensitized to tuberculin and others whose cells were not previously exposed to it. They found that there was no mitosis in cultures of those cases not previously exposed to tuberculin whereas the formerly sensitized cells showed a reaction similar to that obtained with PHA. The authors also reported on the leukaemoid response which could be obtained by administering tuberculin to tuberculous rabbits. In addition, the production of antibody by the transformed lymphocytes was reported by Bach and Hirschhorn (1963), Hirschhorn, Schreibman, Verbo and Gruskin (1964), Forbes and Henderson (1966), Scheurlen (1968) and Pick, Brostoff, Krejci and Turck (1970).

Schrek and Donnelly (1961) - in their study of the morphology and motility of cultured lymphocytes from 20 leukaemic and non-leukaemic patients - observed a small number of large cells with large clear nuclei and prominent nucleoli. The authors noted that the culture proved to be a mixture of bloods from 2 patients with haemochromatosis but they did not elaborate on this observation. André, Schwartz, Mitus and Dameshek (1962) noticed a similarity between the morphology of such cells and those which developed "in vivo" in lymph nodes and spleen following skin homografting in rabbits. Gowans, McGregor, Cowen and

Ford (1962), Bain, Magdalene and Lowenstein (1964), Chapman and Dutton (1965) and Dutton (1965) concluded that the antigenic stimulus in this case might be the foreign tissue. Similarly, the lymphocyte activation in response to anti-lymphocyte serum (Gräsbeck,Nordman and Chapelle, 1963) and antisera to allotypic determinants of immunoglobulins (Sell and Gell, 1965 and Oppenheim, Rogentine and Terry, 1967) all emphasized that the biochemical, the metabolic and the morphological changes occurring in activated lymphocytes under these circumstances were much the same as those observed when lymphocytes were stimulated with phytomitogens. In this context, Rieke (1966) and Adler, Takiguchi and Smith (1969) correlated the inability of lymphoid cells to undergo the mitogen-induced transformation, to their immunological impotence when such cells were derived from neonatally-thymectomized animals. In addition, such inability of blastoid transformation was reported in patients with chronic lymphocytic leukaemia complicated with hypogammaglobulinaemia (Bernard, Geraldes and Boiron, 1964; Elves, Roath and Israëls , 1964; Quaglino and Cowling, 1964; Astaldi, Airo and Sauli, 1965 a; Astaldi, Costa and Airo, 1965 b; Oppenheim, Whang and Frei, 1965 and Elves, Collinge and Israëls, 1967). On the other hand, Coulson and Chalmers (1967), Ling (1968) and Oppenheim (1968) demonstrated that bacterial products activated lymphocytes in much the same fashion as phytomitogens. In this context, Horton, Raisz, Simmons, Oppenheim and Mergenhagen (1972) have stated that antigens present in bacterial culture filtrates of organisms common to the oral cavity did stimulate lymphocytes, from subjects with periodontal disease, to proliferate in vitro.

Möller (1970) supposed that phytomitogens trigger

lymphocytes by binding with, or in close proximity to, immunoglobulin-like antigen receptors. Raff (1970) and Jones, Torrigiani and Roitt (1971) found that B lymphocytes had considerably more cell surface immunoglobulin determinants than T cells. Blomgren and Anderson (1971) noticed that thymocytes demonstrated clear heterogeneity with Con-A, PHA and LM. They supposed that this variation was due to the presence of cortisone sensitive and cortisone resistant lymphocytes which differed in their immunological properties. In this context, Janossy and Greaves (1972) showed that cortisone sensitive cells were considerably activated with Con-A more than resistant cells. Greaves et al. (1972) anticipated that the capacity of PWM to stimulate B cells would be related to its affinity for either immunoglobulin itself or for cell surface binding sites in close proximity to immunoglobulin. On the contrary, Osunkoya et al. (1970) assumed that there was a similarity in the spatial distribution of the receptors for PHA and immunoglobulins on lymphoid cells but the differential susceptibility of the two types of receptors to trypsin indicated that they were two distinct entities. In their previous experiments, Osunkoya (1967) and Osunkoya, Mottram and Isoun (1969) have shown that trypsin destroyed the immunoglobulin receptors but had no direct effect on PHA receptors. Moreover, Greaves et al. (1972) stated that the reason to suppose that phytomitogens trigger lymphocytes by binding with, or in close proximity to, immunoglobulin-like antigen receptors was not convincing. They found that despite the reaction of Con-A, PHA and LM with carbohydrate-containing immunoglobulins, principally IgM, this activity was probably unrelated to mitogenicity since heat treatment of mitogen or absorption with erythrocytes removed the protein precipitating activity without

affecting appreciably the mitogenic potency. Furthermore, high concentrations of IgM did not inhibit PHA-induced lymphocyte activation. The latter authors added that the anti-immunoglobulin light chain antibodies which inhibited antigen-induced proliferation of human lymphocytes did not affect the PHA response. Nevertheless, they supposed that a di - or multi - valent binding of mitogens and subsequent receptor movement (i.e. capping) could distort the distribution of immunoglobulin molecules or otherwise affect adjacent membrane regions bearing immunoglobulins and that this was the prerequisite for activation.

