INTRODUCTION

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 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; 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.

PHYTOMITOGENS AND MITOGENIC FACTORS

Many workers have studied the cell division and the cell cycle in cells readily responsive to stimulants of mitosis; namely, phytomitogens and mitogenic factors. Examples of those phytomitogens are phytohemagglutinin

(PHA), concanavalin A (Con-A), lentil mitogen (LM) and pokeweed mitogen (PWM). Borberg, Yesner, Gesner and Silber (1968); Landy and Chessin (1969); Powell and Leon (1970) and Allan, Auger and Crumpton (1971) observed that lymphocytes were greatly responsive to such phytomitogens. They suggested that the initial interaction of phytomitogens with lymphocytes 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. 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 and 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 resistant lymphocytes which differed in their immunological properties. 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.

Kornfeld and Kornfeld (1969) and Allan et al. (1971) found that the chemical nature of the receptor sites for PHA was glycopeptide. Based on their 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 interesting altered localization of bound

Con-A molecules to one pole of the cell took place.

Rigas and Tisdale (1969) studied the effect of dose difference of the mitogen on the mitotic activity of cultured cells. They found that 5 microgram of purified PHA-P optimally stimulated one million leukocytes. However, the use of 10 microgram resulted in inhibition of mitosis; a result which they attributed to toxic effects. On the contrary, Coulson and Williams (1974) observed that adding more amount of phytomitogen to the culture medium increased the number of mitogen-stimulated lymphocytes. In the same context, Blyden and Handschumacher (1977) have shown that lymphocytes exhibited great responsiveness to factors, other than phytomitogens, known as lymphocyte activating factors (LAF) which have been shown to be 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, Ruco, Uccini, De Franceschi, Baroni and Doria (1976) studied the effect of lipopolysaccharide injection on the response of mouse thymocytes to PHA and Con-A. They found that thymo-

cytes 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.

Jacobsson and Blomgren (1975) proposed that the relative capacity of thymocytes to produce mitogenic factors seemed to parallel their phytomitogen reactivities. They have shown 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. They proposed that the cells, more responsive to phytomitogens, were those that could produce mitogenic factors.

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 Mc Gregor (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), Bernard, Geraldes and Boiron (1964), Hirschhorn, Schreibman, Verbo and Gruskin (1964), Quaglino and Cowling (1964), Astaldi, Airo and Sauli (1965 a), Astaldi, Costa and Airo (1965 b), Oppenheim, Whang and Frei (1965), Forbes and Henderson (1966), Coulson and Chalmers (1967), Ling

(1968), Oppenheim (1968), Scheurlen (1968), Pick, Brostoff, Krejci and Turck (1970) and Horton, Raisz, Simmons, Oppenheim and Mergenhagen (1972).

EFFECT OF PROGESTERONE ON LYMPHOCYTE KINETICS

The effect of female sex hormones on the lymphocyte cell kinetics was studied by many workers and the results of such investigations were contradictory. Clemens, Siiteri and Stites (1979) showed that progesterone at relatively high concentrations (1-20 µg/ml) was found to inhibit mitogen - stimulated cultures of peripheral blood mononuclear cells (P.B.M.C.). In addition, Corsini and Puppo (1982) evaluated if medroxyprogesterone acetate (MPA) had an immunosuppressive action as progesterone and other steroid hormones, and found that the blastogenic response to mitogen showed a significative inhibitory effect only when the drug was used at a concentration of 100 nanogram/ml. In the same context, Stites, Bugbee and Siiteri (1983) suggested that progesterone selectively blocked T cell activation by a direct effect on T cells, whereas cortisol interfered with both monocytes and T cells. In other sites, lymphocyte blastogenic responsiveness to mitogens was slightly reduced in

endometrial cancer patients receiving a high-dose of medroxyprogesterone acetate for 3 months (Gronroos and Eskola, 1984).