In connection with the role of calcium ions in the control of cell kinetics and mitosis, Perris and Whitfield (1967 a, b) and Perris, Whitfield and Rixon (1967) have shown that several treatments which increased the concentration of ionized calcium in the plasma of the rat were accompanied with increased mitotic figures between the cells of the thymus and the bone marrow. Using the same tissues in the same animal, Perris, Whitfield and Tölg (1968) concurred the above-mentioned observations and added that this mitotic surge was quite different in different age groups; being higher in young growing rats. Perris et al. (1968) suggested that the division and growth of other cell types might also be influenced by the concentration of this ion since they observed a striking parallelism between the changes in total body growth rate and the concentration of ionized plasma calcium. In this context, many investigators reported on the enhancement of mitosis in many different cell types, grown in vitro, after increasing the concentration of calcium in the culture media (Hollingsworth, 1941; Tyler, 1941; Heilbrun, 1952; St. Amand, Anderson and Gaulden, 1960; Whitfield and

Rixon, 1962; Whitfield, Brohée and Youdale, 1966; Whitfield and Youdale, 1966; Fautrez-Firlefyn and Fautrez, 1967; Morton, 1968 and Rixon, 1968). Few years later, Alford (1970), Allwood, Asherson, Davey and Goodford (1971), Whitney and Sutherland (1972), Freedman, Martin and Gomperts (1975), Jensen, Winger, Rasmussen and Nowell (1977) and Freedman (1979) emphasized that calcium ion was involved in the responses of lymphocytes to several kinds of mitogen, based on the observation of increased Ca^{++} influx accompanying lectin stimulation of lymphocytes. On the other hand, Borberg et al. (1968) have shown that EDTA could inhibit the binding of I^{131}-labelled PHA to cells and that this inhibition could be overcome by the addition of Ca^{++} to the medium.

Basically in agreement with the above-mentioned findings, Edwards, Rimmer, Atkinson and Perris (1981) found that when rats or mice were immunized with sheep red blood cells, bacterial lipopolysaccharides or bovine serum albumin, a proliferative response could be detected in the bone marrow and spleen. This response was associated with a hypercalcaemic phase. Interestingly, parathyroidectomy - which resulted in a protracted hypocalcaemia - prevented the rise in bone marrow proliferation following antigenic challenge.

Recently, Hesketh, Bavetta, Smith and Metcalfe (1983) noticed an increase in the free cytoplasmic Ca^{++} concentration in thymocytes within a few seconds from the addition of concanavalin A. Based on immunofluorescence studies, they were able to estimate the duration of calcium signal in the mitogenic stimulation and concluded that it lasted only while Con-A was bound to the cell surface and terminated by capping. Izant (1983) exploited an ingenious experiment to reveal the role of calcium ions during

mitosis. Calcium chloride solution was microinjected into dividing cells - in the metaphase stage - and the time consumed to enter anaphase was estimated by 4-5 minutes compared with an average of 16 minutes in cells injected with Ca-free buffer. On the other hand, reducing the intracellular Ca^{++} concentration by injection of ethyleneglycol-bis-β-aminoethylether N,N'-tetraacetic acid (EGTA), increased the lag between injection and anaphase to 20 minutes or more. The author added that calcium solutions did not promote precocious chromatid separation indicating that the enhancement of metaphase termination was not due to a direct induction of centromere separation. Moreover, Ralph (1983) proposed that cyclic AMP normally controlled the cell cycle at a point in G1 phase only by virtue of its ability to exclude calcium required by the cells to progress past this point into S phase. The author found that increasing the influx of calcium by other routes induced by various factors could bypass the inhibitory effect of cyclic AMP and subsequently stimulated growth. Such a deduction was confirmed by McQuillan, Wojcik and Ralph (1984) and Metcalfe (1984) who showed that the net efflux of Ca^{++} occurred during growth inhibition and vice versa. However, Kaibuchi, Takai and Nishizuka (1985) proved that the situation was not as simple as this and were able to record a failure of mitogenesis in macrophage-depleted human peripheral lymphocytes despite the availability of PHA and calcium ions. They suggested that the macrophage-stimulated protein kinase C together with calcium ion mobilization were essential for receptor occupation by PHA.

HYPERCALCAEMIA AND LYMPHOPROLIFERATIVE DISORDERS.

The pathogenesis of cancer hypercalcaemia is an enigma that continues to challenge clinical investigators.

Despite the difference in the causative agent of hypercalcaemia, the morbid consequences of excessive osteolysis are usually similar.

Breast cancer was considered to be the most common malignant disease associated with elevated calcium level of the blood although a variety of other solid tumours were involved (Myres, 1960 and Lafferty, 1966). Among the haematologic malignancies, Cannelos (1974) considered the multiple myeloma as the most frequently culpable although hypercalcaemia was reported in both acute leukaemia and malignant lymphoma. Benevenisti, Sherwood and Heinemann (1961), David, Verner and Engel (1962), Green (1964), Jorden (1966), Zuffa, Mensatoris and Horvath (1967), McKee (1974), Johansson and Warner (1975), Norrby and Virkrot (1975), Mundy, Turcotte, Bardelin and Rick (1976) and Wang, Steier, Aung and Tobin (1978) reported the association of hypercalcaemia with chronic lymphatic leukaemia. Mundy, Luben, Raisz, Oppenheim and Buell (1974) suggested that an osteoclast activating factor (OAF) might be secreted by the malignant cells and be responsible for the associated hypercalcaemia. Shortly after, Norrby and Virkrot (1975), Raisz, Luben, Mundy, Dietrich, Horton and Trummel (1975) and Mundy et al. (1976) concurred the above assumption and Wang et al. (1978) were able to show that OAF had a molecular weight of 18,000 and its activity was destroyed by pronase and trypsin. In this context, Horton et al. (1972) and Raisz (1974) have reported that normal leukocytes were also able to release such OAF after stimulation by antigen or PHA in vitro.

Vitamin D and its metabolites (Gordon, 1966 and Raisz and Trummel, 1972), prostaglandins (Robertson, Baylink, Marim and Adkison, 1975 and Seyberth, Serge,

Morgan, Sweetman, Potts and Oates, 1975) and ectopic parathyroid hormone secretion (Sherwood, O'Riordan, Auerbach and Potts, 1967) together with OAF were considered to act humorally to stimulate osteoclasts to resorb bone and release calcium. In addition, Auerbach (1975) assumed that the prolonged immobilization due to disease - whether malignant or otherwise - might be of possible etiologic importance in the associated hypercalcaemia.

In connection with the role of vitamin D and its metabolites in malignancy-associated hypercalcaemia, the recent study undertaken by Ralston, Cowan, Robertson, Gardner and Boyle (1984) has shown that immunoreactive parathyroid hormone (PTH) concentrations were suppressed and the patients had detectable 1,25 dihydroxycholecalciferol concentrations than normocalcaemic cancer patients. The authors suggested that the active vitamin D metabolite might contribute to the pathogenesis and maintenance of the hypercalcaemia by stimulating bone resorption and/or by increasing the calcium absorption from the intestine. However, the authors assumed that the measurement of plasma 1,25 dihydroxycholecalciferol concentration did not provide a wholly reliable method for distinguishing the hypercalcaemia of malignancy from primary hyperparathyroidism. In the same context, Rosenthal, Insogna, Godsall, Smaldone, Waldron and Stewart (1985) detected the same elevation of plasma 1,25 dihydroxy-vitamin D in three patients with hypercalcaemia associated with malignant lymphoma. Moreover, the authors reported on a suppressed immunoreactive PTH level. Surgical excision of a solitary splenic lymphoma in one patient and medical therapy in another resulted in rapid normalization of the serum calcium and plasma 1,25 dihydroxy-vitamin D levels, a result which led the

authors to emphasize that the vitamin D metabolite might act as a humoral or systemic mediator of hypercalcaemia. In contrast, Hove, Horst, Littledike and Beitz (1984) have shown that there was an inverse proportion between the blood calcium level and the level of plasma 1,25 dihydroxy-vitamin D. The authors also concluded -from their study on calves and goats- that the concentration of calcium in plasma had the major regulatory role on the plasma level of 1,25 dihydroxy-vitamin D. In addition, Law, Bollman, Kumar and Heath (1984) proved that the plasma 1,25 dihydroxy-vitamin D levels in familial benign hypercalcaemia (hypocalciuric hypercalcaemia) were significantly lower than the control values. They, therefore, concluded that the hypercalcaemia of malignancy might be due to another factor rather than being tentatively a result of elevated plasma vitamin D metabolites.

Although elevation of PTH level in the serum may suggest the etiology of the hypercalcaemia of malignancy, demonstration of the production of PTH by tumor cells provides further insight into this phenomenon. Zidar, Shadduck, Winkelstein, Zeigler and Hawker (1976) reported a patient with acute myelogenous leukaemia who had the syndrome of hypercalcaemia and ectopic PTH production. The authors "in vitro" incubated leukaemic cells from the peripheral blood and noticed an increased level of PTH after 24 and 48 hours of incubation. In addition, freeze-thawing of the patient's cells yielded immunoreactive PTH. In this context, Ramsay, Brown, Nesbit, Coccia, Krivit and Krutzik (1979) have shown that leukaemic cells obtained from the cerebrospinal fluid also demonstrated the production of PTH in culture. They relied on this observation to support the hypothesis that the leukaemic cells, regardless of their

environment, had the capability to produce PTH. Again, they correlated the inappropriate serum levels of PTH in leukaemic patients to a loss of control on the hormone secretion and/or to the ectopic secretion by malignant cells.