Concerning the effect of progesterone on DNA synthesis, Johannisson, Langren and Hagenfeldt (1977) suggested that progesterone, released in utero, might interfere with DNA synthesis of the epithelial cells, which might result in a disturbed metabolic function of the endometrium at the time of implantation. Clemens et al. (1979) investigated the kinetics of progesterone-mediated suppression of thymidine incorporation and DNA synthesis at various times during lymphocyte activation. They found that if progesterone was present only at the beginning of culture, subsequent DNA content, but not thymidine incorporation, was suppressed. However, if added late during the activation process, progesterone reduced thymidine incorporation independent of cellular DNA content. Moreover, if the hormone was present during the entire culture period, both were suppressed. Similarly, Ogawa, Sueda and Matsui (1983) showed that cortisone and progesterone significantly suppressed ^{3}H-thymidine incorporation in phytohaemagglutinin-stimulated peripheral blood mononuclear cells.

On the contrary, other studies have shown an increased mitotic activity in uterine fibromyomas in patients using exogenous hormones. Tiltman (1985) compared the mitotic activity of fibromyomas in three groups of patients. The first group was using progesterone (medroxyprogesterone acetate) only, the second group had never used any exogenous hormone, and the third group was using a combined oestrogen/ progesterone oral contraceptives. The results showed that the first group had a significantly higher mitotic activity in fibromyomas than the patients from the other two groups.

EFFECT OF OESTROGEN ON LYMPHOCYTE KINETICS

Ablin, Bruns, Guinan, Al-Sheik and Bush (1974 a) noted a significant reduction in lymphocyte proliferation as measured by the incorporation of ^{3}H-thymidine of PHA-stimulated normal human peripheral blood lymphocytes when cultured in the presence of diethylstibesterol diphosphate (a synthetic oestrogen). Similarly, Ablin, Bruns, Guinan, Sadoughi and Bush (1974 b) found that the "in vitro" blastogenic transformation of T lymphocytes was inhibited by oestrogen. In addition , Wyle, Kent and Geist (1975) demonstrated the suppres-

sive effect of oestradiol on tuberculin-and PHA-stimulated peripheral blood leukocytes. Again, ablin, Bruns, Guinan, Al-Sheik and Bush (1976) observed a similar inhibition when adding autologous serum, obtained from patients with prostatic carinoma having oestrogen therapy, to normal blood culture. Moreover, Clemens et al. (1979) stated that oestradiol at a relatively high concentration (1-20 µg/mL) was found to inhibit mitogen-stimulated cultures of P.B.M.C. In contrast, Gerschenson , Depaoli and Murai (1981) and Gerschenson, Gorski and Prescott (1984) noted that the addition of 17-B oestradiol to primary cultures of rabbit uterine cells resulted in the induction of DNA synthesis. This phenomenon was present only in cells cultured at low density. Culture medium taken from high density cultures, however, showed an inhibited DNA synthesis; suggesting that those cells produce a soluble oestrogen-inhibiting factor.

Antibody production by the transformed cells was found to be affected by steroid sex hormones. Clemens et al. (1979) showed that testosterone and oestradiol induced immunomodulation of the lymphoid system in vivo and in vitro. Peck, Burgner and Clarck (1973)

described the entery of the hormones to the inside of the cell as being a process of passive diffusion. However, Milgrom, Atger and Baulieu (1973) postulated that the hormones enter the cells by a process of facilitated diffusion. Specific binding sites for steroids have been demonstrated on the plasma membrane of human lymphocytes (Tubiana, Derre, Carcassonne and Martin, 1984), and steroid binding on their membrane "receptors" induced cellular functions such as cyclic AMP uptake (Chew and Rinard, 1974).

Hulka, mohr and Lieberman (1965) proved that antibody production by rabbits treated with oestradiol or 17-hydroxyprogesterone appeared to fall within the normal range. However, progesterone and medroxyprogesterone consistently depressed antibody production below the normal range, as did cortisone. Inspite of that, the authors found that neither oestradiol nor progesterone prolonged graft survival. In addition, they suggested that the direct suppressive action of oestrogens on the reticuloendothelial system might be due to a depression of oxygen uptake by lymphocytes "in vitro" in the presence of these compounds.

Concerning the effect of oestrogen on DNA

synthesis, Mcmanus and Welsch (1984) found that the administration of oestrogen or thyroxine significantly increased the labelling index of ^{3}H-thymidine incorporation in human breast ductal epithelium maintained in athymic nude mice. In the same connection, oestrogen administration was shown to increase the net DNA synthesis in cultured human breast cancer cells; the stimulation was most evident after 36 hours of hormone treatment (Aitken and Lippman, 1982). conversely, tamoxifen (antioestrogen) produced a substantial inhibition of net DNA synthesis after 36 hours of hormone treatment. Moreover, the cell kinetic effects of oestrogen and antioestrogens on human breast cancer cell cycle have been discussed by Osborne, Boldt and Estrada (1984). They showed that tamoxifen effect was reversed with the addition of 17B-oestradiol.

Diethylstiblesterol (DES) is one of the few known causes of prenatally induced cancer in humans (Herbst, Ulfelder and Poskanzer, 1971). However, whether its mode of action is by direct interaction with the chromosomal material, by interference with cell division, or by a hormonal effect on cell growth is still not clearly understood. In addition, DES was

shown to induce unscheduled DNA synthesis in HeLa cells (Martin, McDermid and Garner, 1978), cell transformation in Syrian hamster embryo cells (Barrett, Wong and McLachlan, 1981), and sister chromatid exchanges in human lymphocytes (Hill and Wolff, 1982). Again, DES has been reported to induce chromosomal aberrations in mouse bone marrow (Ivett and Tice, 1981). An increasing amount of evidence suggests that at least one part of the carcinogenicity of DES is due to the interference with spindle formation resulting in aneuploidy and polyploidy (Rao and Engelberg, 1967; Ishidate and Odashima, 1977; Sawada and Ishidate, 1978; Danford and Parry, 1982; Parry, Danford and Parry, 1982 and Tsutsui, Masizumi, McLachlan and Barrett; 1983). In addition, the induction of aneuploidy following in vivo exposure to DES has been reported by Chrisman (1974), Chrisman and Hinkle (1974) and Henderson and Regan (1985).

On the other hand, diethylstilbesterol was reported to produce uterine cancer on prolonged administration (Gusberg and Hall, 1961), and vaginal carcinomas through transplacental effects on the offspring of women who received the durg during pregnancy (Herbst, Kurman and Scully, 1972).

In concept of the direct effect of sex hormones on the biology of the lymphatic structures, Schacher, Browne and Selye (1937) noticed a definite thymic involution as well as a decrease in the size of the spleen and lymph nodes following oestrone administration to adrenalectomized rats. Moreover, oestradiol was shown to produce a significant decrease in thymus weight, whereas progesterone administration caused no alteration in the thymus gland.

Many years later, Andersson and Muntzing (1971) concurred the above results and recorded a marked reduction in the weight of the spleen following long-duration oestrogen treatment in male rats. The latter authors attributed this weight loss to a histochemically-demonstrated atrophy of the white pulp. On the contrary, Weaver (1955), Luz, Marques, Ayub and Correa (1969) and Sobhon and Jirasattham (1974) found no detectable changes in the lymph nodes or the spleen in comparison to the thymus.

On the other hand, the absolute number of the peripheral blood lymphocytes was shown to decrease

after the administration of oestrogen to dogs (Crafts, 1941) and to rats (Sobhon and Jirasattham, 1974). In human beings, similar results were obtained by Nelson and Hall (1965) in their study on the morphological changes in external iliac lymph nodes during early pregnancy and the puerperal period. They recorded a regression of the germinal centres in the early pregnancy and the effect was accentuated as pregnancy progressed. Moreover, they noticed a complete absence of the germinal centres during the first three days of puerperium; a result which persisted for 4 weeks postpartum.